SECTION 5: Improving propagation through spectral manipulation

5.0. INTRODUCTION

Propagation from vegetative cuttings is an effective method of rapidly multiplying numbers of plants and forms an important part of the hardy nursery stock industry. Cuttings are susceptible to dehydration once collected. Sunlight, while important for plants, also contributes to the dehydration of cuttings via heating of the plant material (which increases evaporative demand) and via light-induced stomatal opening (a predominantly blue light response). To prevent dehydration, cuttings are kept in humid environments, often achieved with misting systems. However, while light is one of the major factors driving dehydration, little attention other than simple shading is given to the light environment. In this section we examine the potential for using LED lights to create a light spectrum that is optimised to maximise cutting survival and speed of rooting. Optimising strike rates has the potential to greatly improve the efficiency of propagation businesses especially those focusing on difficult to root species.

Avoiding dehydration is vital for efficient rooting of cuttings but the hormonal status of cuttings is also very important for effective, rapid rooting. Application of hormones to cuttings is common practice in the industry due the great improvements in rooting that can be achieved. However, light quality is also known to alter the synthesis and transport of hormones (Friml 2003). Spectral manipulation is, therefore, expected to alter the speed and efficiency of rooting via its effects on endogenous hormone concentrations of cuttings.

In the trials reported in this section we examine the effect of different red, blue, and far-red light spectra on the survival and rooting of cuttings from several species. We have also begun to examine the effects of light quality on the hormone status of cuttings using tomato as a model system.

Species examined include:
Photinia
Eleagnus
Rhododendron
Santomlina
Iberis
Clematis,
Lavender
Thyme
Tomato *(model system for hormone analysis)*

5.1. METHODS

5.1.1. Plant material

All the plant material for these experiments were provided by commercial propagators (see Table 5.1). All cuttings were trimmed to size, placed in moist substrate. Following grower instructions some cuttings were treated with 1.5% Rhyzopon rooting powder prior to sticking, Table 5.1 shows which cuttings were treated. Trays were placed under the light treatments and enclosed under a clear polythene tent and misted to maintain high humidity. The temperature and humidity within the tents were monitored and the plants misted as required to reduce dehydration which was especially important during the first week after collection. The tents were opened once per week to vent the system to maintain plant health.

Photinia, Elaeagnus, and Rhododendron material.

Field-grown cutting material of photinia (Red Robin), *Elaeagnus ebbingei,* and a dwarf rhododendron (Scarlet Wonder) were supplied by New Place Nurseries (Mr John Hedger). Photinia and rhododendron material was delivered on 10th September 2014. Elaeagnus material was delivered on the 23rd September 2014. Cuttings were dipped in 1% Rhizopon AA to promote rooting and planted in a 2:1 peat:perlite mixture. Plants were irrigated using the ebb and flood systems as required.

Santolina 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.

Iberis

Iberis 'Absolutely Amethyst' cuttings were supplied by Kernock Park plants. Cuttings were collected week 15 of 2016. 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. Iberis cuttings were collected from stock plants grown under the Unlit and Supplemental treatments (see light treatments section).

Table 5.1. Details of the cutting material, cutting supplier, history of the mother stock plants, type of substrate used, use of rooting powder and which year of the study the experiments were performed.

Crop	Supplier	Stock plant location	Substrate used	Rooting powder	Year of project
Photinia (Red Robin)	New Place Nurseries	Field collected	50:50 M2:Perlite mixture	Rhizopon	1
Eleagnus ebbingei	New Place Nurseries	Field collected	50:50 M2:Perlite mixture	Rhizopon	1
Rhododendron (Scarlet Wonder)	New Place Nurseries	Field collected	50:50 M2:Perlite mixture	Rhizopon	1
Santolina (Lemon Fiz)	Kernock Park Plants	Glasshouse	Elle plugs	None	2
Iberis	Kernock park plants	Glasshouse	50:50 M2:Perlite mixture	None	2
Clematis (The President)	Micropropagation Services	Field collected	50:50 M2:Perlite mixture	None	2
Thyme vulgaris (Silver Posie)	Kernock park plants	Glasshouse	Elle plugs	None	3
Lavandula provençal (Antibes)	Kernock park plants	Glasshouse	Elle plugs	None	3

Clematis

Tip and nodal cuttings of clematis 'The President' were provided by Micro Propagation services. Cutting material was collected from field grown plants during 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.

Lavender

Cuttings of *Lavandula provençal* (Antibes) were provide by Kernock Park plants. Cuttings were received on the 24th January 2017 and planted on the 25th January 2017 at STC. Cuttings were planted in ellegard plug, 100 cell trays.

Thyme

Cuttings of *Thyme vulgaris* (Silver Posie) were provided by Kernock Park plants. Cuttings were received on the 24th January 2017 and planted on the 25th January 2017 at STC. Cuttings were planted in ellegard plugs in 100 cell trays.

5.1.2. Light treatments

All light measurements reported were made within the polythene structures once condensation had built up so the measurements were representative of the experimental conditions.

Post-excision light treatments (photinia, elaeagnus, and rhododendron)

Cuttings were placed under eight light treatments examining the influence of red:blue ratio and red:far-red ratio on performance. For the red:blue experiments the blue percentage was varied from 100% blue to 100% red (referred to as 0% Blue) with two intermediate treatments of 33% and 66% blue. The intensity in each treatment was 70 µmol m^{-2} s^{-1} (Appendix: Light treatments 10). The red:far-red treatments had a red:blue (89% red: 11% blue) background of 70 µmol m^{-2} s^{-1} and four far-red intensities 0, 15, 30, 48 µmol m^{-2} s^{-1} (Appendix: Light treatments 10).

Pre-excision light treatments (Santolina)

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 51µmol m^{-2} 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).

Post-excision light treatments (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 (Appendix: Light treatments 11). Four red: blue treatments were included 100% blue, 61% blue, 33% blue and 9%blue. An additional 9% blue light treatment that also included 15µmol m^{-2} s^{-1} far-red light with was included to assess the influence of far-red on rooting. A white LED light treatment was included that contained 9% blue light and 17% green light was included to examine the effect of green light. For these six treatments total photon irradiance varied between 80 and 93µmol m^{-2} s^{-1} between treatments. Three red:blue (11%blue) treatments with differing photon irradiances 36.5, 65.3 and 84 µmol m^{-2} s^{-1} were used to examine the influence of daily light integral (2.1, 3.8 and 4.8 mol m^{-2} d^{-1}) on rooting efficiency.

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 5.2 for details.

Pre-excision light treatments (Iberis)

The iberis cuttings were collected from mother stock plants grown through the winter under two different light treatments **1) Unlit** - control treatment exposed to only natural light , **2) Supplemental** - natural light plus LED light provided at 51µmol m^{-2} s^{-1} (Philips GreenPower top lights 6% blue: 94% red) for 12 hours per day.

Table 5.2. Number of santolina cuttings from each pre-excision treatment (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

Post-excision light treatments (Iberis and Clematis)

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 (Appendix: Light treatments 12). Four red:blue treatments were included 100% blue, 61% blue, 33% blue and 15%blue. Two additional 15% blue light treatment that also included 9 or 15µmol m^{-2} s^{-1} far-red light were included to assess the influence of far-red on rooting. A white LED light treatment was included that contained 9% blue light and 17% green light was included to examine the effect of green light. For these six treatments total photon irradiance varied between 80 and 93µmol m^{-2} s^{-1}. Two red:blue (11%blue) treatments with differing photon irradiances 37.8 and 73 µmol m^{-2} s^{-1} were used to examine the influence of daily light integral (2.18, 4.23 mol m^{-2} d^{-1}) on rooting efficiency.

Post-excision light treatments (Lavender and Thyme)

The post excision light treatments were performed in the LED4CROPS facility using Philips GreenPower Research LED modules (red, blue and far-red modules). Four light treatments were set up to examine the influence of red:blue ratio on survival and rooting (Appendix:

Light treatments 12). The four red:blue treatments included were 100% blue, 52% blue, 30% blue and 0%blue (100% red).

5.1.3. Assessments of propagation efficiency

At the end of each trial the number of dead (D), living (L) and rooted (R) cuttings were determined. Percentage survival (%S) was calculated as:

$$\%S = (L+R) / (L+R+D)$$

Survival corrected rooting ($\%R_S$) was calculated as

$$\%R_S = R / (R+L)$$

To assess root development substrate was carefully washed from the roots of the cuttings. The number of primary roots growing out of the stem was counted and the length of the longest root was determined.

Root number was determined by carefully washing soil from the roots. The number of primary roots (those growing out of the stem) was counted.

5.2. RESULTS

5.2.1. Photinia ‘Red Robin’

Under the different red: blue light treatments the survival of photinia cuttings varied considerably between treatments (Figure 5.1A). Survival increased by ~20% as the blue light percentage increased from 0% to 15% but decreased as the blue percentage increased further. Survival was similar in cuttings grown under 0% blue (100% red) and 30% blue but all cuttings died when propagated under 100% blue light. The additionof far-red light under to background of red: blue (11% blue light) was observed to have a weak

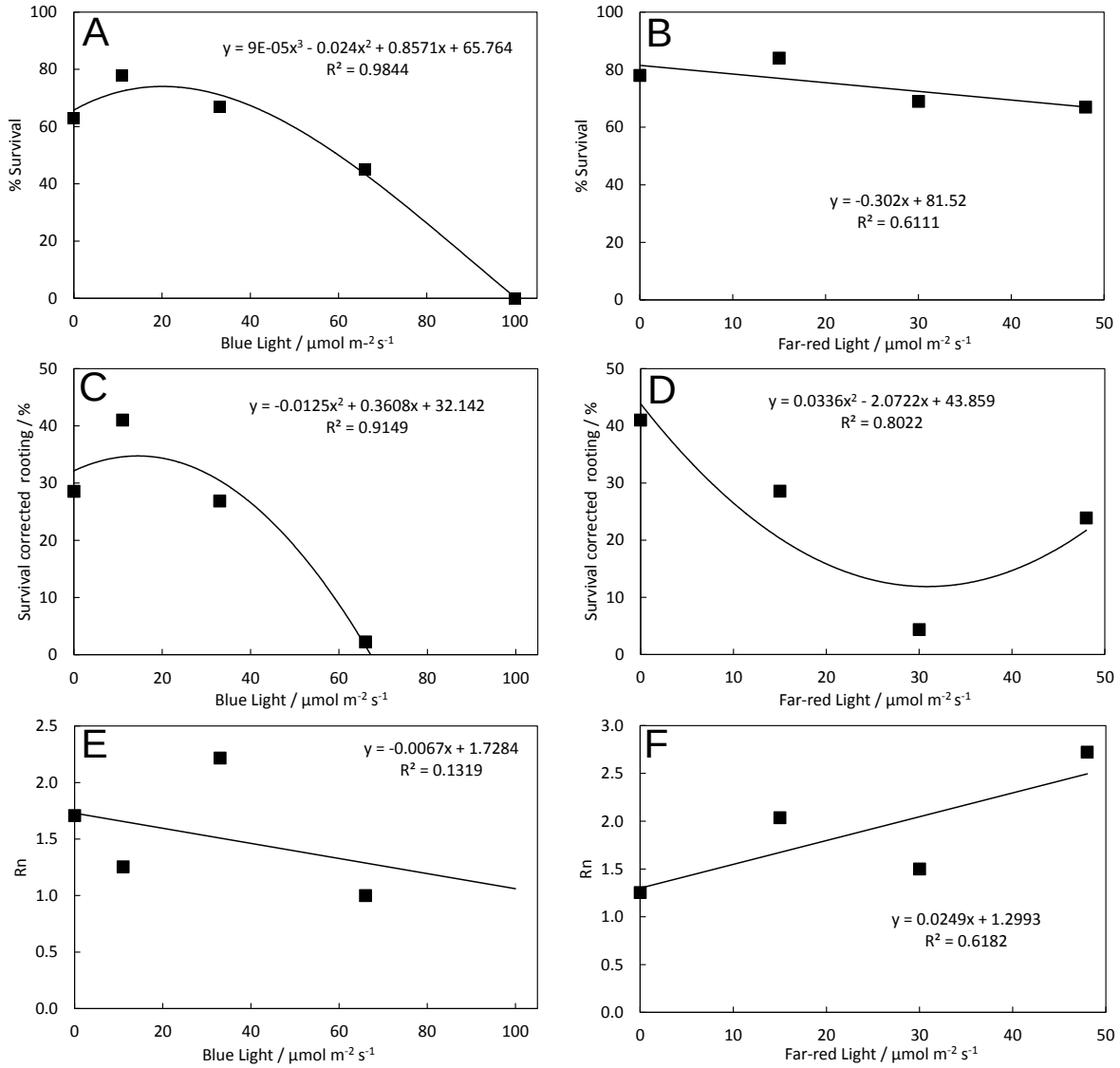

Figure 5.1. The influence of blue light percentage (left side) and far-red (right side) on the percentage survival (A,D), survival corrected rooting percentage (B,E) and root number (Rn: C, F) of Photinia (red robin) cuttings.

negative influence on cutting survival, with greater than 15 µmol m^{-2} s^{-1} of far-red causing a slight reduction in cutting survival (Figure 5.1B). The survival corrected rooting was lower than 41% of cuttings across all the photinia trials (Figure 5.1C and Figure 5.1D). Percentage rooting was greatest under 11% blue light (41%). Rooting percentages decreased under 100% red light (29%) and at higher percentages of blue light. Percentage rooting was lowest under 60% blue light where only 2% of cuttings formed roots. The inclusion of far-red in the spectrum resulted in a decrease in the percentage of cuttings that formed roots though the numbers of roots that formed was variable between treatments. For the red: blue treatments there was no significant relationship between numbers of roots and blue percentage (Figure 5.1E). For the far-red treatments an increase in far-red resulted in an increased number of roots (Figure 5.1F). However, due to the variation observed between treatments it may be the case that this trend is an artefact of measurement uncertainty. While the rooting percentages were low in these trials the cuttings were observed to produce a considerable amount of callus, the precursor to root formation in photinia (Figure 5.2). Callus was observed to continue to grow throughout the experiment with fresh callus observed even after three months. The greatest amount of callus was observed in the 33% blue treatment and lowest amount was observed in the 66% blue light treatment (no cuttings survived in the 100% blue treatment). Far-red treatments also appeared to result in an increase in callus formation. However, the 0 µmol m^{-2} s^{-1} far-red treatment is inconsistent with the data from the red: blue treatments. Based on the data in Figure 5.2B 11% blue light should generate a callus with a mass of greater than 2g. If this was the case the data may indicate little or no effect of far-red on callus formation.

The cuttings that survived were transplanted in to 11 cm pots and grown on under four different red:blue treatments (100%blue, 66% blue , 33% blue and 0% blue). The different light treatments resulted in considerable difference in plant morphology (Figure 5.3). The plants grown under 15 % blue light were the tallest plants and least compact. The plants grown under the other treatments were similar in height but different in appearance. The 100% blue plants were less compact than all but the 15% blue treatments and the leaves were flatter and more horizontal than the other treatments. The 66% and 33% were similarly compact but overall the 33% treatment produced the best looking plants.

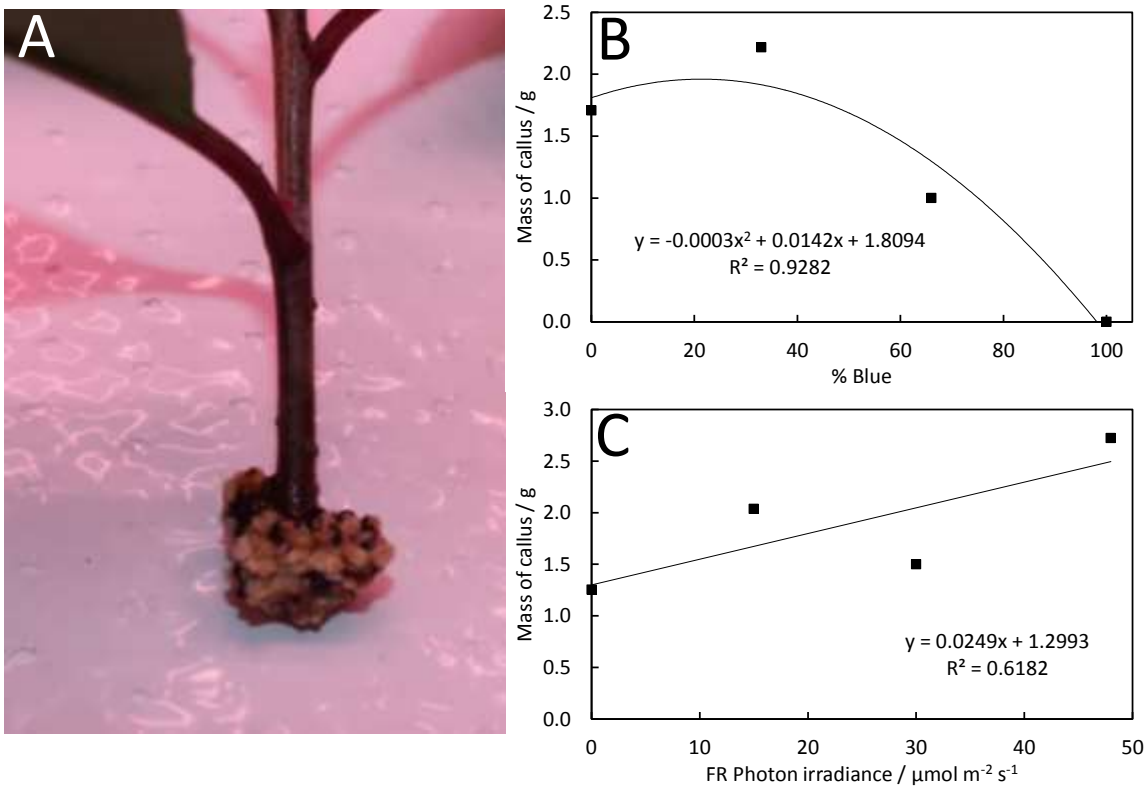

Figure 5.2. **A)** An example to the callus formation observed in the unrooted photinia cuttings. **B)** The influence of blue percentage on mass of callus produced by photinia cuttings. **C)** The production of callus in photina cuttings grown under a standard red: blue (11% Blue) with different quantities of additional far-red light.

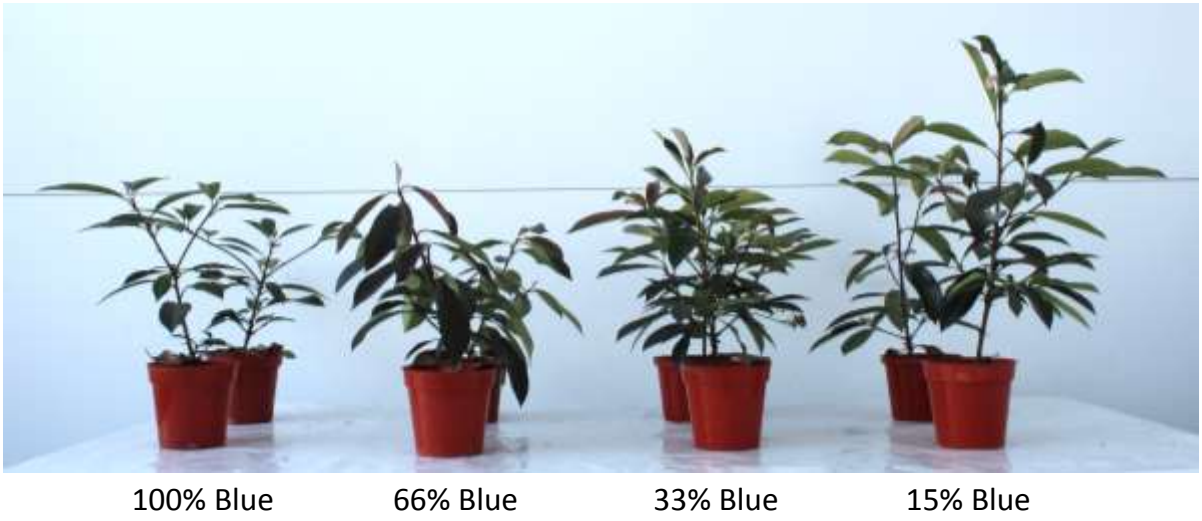

Figure 5.3. Photinia plants grown under 4 different light treatments.

5.2.2. Eleagnus

The eleagnus cuttings were assessed for wilting 2 weeks after transfer to the different light treatments. Each plant was scored 1 to 3 for wilting with 1 being no wilting and 3 being severe wilting (Figure 5.4). Wilting increased as the blue light percentage increased. In the 100% blue light treatment the wilting was so extensive that the plants shed their leaves, with a large proportion of the plants losing all their leaves after 1 week. Far-red was also observed to increase wilting, though leaf shedding was less prevalent in these treatments than the 100% treatment.

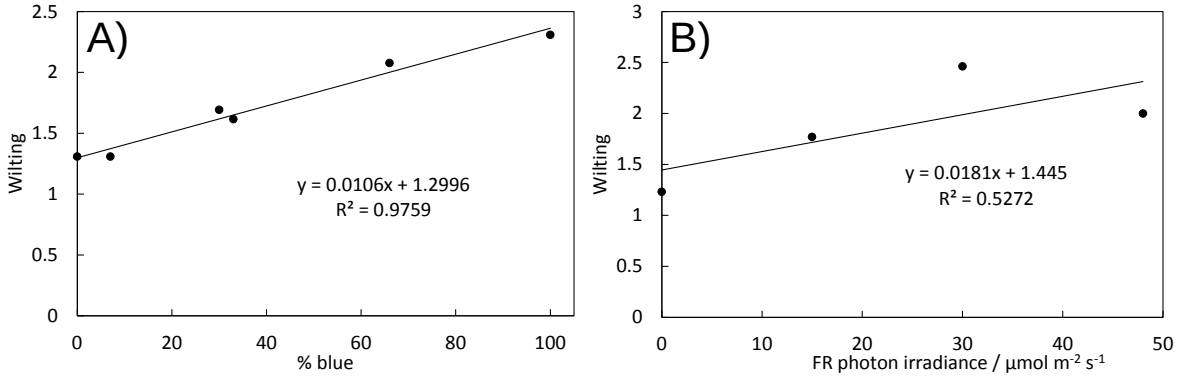

Figure 5.4 Observed wilting of eleagus cuttings 2 weeks after exposure to the different light treatments. **A)** Influence of different red blue treatments and **B)** the influence of different far-red treatments.

The survival of eleagnus cuttings was also strongly influenced by the blue light percentage (Figure 5.5A) and between the 33% and 66% blue light treatments survival dropped from 57% to 15%. Survival was also greatly reduced by the presence of far-red light in these experiments with survival dropping from 61% to 14% as the far-red photon irradiance increased from 0 to 48 µmol m^{-2} s^{-1} (Figure 5.5B).

The survival corrected rooting percentages were variable between the different red: blue treatments (Figure 5.5C) but no overall trend in response to blue percentage was observed. Far-red light had a negative impact on survival corrected rooting in this trial (Figure 5.5D). % rooting decreased from 26 to 6% as the far-red photon irradiance increased from 0 to 15 µmol m^{-2} s^{-1}. At the highest far-red intensity the survival corrected rooting increased, though this was probably as a result of the cutting low survival in this treatment.

Numbers of roots was also influenced by light quality. In the red: blue treatments the most roots were produced under the 66% blue light treatment and fewest under the blue light treatment. Even a small amount of blue light (11%) resulted in a doubling of the numbers of

roots produced compared with the 100% red light treatment. Far-red on the other hand had a negative impact on numbers of roots. The no far-red treatment produced ~13 roots per cutting while the treatments with far-red produced only ~6 roots per cutting.

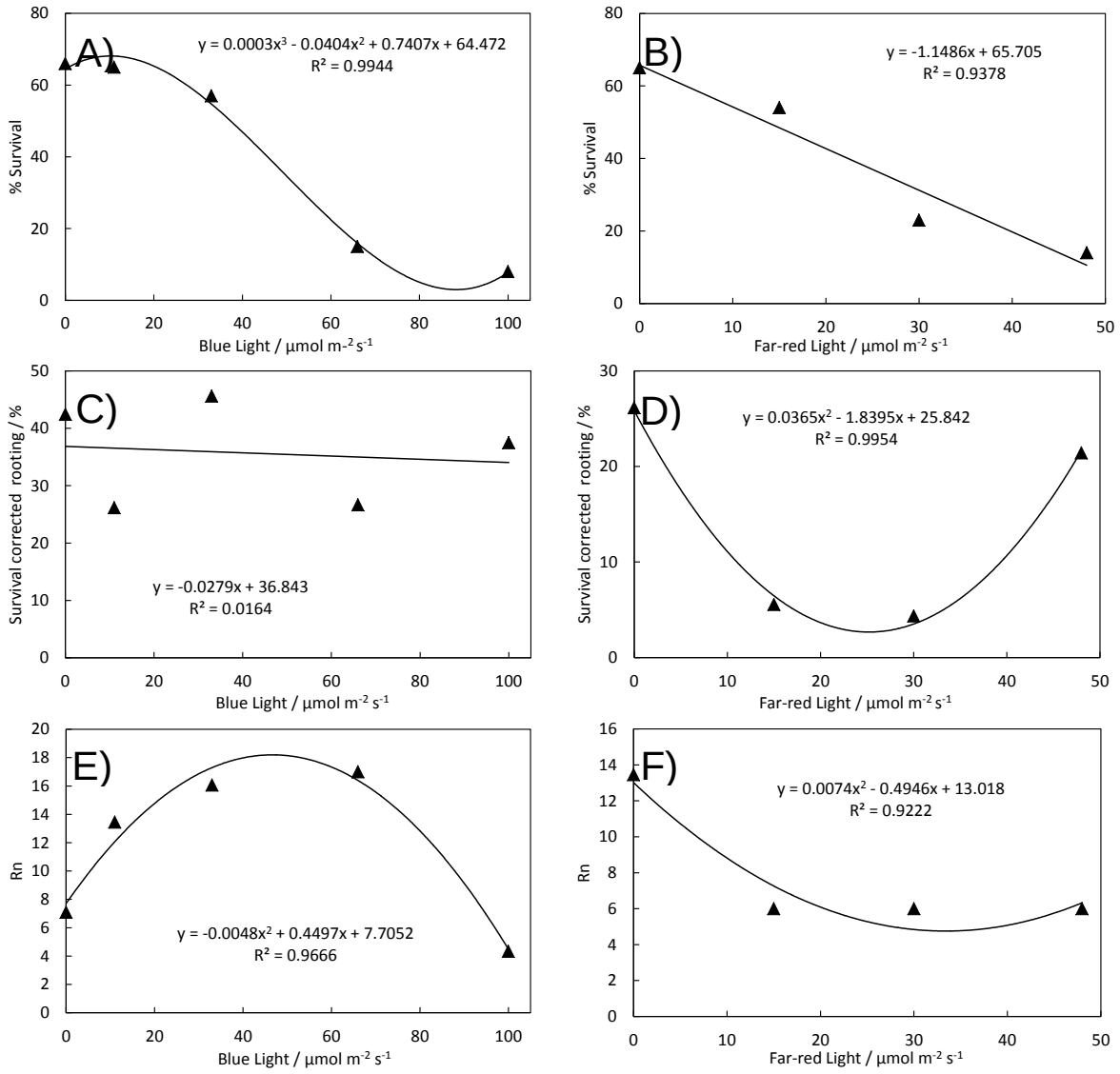

Figure 5.5. The influence of blue light percentage (left side) and far-red (right side) on the percentage survival (**A,B**), survival corrected rooting percentage (**C,D**) and root number (Rn: **E, F**) of Eleagnus (red robin) cuttings.

Following on from the propagation trial a selection of the rooted eleagnus liners were potted up and grown on under four different light treatments, 100% blue light, 66% blue light, 33% blue light and 15% blue light. The plants showed considerable differences in size and morphology (Figure 5.6). The most compact plants with the shortest internodes were those grown under 66% blue light (Figure 5.7). The plants grown under 33% blue light were less

compact but also grew more rapidly and produce longer stems than those grown under 66% blue. The plants grown under 100% blue light exhibited signs of etiolated growth especially at the top of the plant, which was quite stretched in appearance. The plants grown under 15% blue light produced the most new growth (Figure 5.7) though the morphology of these plants was least compact (longest internodes).

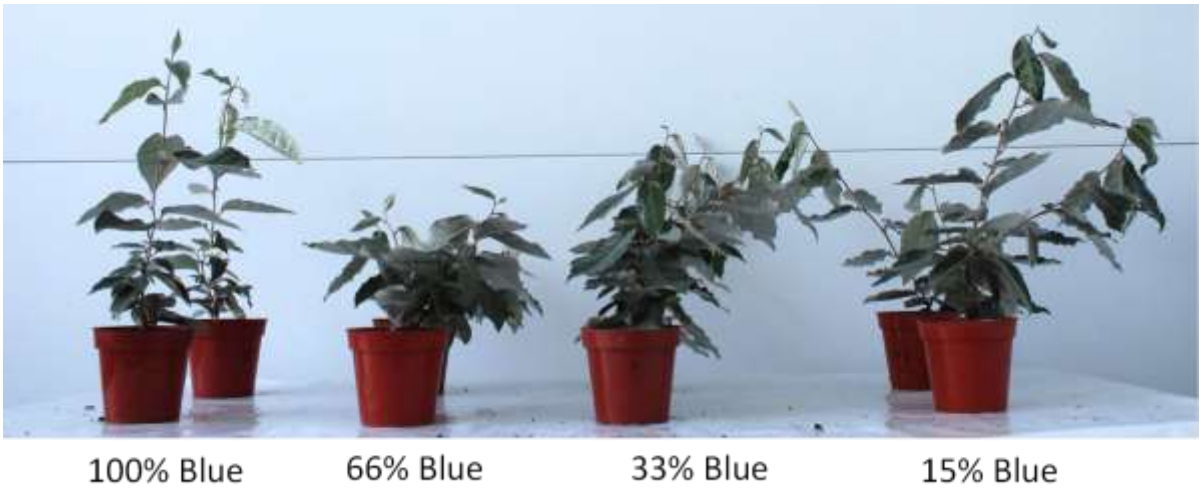

Figure 5.6. The morphology of eleagnus plants grown under four differetn red:blue mixtures of light.

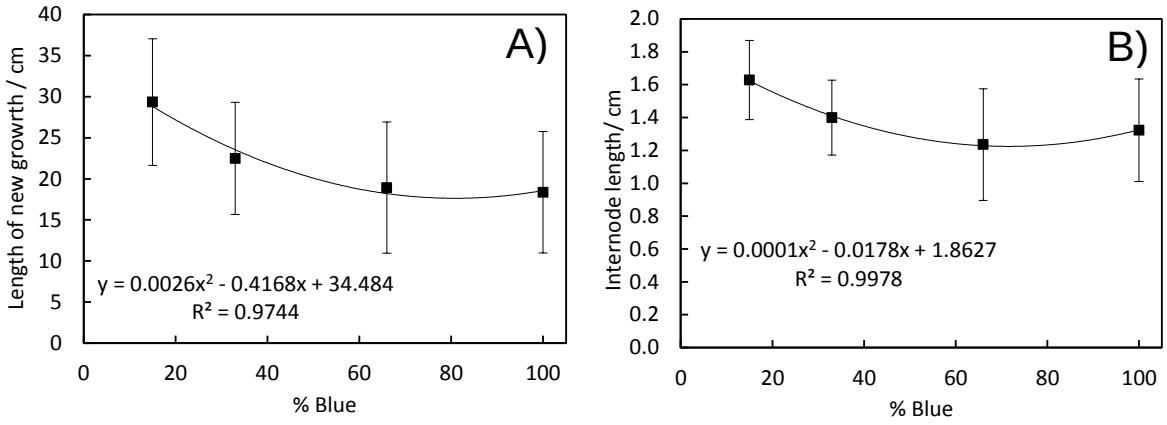

Figure 5.7. A) The length of new growth and **B)** the mean internode lengths of the eleagnus growth under the four red: blue light treatments. .

The colouration of the leaves also differed between the four treatmetns (Figure 5.8). The plants grown under 100% blue light had much paler green leaves than plants from the other treatments. The darkest green leaves were observed in the plants grown under 66% blue light. The density of white scales also varied between treatments with the greatest density found on the leaves grown under 33 % blue light.

Figure 5.8. Differences in leaf colouration of eleagnus plants grown under different red:blue light spectra.

During the this stage of the trial we encountered an infestation of eleagnus leaf miner (Figure 5.9) which was not identifed early, partially due to the light environment making it difficult to idetify pest problems before they are in an advanced state.

Figure 5.9. A) Infestation of Eleagnus sucker (*Cacopsylla fulguralis*), a member of the psyllid family, on the eleagnus plants. The white strings are waxy tubules produced by the nymphs for defence. **B)** Final instar nymph and **C)** adult of eleagnus sucker.

5.2.3. Rhododendron

For the rhododendron cuttings examined survival was observed to increase as the blue light percentage increased from 0% (33% survival) to 33% (81% survival) but at higher blue light percentages examined (66% and 100% blue) no cuttings survived (Figure 5.10A). Far-red light was found to have a slight negative influence on cutting survival (Figure 5.10B) with survival dropping from 64% to 50% as far-red photon irradiance increased from 0 to 48 µmol m^{-2} s^{-1}. Under the red: blue treatments survival corrected rooting was observed to increase rapidly as blue light percentage increased (Figure 5.10C) with over 90% of the cuttings rooting in the 33% blue light treatment but very few rooting in lower blue percentages. Red: blue light treatments were found to have little influence on root number (Figure 5.10E). As none of the cuttings from the higher blue light treatments survived percentage rooting and root number could not be assessed making it difficult to assess the influence of blue percentage on root number. In the far-red treatments, increasing far-red photon irradiance from 0 to 30 µmol m^{-2} s^{-1} resulted in an increase in survival correct rooting percentage from 1% to 38%, though no cuttings rooted at the highest far-red intensity (Figure 5.10D). The numbers of roots produced under the far-red treatments decreased as far-red intensity increased. When a non-linear regression line (Figure 5.10F) fitted to the three data points is extended to the 48 µmol m^{-2} s^{-1} it reaches a value of zero. This may partially explain why survival corrected rooting percentage is zero under the highest far-red treatment.

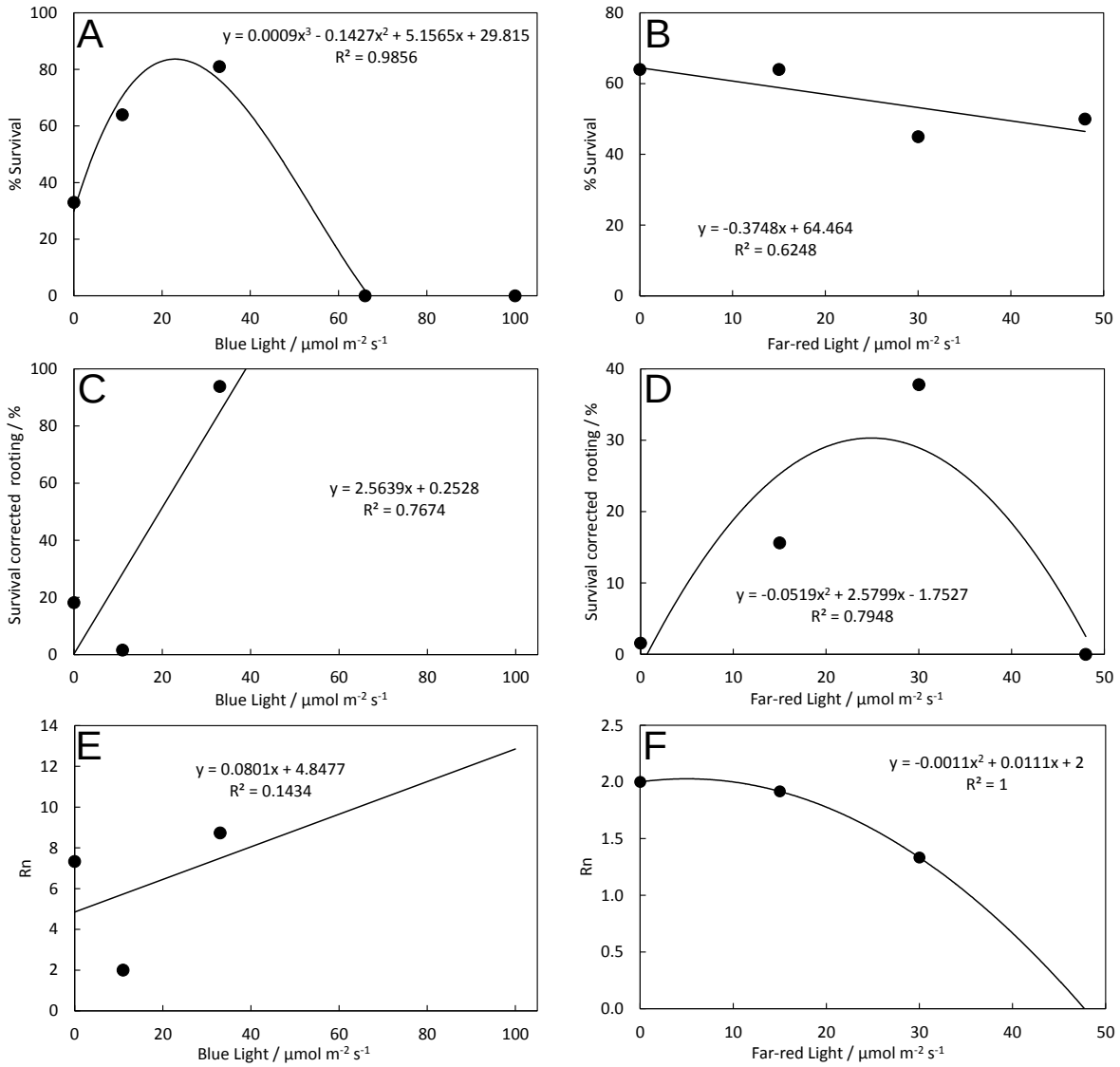

Figure 5.10. The influence of blue light percentage (left side) and far-red (right side) on the percentage survival (**A**, **B**), survival corrected rooting percentage (**C**, **D**) and root number (Rn: **E**, **F**) of rhododendron cuttings.

5.2.4. 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 mother stock light pre-treatments for cutting production. Overall appearance and quality of the cuttings collected from the three supplemental pre-excision treatments (unlit, supplemental and day length extension) 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 slightly paler shade of green than those from the other two treatments and were generally 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 5.11). 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, though in cases were the leaves remained green the fungus was thought to be the cause of death.

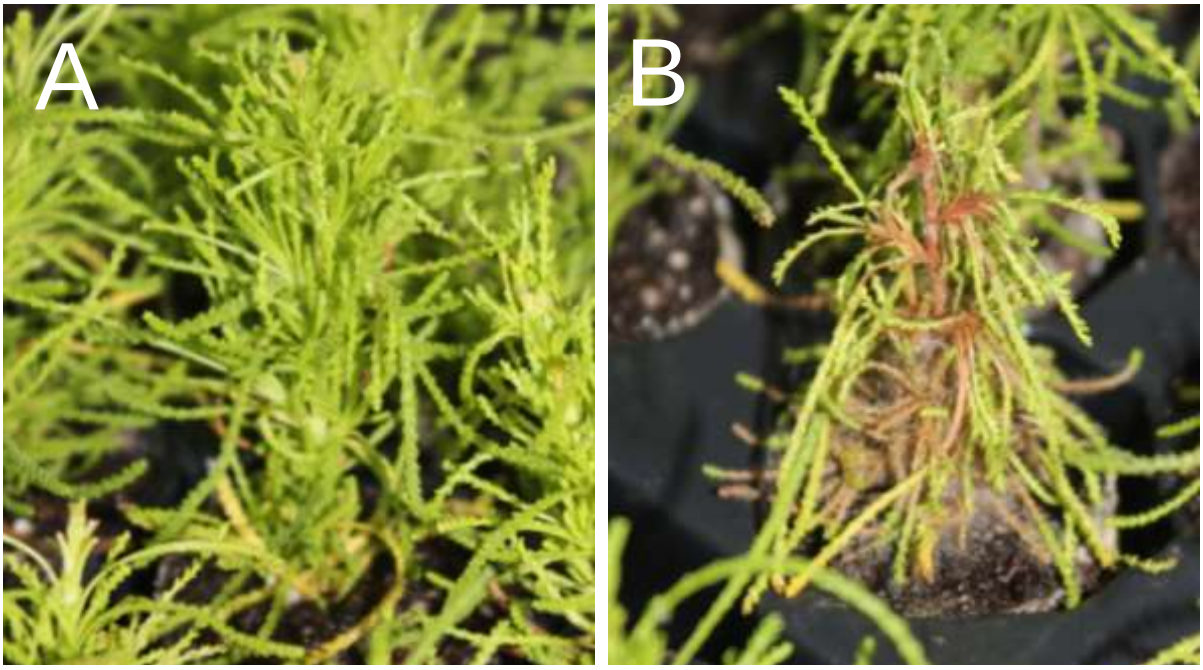

Figure 5.11. Photographs of **A)** a healthy santolina cutting and **B)** a dying santolina cutting. Note that on the dying cutting the stem is turning browning first and that many of the leaves remain healthy looking at their tips.

The influence of pre-treatment on Santolina cutting survival and rooting

The influence of pre-excision light treatments on cutting survival and rooting, determined from the four treatments where cuttings from all pre-excision treatments were available (see Table 5.2), is shown in Figure 5.12. 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).

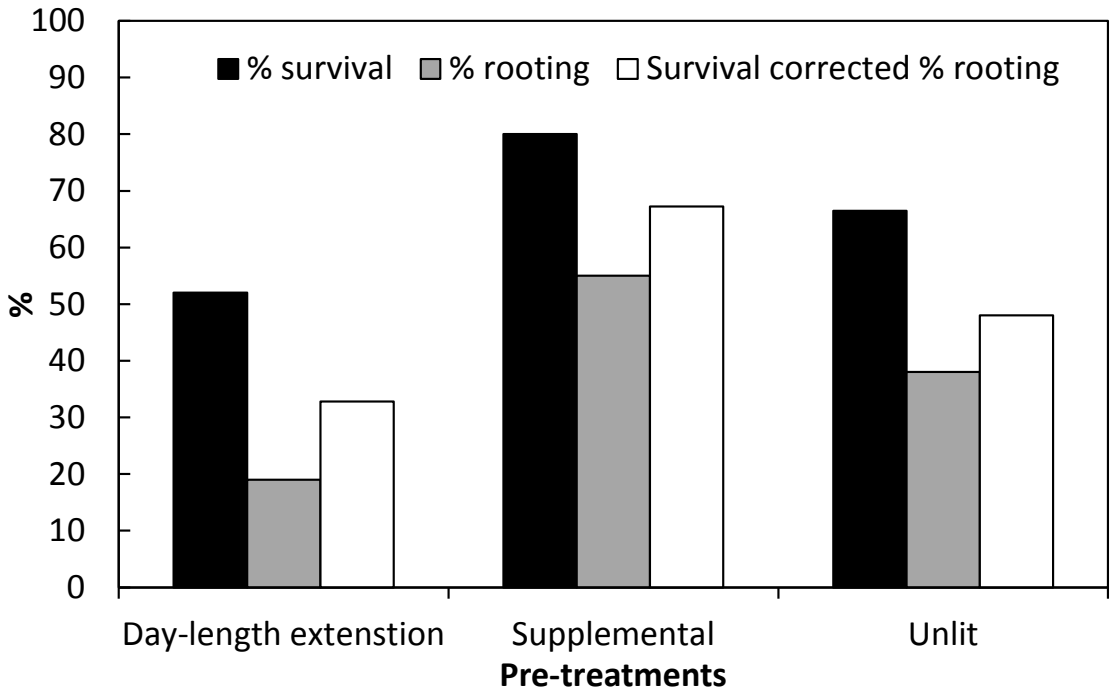

Figure 5.12. The influence of pre-excision treatment provided to the mother stock plants on the percentage of survival and rooting for the Santolina cuttings. Values were calculated using data from the four light treatments where cuttings from all three pre-excision treatments were present, see Table 5.2.

Influence of pre-excision and post-excision treatment on santolina cutting rooting

Following exposure to the red: blue LED treatments differences in the responses of cuttings between both post-excision treatment and pre-excision treatments were visible in the plant material. After 11 days exposure to the treatments (Figure 5.13) plant stress (browning of the leaves) was observed to increase as blue percentage increased from 0% (100% red) through to the 60% blue light treatment.

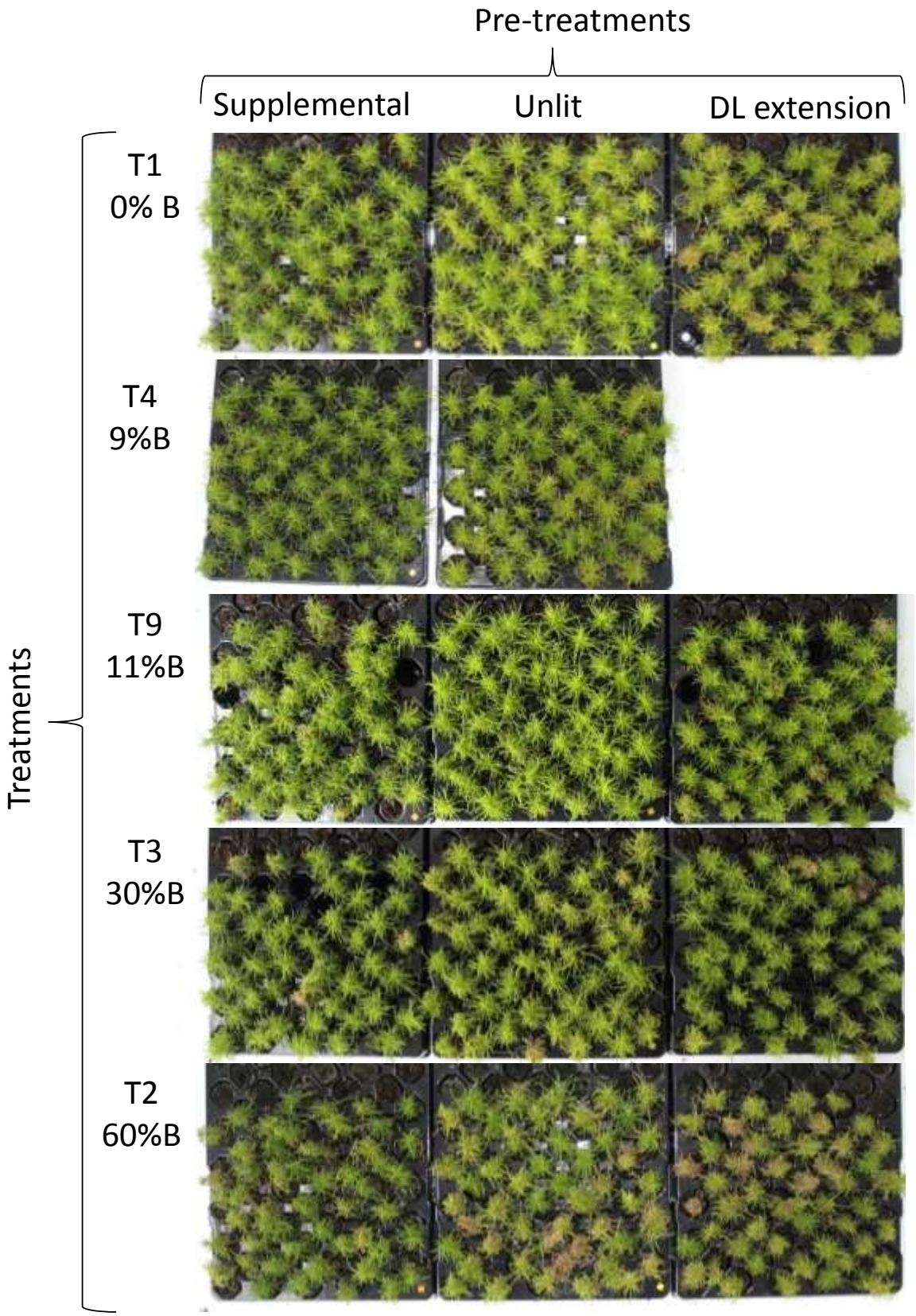

Figure 5.13. Influence of pre-excision treatments and blue percentage of light-treatments on the appearance of santolina cuttings after 11 days (2nd February 2016) in the LED4CROPS facility.

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 5.14) 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 stress) 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 pre-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 5.15). 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 percentage was increased from 0 to 61%

To assess the number of cuttings that rooted we determined the survival corrected rooting percentage (Figure 5.15B). 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 percentage increased from 0 to 61 %. 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 5.15C). Root length was found to be influenced by neither pre-excision treatment nor blue percentage of the light treatment.

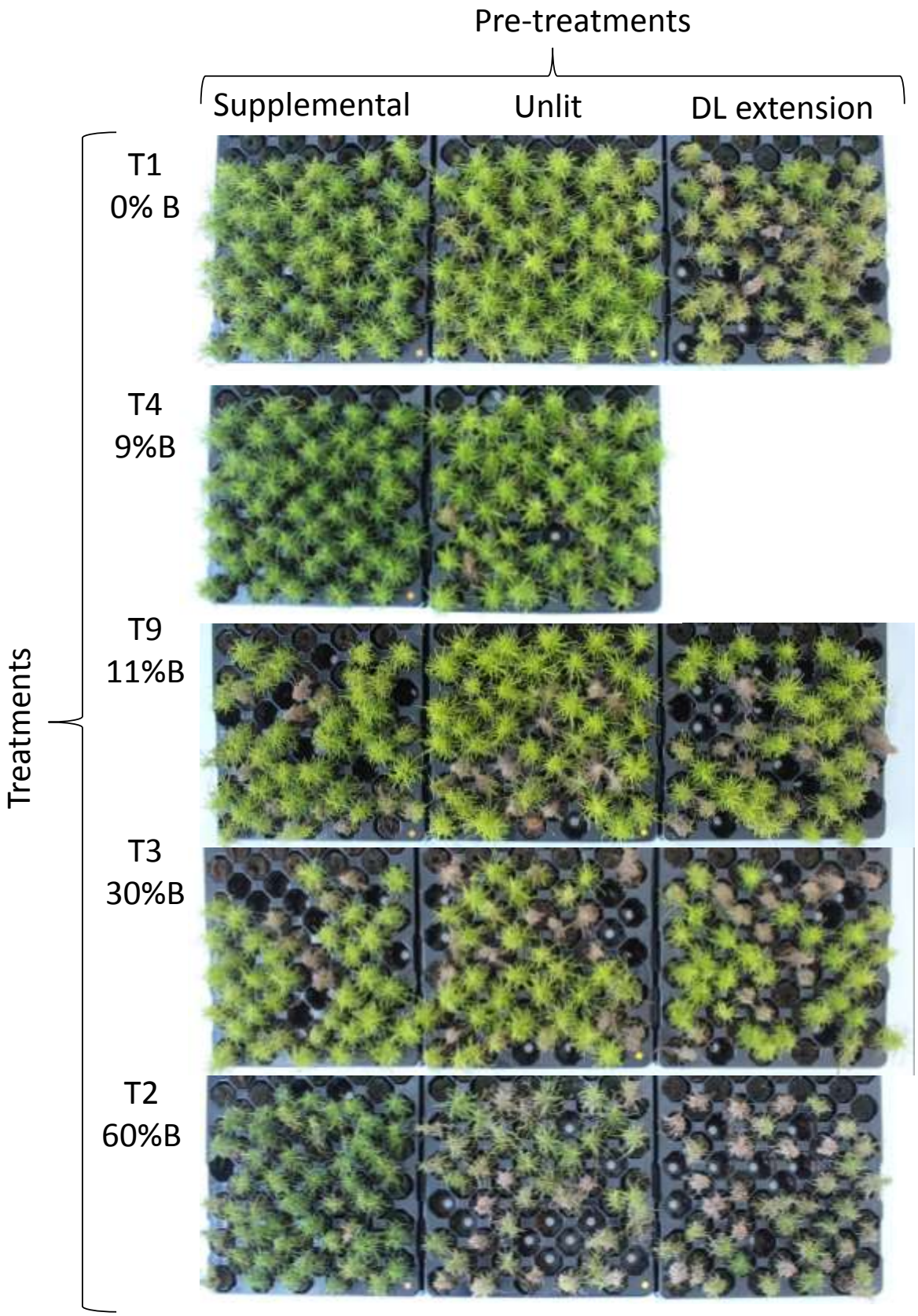

Figure 5.14. Influence of pre-excision treatments and blue percentage of light-treatments on the appearance of santolina cuttings after 27 days (18nd February 2016) in the LED4CROPS facility.

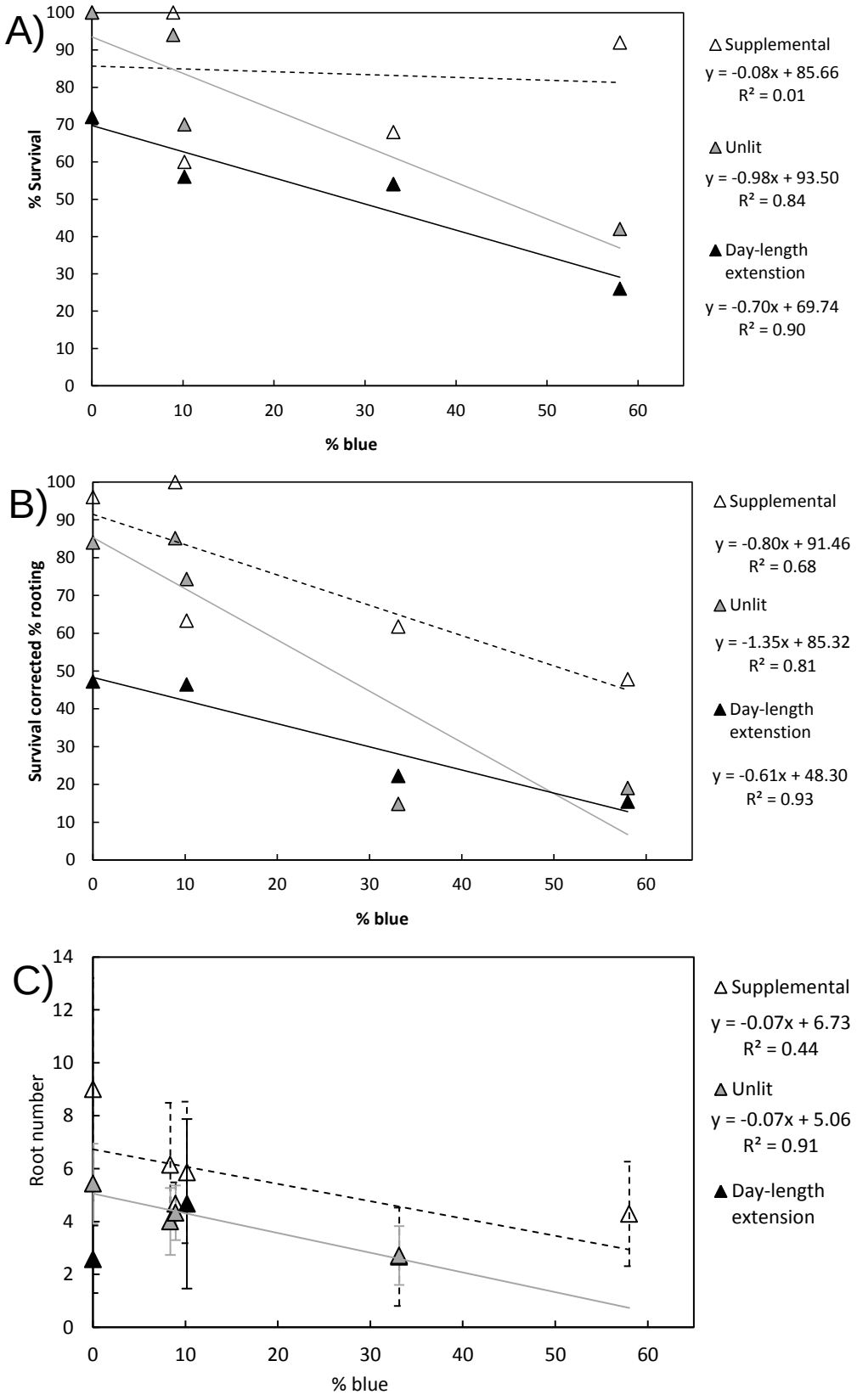

Figure 5.15. The influence of blue light intensity on **A)** the percentage survival, **B)** the survival corrected rooting percentage and **C)** the number of roots produced by the santolina cuttings from the three pre-excision light treatments (supplemental lighting = 51µmol m^{-2} s^{-}, Unlit and day-length extension lighting = 5 µmol m^{-2} s^{-}).

Influence of light intensity on santolina 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 68µmol m^{-2} s^{-1}, of a red blue mix containing 11% blue light. The plants in both of the light treatments remained healthy and showed no signs of stress even after 27 days (Figure 5.16). Cutting survival was 100% in the 33 µmol m^{-2} s^{-1} and 98% in the 68µmol m^{-2} s^{-1} treatment. Survival corrected rooting was 100% in the 33 µmol m^{-2} s^{-1} and 98% in the 68µmol m^{-2} s^{-1} treatment. Number of roots produced was higher in the 68µmol m^{-2} s^{-1} treatment (5.9 roots) than in the 33µmol m^{-2} s^{-1} treatment (4.0 roots) but root lengths were similar between treatments.

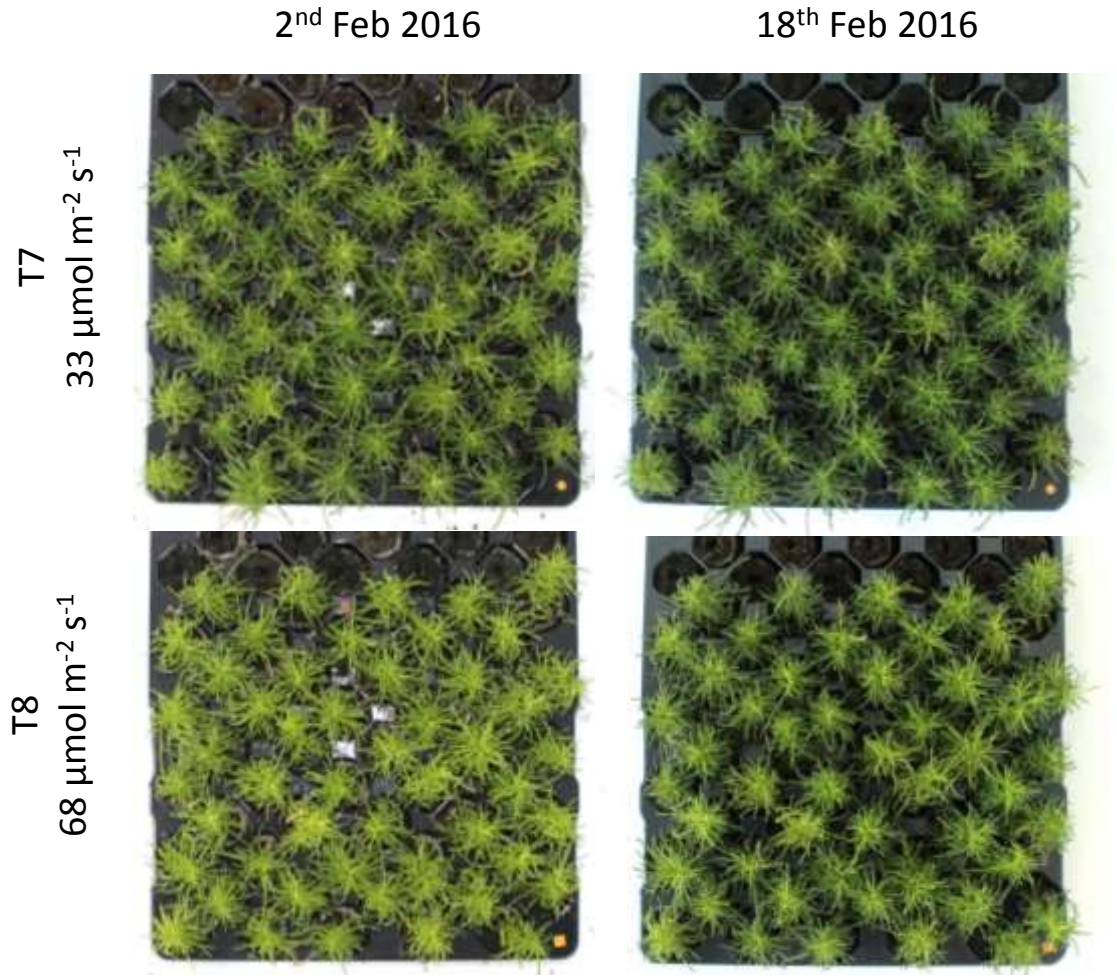

Figure 5.16. Influence of light intensity on the appearance of santolina cuttings after 11 days (2nd February 2016) and 27 days (18th February 2016) in the LED4CROPS facility.

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 5.17) and cutting survival was 96% and the survival corrected rooting was 92%.

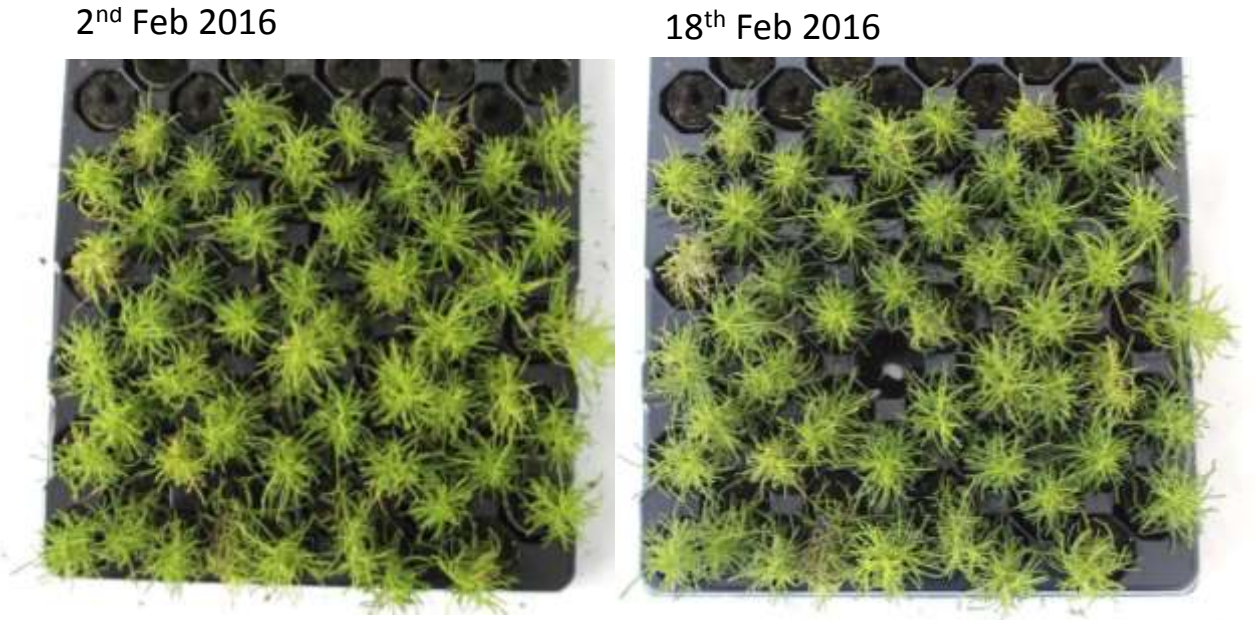

Figure 5.17. Influence of white light on the appearance of santolina cuttings after 11 (2nd February 2016) and 27 days (18th February 2016) in the LED4CROPS facility.

Influence of far-red light on santolina cutting rooting

In 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 µmol m^{-2} 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 5.18). 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 5.19). The supplemental pre-excision treatment cuttings

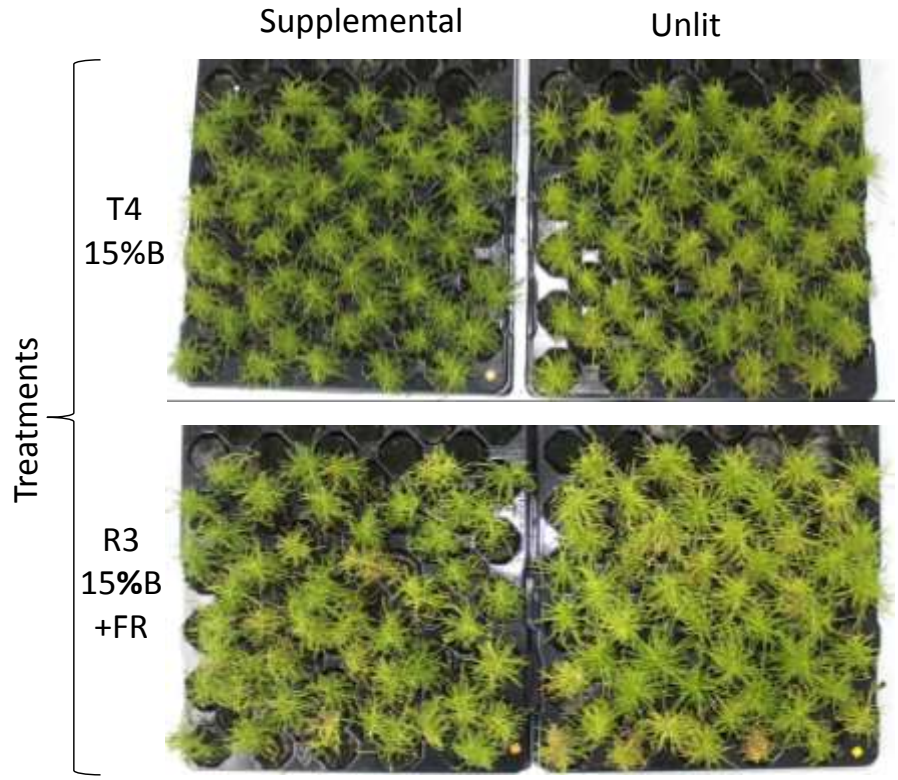

Figure 5.18. Influence of pre-treatment and far-red light treatment on the appearance of santolina cuttings after 11 days (2nd February 2016) in the LED4CROPS facility.

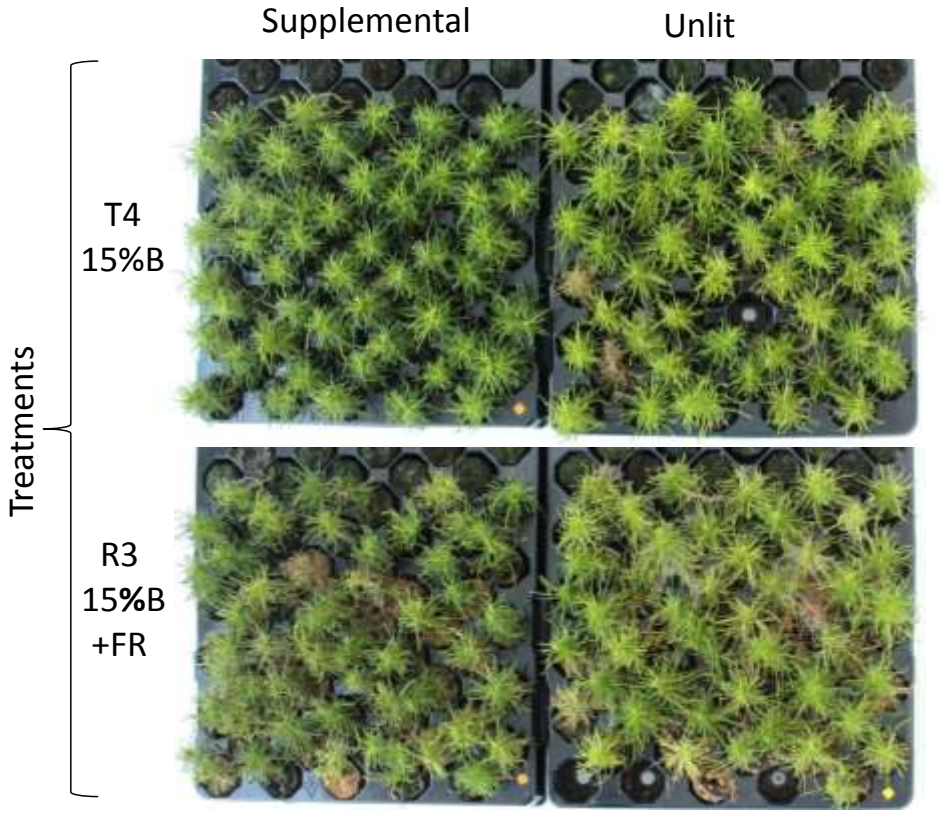

Figure 5.19. Influence of pre-treatment and far-red light treatment on the appearance of santolina cuttings after 27 days (18th February 2016) in the LED4CROPS facility.

had a 100% survival and rooting when exposed to the no far-red treatment (Figure 5.20). 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 5.20). 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 plant responses to pre-excision and post-excision light treatments.

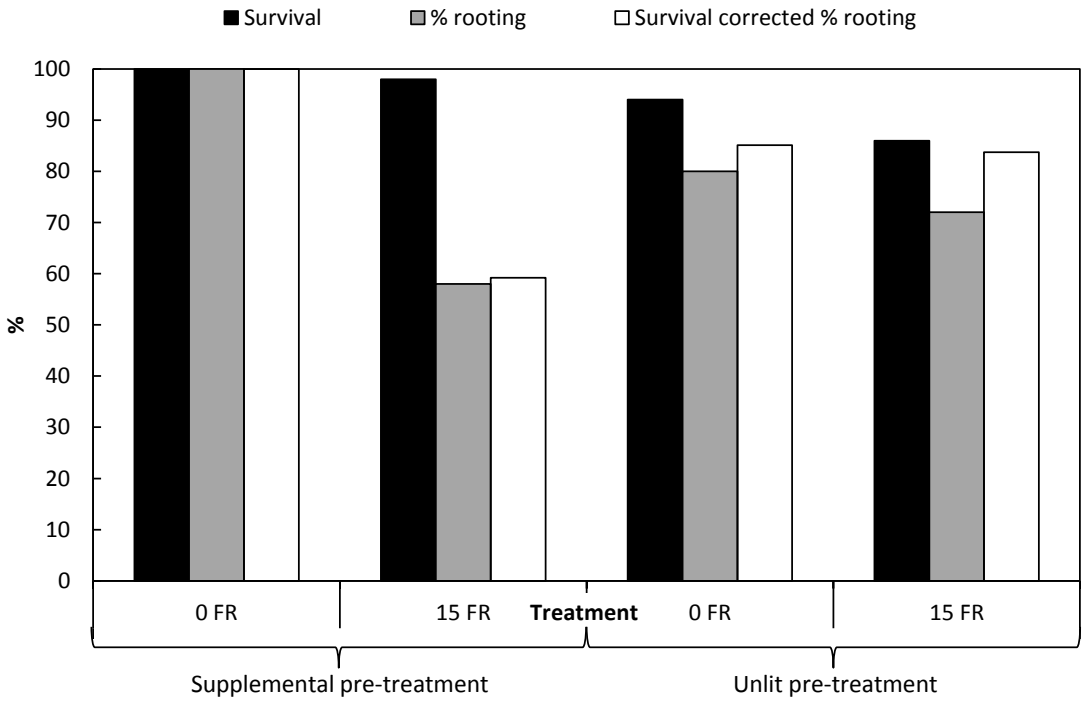

Figure 5.20. Influence of pre-excision light treatment (supplemental light of 54 µmol m^{-2} s^{-1} versus an unlit control) and post excision far-red light treatment on santolina cuttings percentage survival, percentage rooting and survival corrected rooting percentage.

5.2.5. Clematis ‘The President’

The influence of light quality 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 5.21). The shoots of the tip cuttings grew during the trial, especially those in the 100% red and

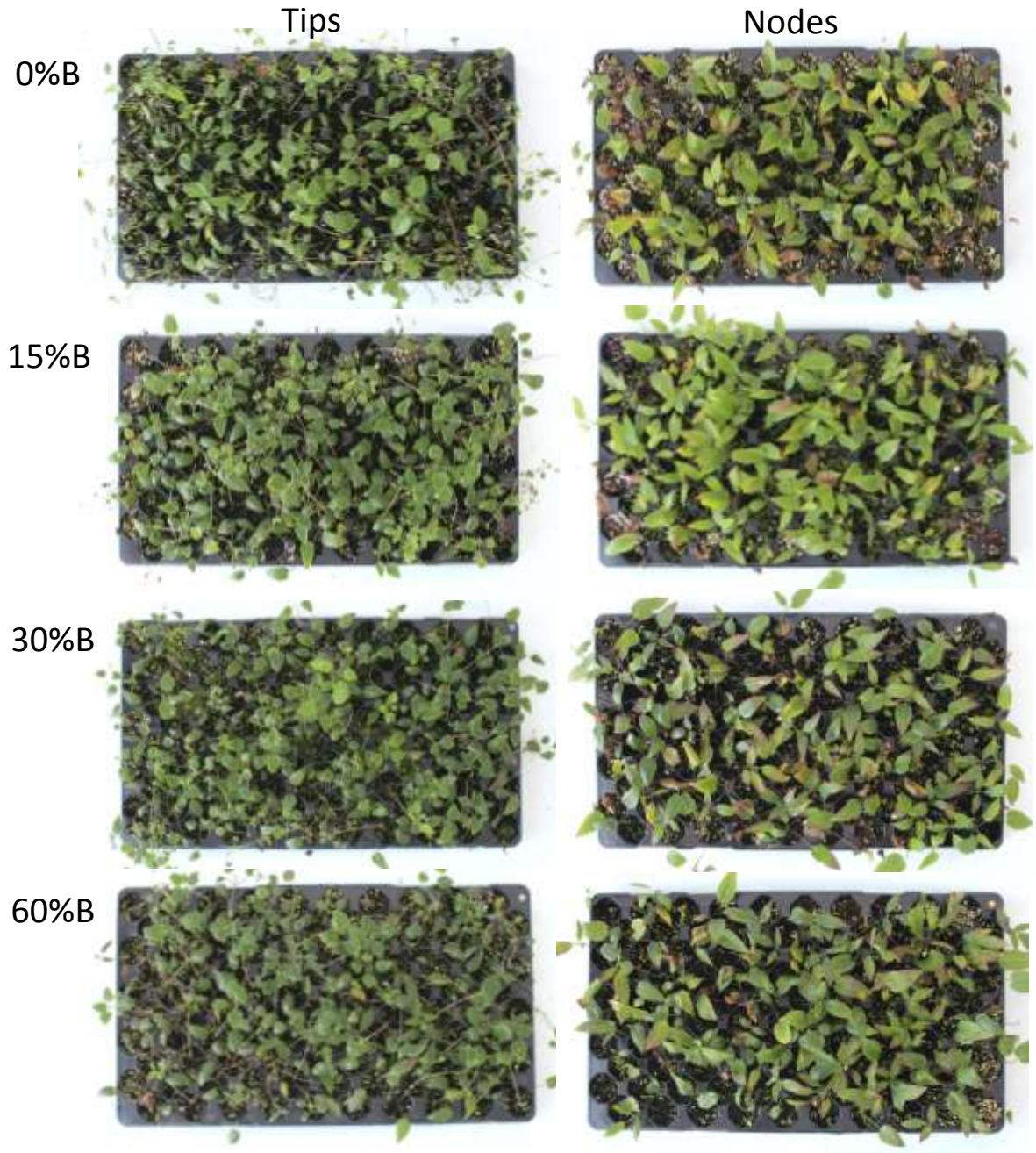

Figure 5.21. Photographs of the clematis tip and nodal cuttings grown in the different red blue light treatments. Photographs taken after 5 weeks of exposure to the treatments.

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 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 5.22), presumably due to dehydration caused by stomatal opening. Rooting percentage was observed to decrease as blue light percentage increased (Figure 5.22C). Tip cuttings had on average 13% higher strike rates that 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. Tip cuttings produced more roots than node cuttings (Figure 5.22E). Root number of the tip cuttings decreased linearly as blue light percentage increased dropping from 7 roots under the 100% red light treatment to 4 roots under the 60% blue light treatment. Root number of the nodal cuttings was also observed to decreases as blue light percentage increased but to a lesser extent, possibly as these cuttings generally produced fewer roots.

The influence of far-red light on rooting was only determined for the nodal cuttings. Far-red light had a small negative influence on cutting survival rates. Survival correct rooting was found to decrease rapidly as far-red intensity increased (Figure 5.22D). 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 5.22F).

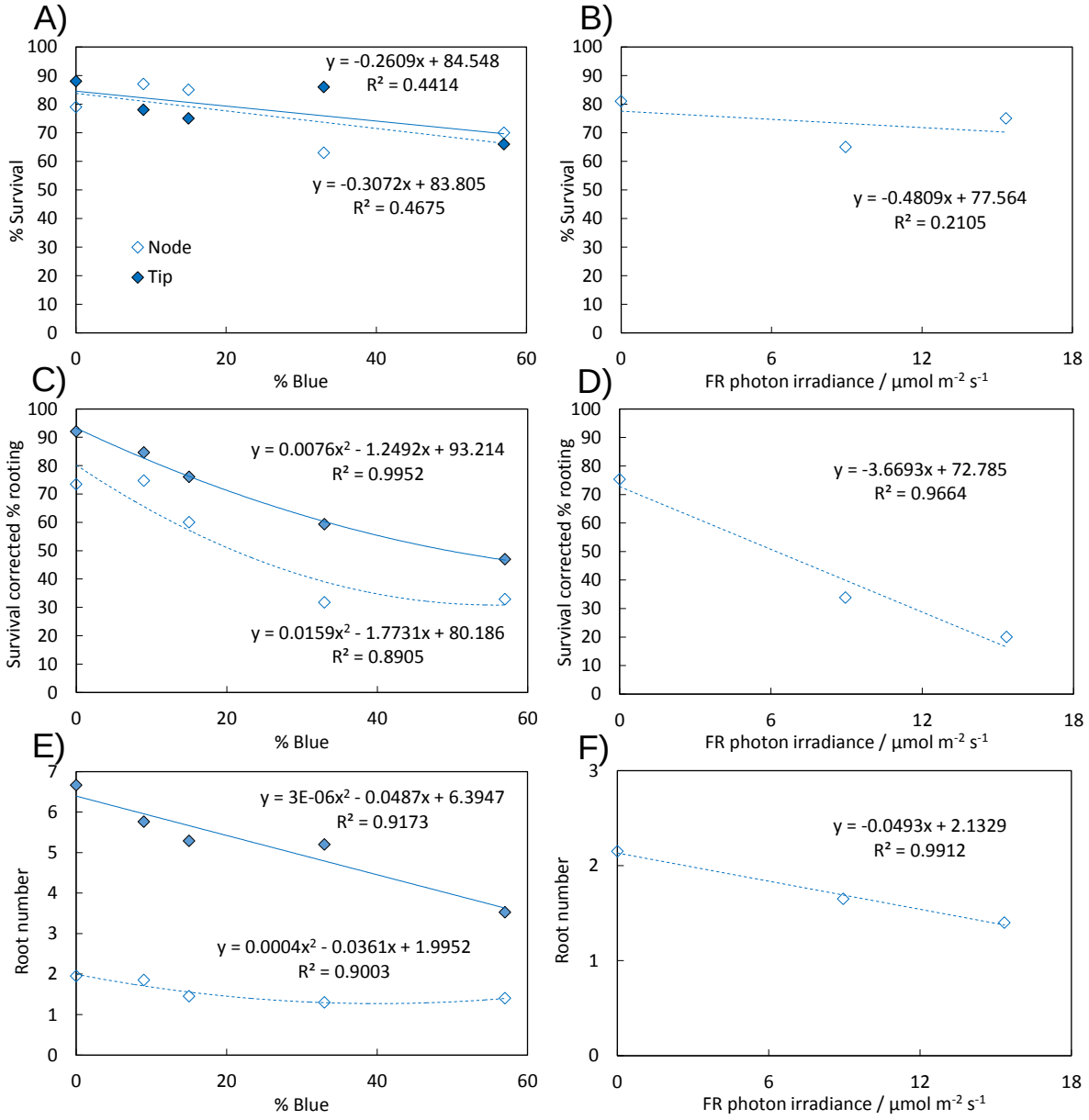

Figure 5.22. The influence of blue light percentage (left side) and far-red photon irradiance (right side) on cutting percentage survival (**A & B**), survival corrected rooting percentage (**C & D**) and number of roots produced per cutting (**E & F**) of clematis nodal (open symbols and dashed lines) and tip cuttings (filled symbols and solid lines). Measurements made after five weeks exposure to the different light treatments.

5.2.6. Iberis ‘Absolutely Amethyst’

The influence of red: blue light spectra on rooting 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 5.23). 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 5.23). 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: Figure 5.24). 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 cuttings had greater stored resources.

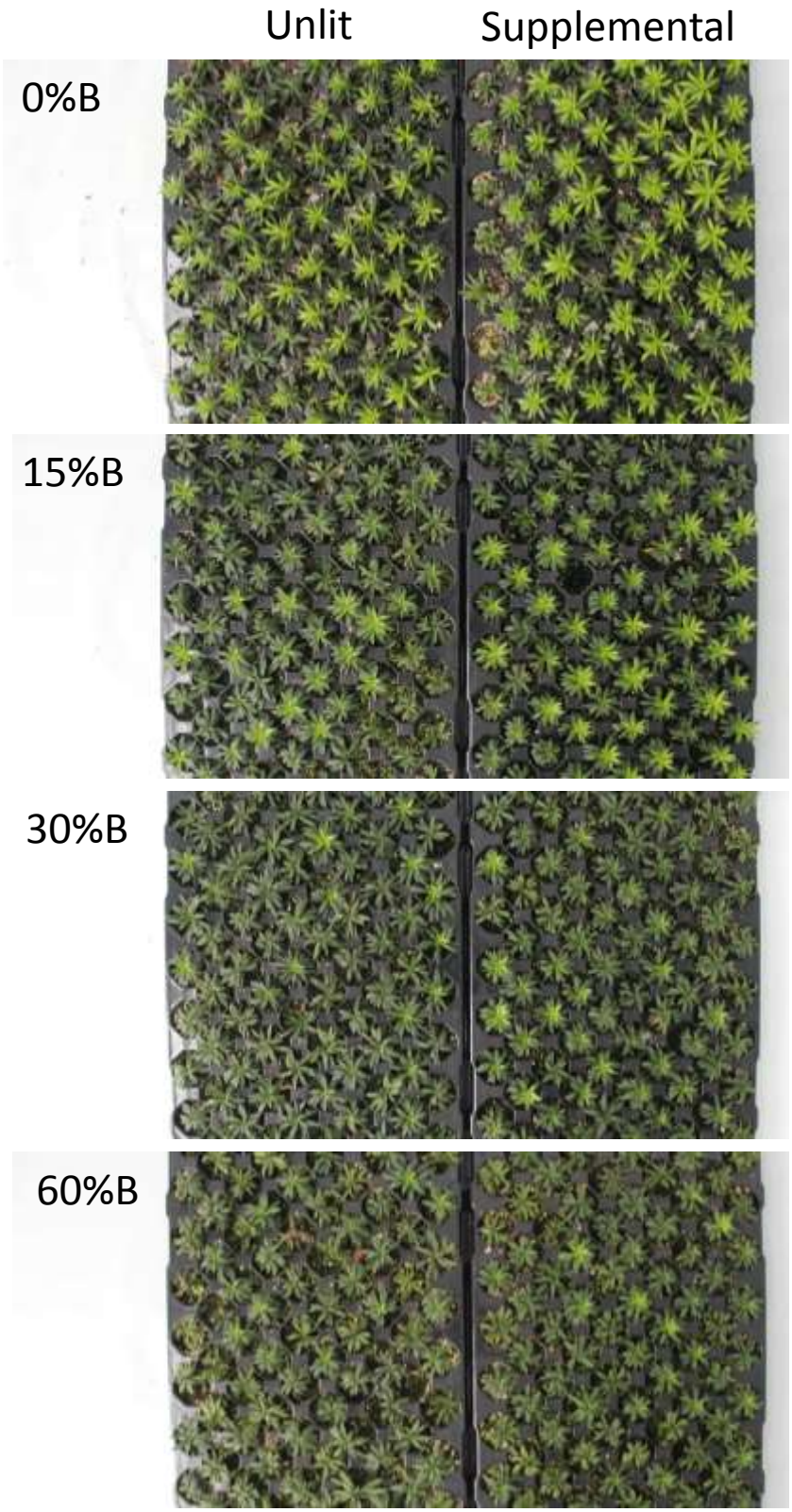

Figure 5.23. Iberis 'Absolutely Amethyst' cuttings grown under different light treatments for 4 weeks. Mother stock plants were grown with (supplemental) and without (unlit) supplemental LED lighting through the winter months at Kernock Park Plants.

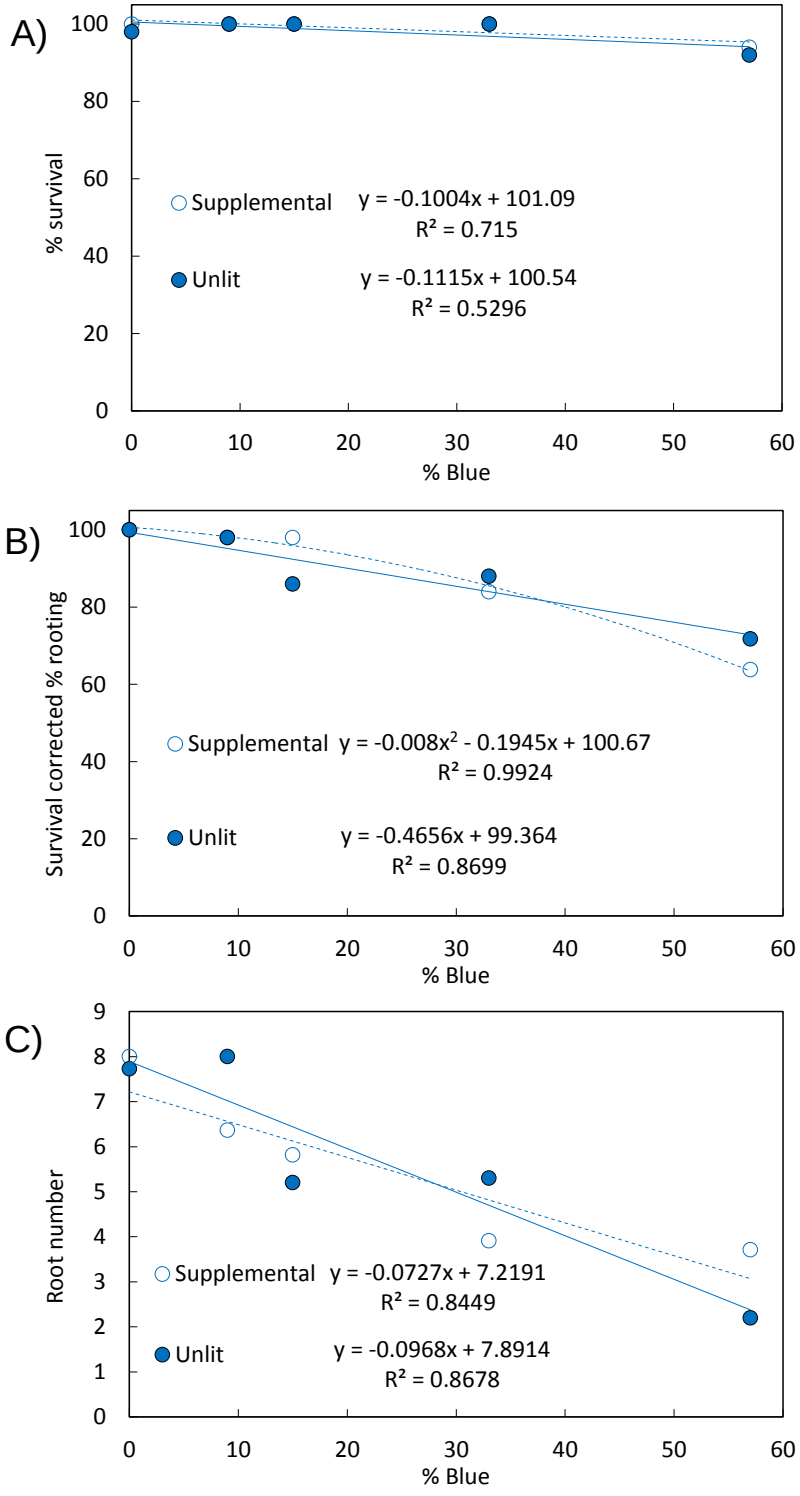

Figure 5.24. The influence of blue light percentage on the **A)** survival, **B)** survival corrected rooting percentage and **C)** number of roots produced per cutting for iberis 'Absolutely Amethyst' cuttings. Mother stock plants were grown with (open symbols and dashed lines) and without (closed symbols and solid lines) supplemental LED lighting through the winter months.

5.2.6. Lavender

Following transfer to the four light treatments the lavender cuttings showed signs of dehydration under the higher blue percentage treatments within 24 hours. Figure 5.25 shows the cuttings from the four treatments after 48 hours of exposure to the light treatments with those under 100% blue light, in particular, wilting. After the first week the majority of cuttings had regained turgidity and were more tolerant to dehydration. Cutting survival and rooting was assessed after 22 days under the light treatments. Under the 100% red light treatment cutting survival was 82% but it decreased rapidly as the blue light percentage increased with only 34% of cuttings surviving under the 100% blue treatment (Figure 5.26). Survival corrected rooting was also found to decrease rapidly as blue light percentage increased. Under the 100% red light treatment 88% of the surviving cuttings rooted and this decreased to 51% under the 50% blue light treatment. Further increases in the blue percentage had little impact of rooting with 49% of the surviving cuttings rooting under the 100% blue light treatment. While the plants grown under 100% red light had the highest survival and rooting rates there were some negative impacts on morphology, with leaves showing strong longitudinal curling (Figure 5.27). This curled morphology was less pronounced in the treatments containing blue light. The plants from the 100% blue light treatment in addition to having low survival and rooting also had slower rooting. This was reflected in the reduced shoot growth and reduced root development (fewer roots were observed on the outer surface of the soil plugs) under these treatments.

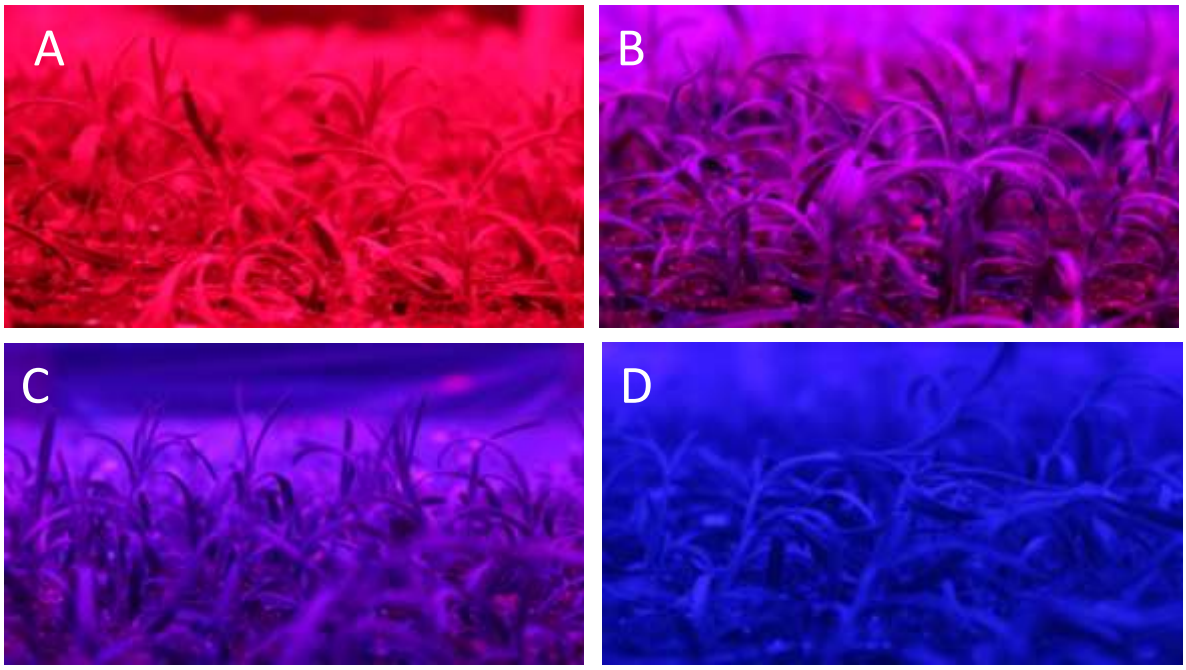

Figure 5.25. Lavender provençal (Antibes) cuttings after 48 hours exposure to different red:blue ratios **A)**100% red, **B)** 70% red 30% blue, **C)** 50% red 50% blue, **D)** 100% blue.

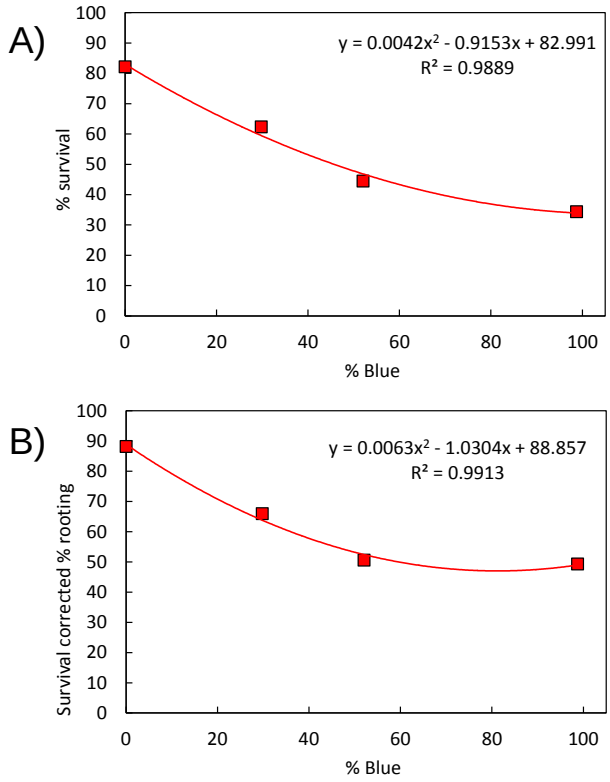

Figure 5.26. The influence of blue light percentage (% blue) on **A)** the percentage survival and **B)** the survival corrected rooting percentage of the lavender cuttings.

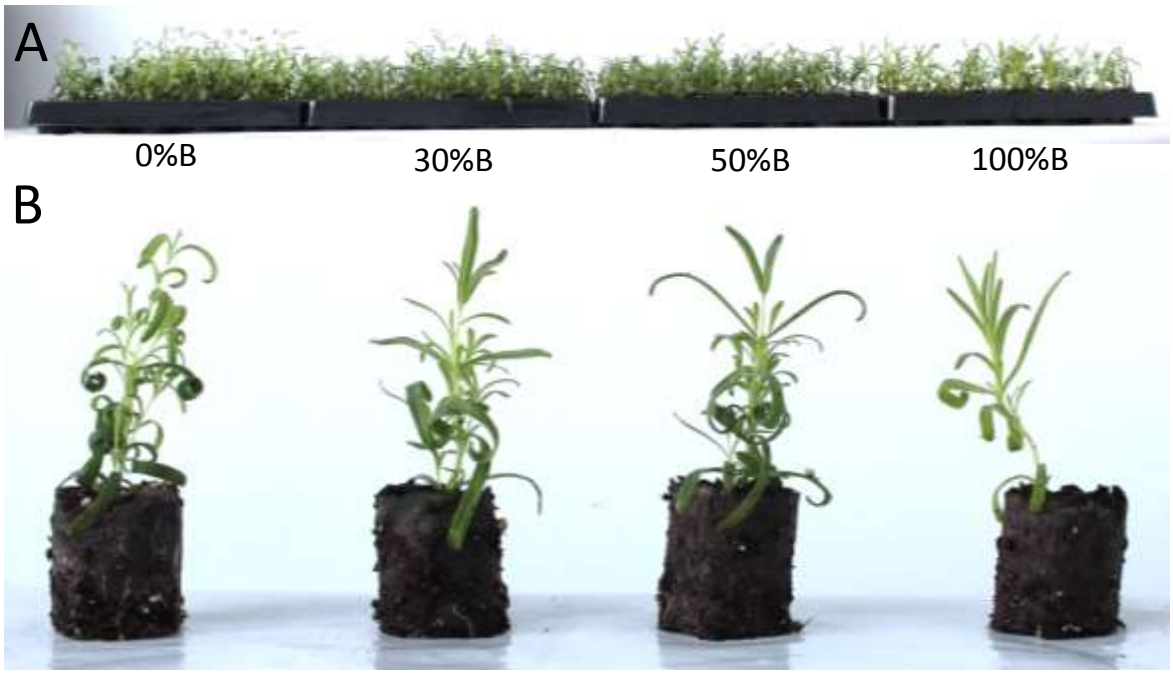

Figure 5.27. Images of the rooted Lavender cuttings taken on the 16 Feb 2017, 22 days after sticking. **A)** Image of the trays showing the differences in cutting vigour between the treatments. **B)** Representative rooted cuttings from the four treatments. Note the leaf curling observed in the 100% red treatment (0% blue).

5.2.7. Thyme

The survival and rooting of thyme cuttings was assessed after 22 days exposure to the light treatments (Figure 2.28). Under the 100% red light treatment cutting survival was 81% but it decreased rapidly as the blue light percentage increased with only 14 % of cuttings surviving under the 100% blue treatment. Survival corrected rooting was also found to decrease rapidly as blue light percentage increased. Under the 100% red light treatment 95% of the surviving cuttings rooted and this decreased to 18% under the 100% blue light treatment. While the plants under 100% red light had the highest survival and rooting rates there were some negative impacts of this light treatment on morphology, with shoots elongating and producing long internodes (Figure 5.29). This etiolated morphology was less pronounced as blue percentage increased. While some of the differences in growth (height of cuttings) were associated with the influence of light quality on morphology some were associated with differences in rooting. As the 100% red light treatments had rooted more extensively (and presumable also rooted sooner) these plants would have more resources for growth.

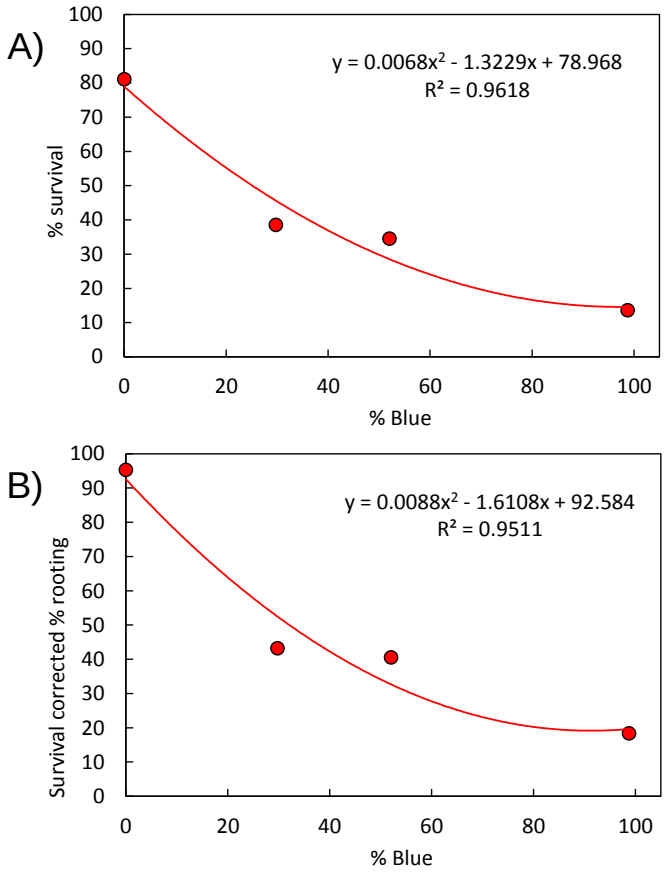

Figure 5.28. The **A)** cutting percentage survival and **B)** survival corrected rooting percentage of Thyme cuttings exposed to light treatments with different red: blue treatments.

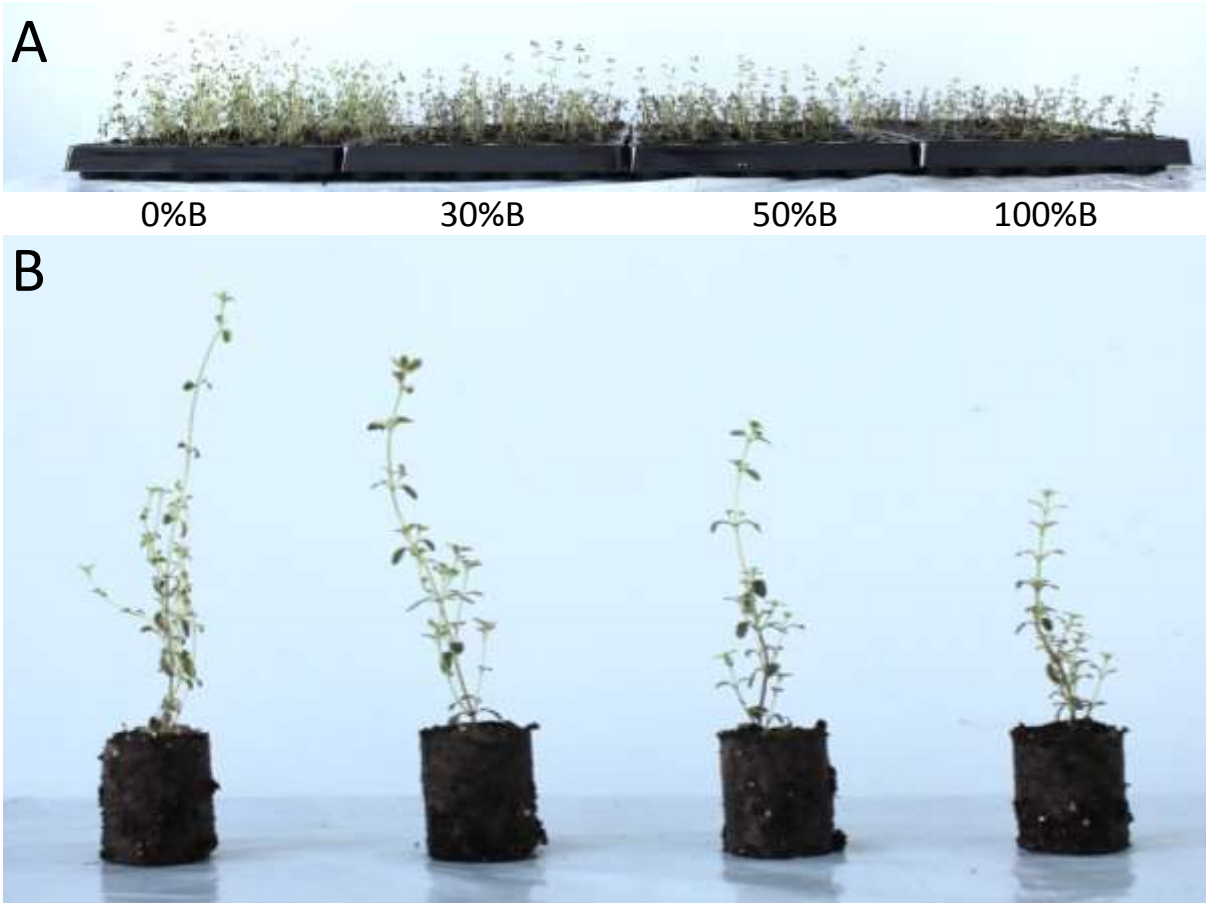

Figure 5.29. Images of the rooted thyme cuttings, photographs taken on the 16 February 2017 three weeks after sticking. **A)** Image of the trays showing the differences in vigour of the cuttings between the four light treatments. **B)** Representative rooted cuttings from the four treatments.

5.3. Tomato – A model system for assessing the influence of light spectra on hormones and adventitious rooting.

5.3.1. Methods

Plant material

Plant material for these rooting studies was collected from a 'sunstream' tomato crop being grown in the LED4CROPS high-wire glasshouse. The crop was grown following commercial practice under the guidance of Mr Gerry Andrew. At weekly intervals unwanted side shoots were removed from the crop and stored in black plastic bags until the side shoots could be processed into cuttings (all cutting processed within 3 hours of collection). The tips of the side shoots were trimmed to a consistent length ~4 inches in length, and placed in fully hydrated jiffy 7 plugs (50 per treatment). Four red: blue light treatments were examined 0% blue (100% red), 15% blue, 55% blue and 100% blue. To minimise transpiration the cuttings were enclosed in a polythene tent. After 7 days rooting assessments were performed. These experiments were repeated on several occasions; endogenous hormones, however, were only assessed on one occasion.

Hormone analysis

Hormone analysis was performed on leaves and the bottom 3cm of the cutting stem. Samples were collected at three time points 2-4 hours after removal of the side shoots from the donor plants, 24 hours after collected and 48 hours after collection. An additional leaf sample was collected 7 days after collection when cuttings had rooted. In order the stream line the sample collection and processing cuttings were collected and planted at staggered intervals so all samples could be collected on two sampling dates. After collection samples were stored in plastic bags on ice. Samples were transferred to a -20°C freezer within 2 hours of collection. The following day samples were transported to Lancaster University where the plant tissue was freeze dried. After freeze drying the samples were ground to powder using a ball mill. When necessary samples were combined to achieve the minimum mass for the hormone analyses. For each time point, and tissue five replicates measurements were made. Hormone analysis was performed by the Department of Plant Nutrition, CEBAS-CSIC, Murcia, Spain. A full list of the hormones that were measured is provided in Table 5.3.

Table 5.3. List of the plant hormones that were measured by CEBAS-CSIC, Murcia, Spain.

Type of hormone	Acronym	*Full chemical name*
Ethylene biosynthesis	ACC	*1-Aminocyclopropane-1-carboxylic acid*
Cytokinins	*t*Z	*trans-Zeatin*
	ZR	*Zeatin riboside*
	iP	*Isopentenyladenine*
Gibberellins	GA1	*Gibberellin A1*
	GA3	*Gibberellin A3 or Gibberellic acid*
	GA4	*Gibberellin A4*
Auxin	IAA	*Indole-3-acetic acid*
Other	ABA	*Abscisic acid*
	JA	*Jasmonic acid*
	SA	*Salicylic acid*

2.3.2. Results

Blue light effects on tomato rooting

Prior to performing the hormone analysis we first assessed the effects of different red: blue ratios of the survival and rooting of tomato cuttings to ensure tomato responses were similar to the other species examined. Survival of cuttings and rooting was near to 100% in all the light treatments examined even when cuttings wilted due to dehydration. This was partly due to the rapid rooting speed of tomatoes which kept the trials short (this makes them a good model species) and partly due to their high tolerance to dehydration. While rooting was high in all treatments speed of rooting was decreased under higher blue percentages. As observed in other species the number of roots per cutting decreased as the blue percentage increased (Figure 5.30).

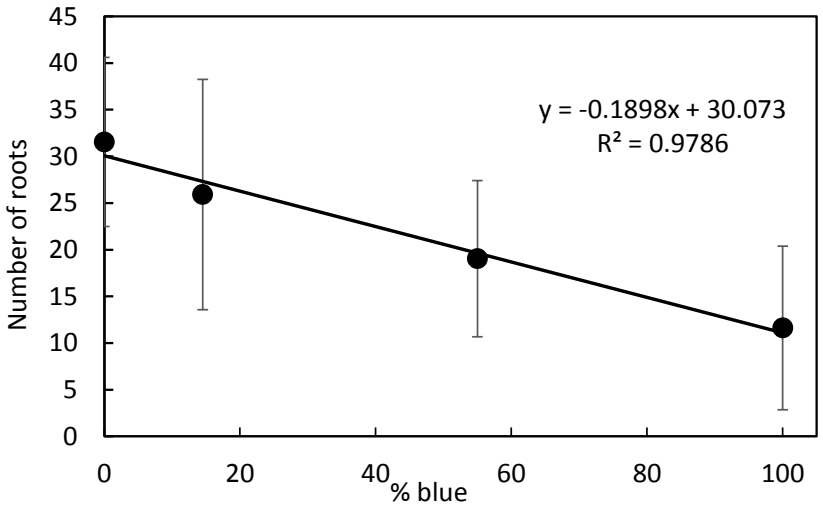

Figure 5.30. Influence of red: blue ratio on the number of roots produced by tomato cuttings.

Hormone concentrations

The concentrations of the hormones varied considerably over the course of the experiment (Figures 5.31, 5.32, 5.33). The change in concentration of individual hormones also varied considerably between different tissues for example over the first 48 hours GA1 concentration increased in the stem but decreased in the leaves. For several hormones (GA4, IAA, tZ, ZR, JA and SA) the concentration in the stem was significantly higher at the first sampling point and rapidly drops over the first 24 hours. While this at first glance indicates that these hormones were elevated in the plant before harvest but this is probably not the case. It is more likely that the hormone concentrations peaked in response to the wounding that occurs during excision. Gradual changes in concentration were also observed over the first 48 hours, GA1, ACC and ABA increased gradually possibly in response to increasing drought stress. In the leaf tissue an additional sample was collected one week after excision (168 hours). At this time point all the cuttings would have rooted. Between 48 and 168 hours the concentration of ACC, tZ, and iP decreased and the concentration of Zr and GA1 increased. For ABA the concentration increased at 48h for treatments with 0 and 15% blue light but for treatments with 55% and 100% blue light concentration increased more slowly. While the large changes in concentration over time provided interesting insights in to the processes that take place between excision and root development the main focus of this trial was to examine the influence of light quality on hormones. In the majority of cases the temporal changes in concentration are greater than those caused by light quality, however, light quality does appear to influence at least some of the hormones. The influence of light quality was most obvious in the stem samples after 48h (Figure 5.35). The concentration of IAA (auxin) in the stem at 48 hours decreased five-fold, non-linearly, as the blue light intensity increased from 0% blue (100% red light) to 100% blue. In contrast GA4 concentration increased five-fold as the blue percentage increased (much of this increase occurred between 55 and 100% blue). ABA and tZ concentrations decreased linearly as blue % increased. While the concentrations of the other hormones changed with blue light percentage the trends did not appear to correlate with changes in rooting (for example JA ZR iP and SA concentrations initially decreased as blue percentage increased but increased again at higher blue percentages).

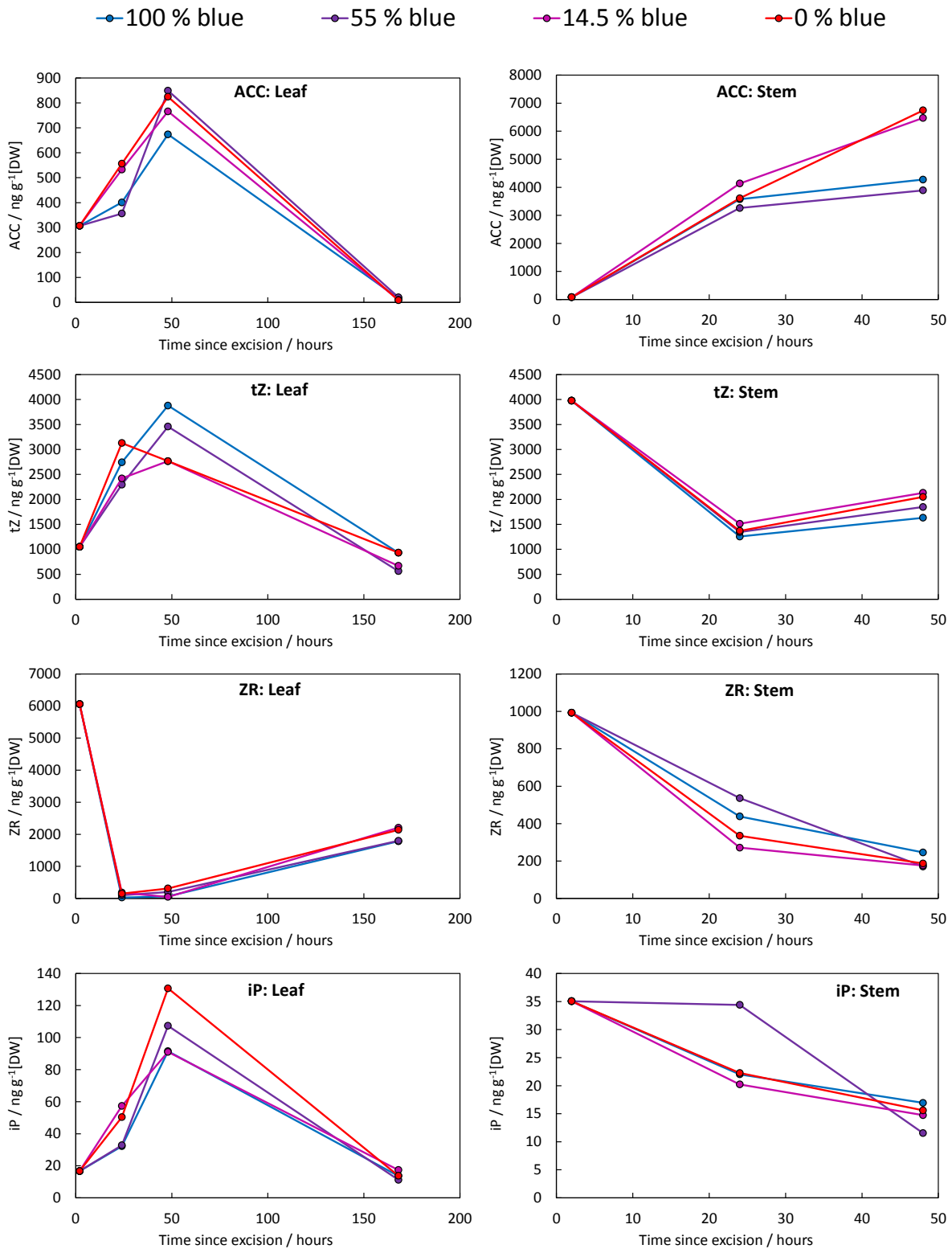

Figure 5.31. Changes in the concentration of ACC and three types of cytokinin (tZ, ZR and iP) through time since excision in the leaves (left hand graphs) and the bottom 3cm on the stem (right hand graphs).

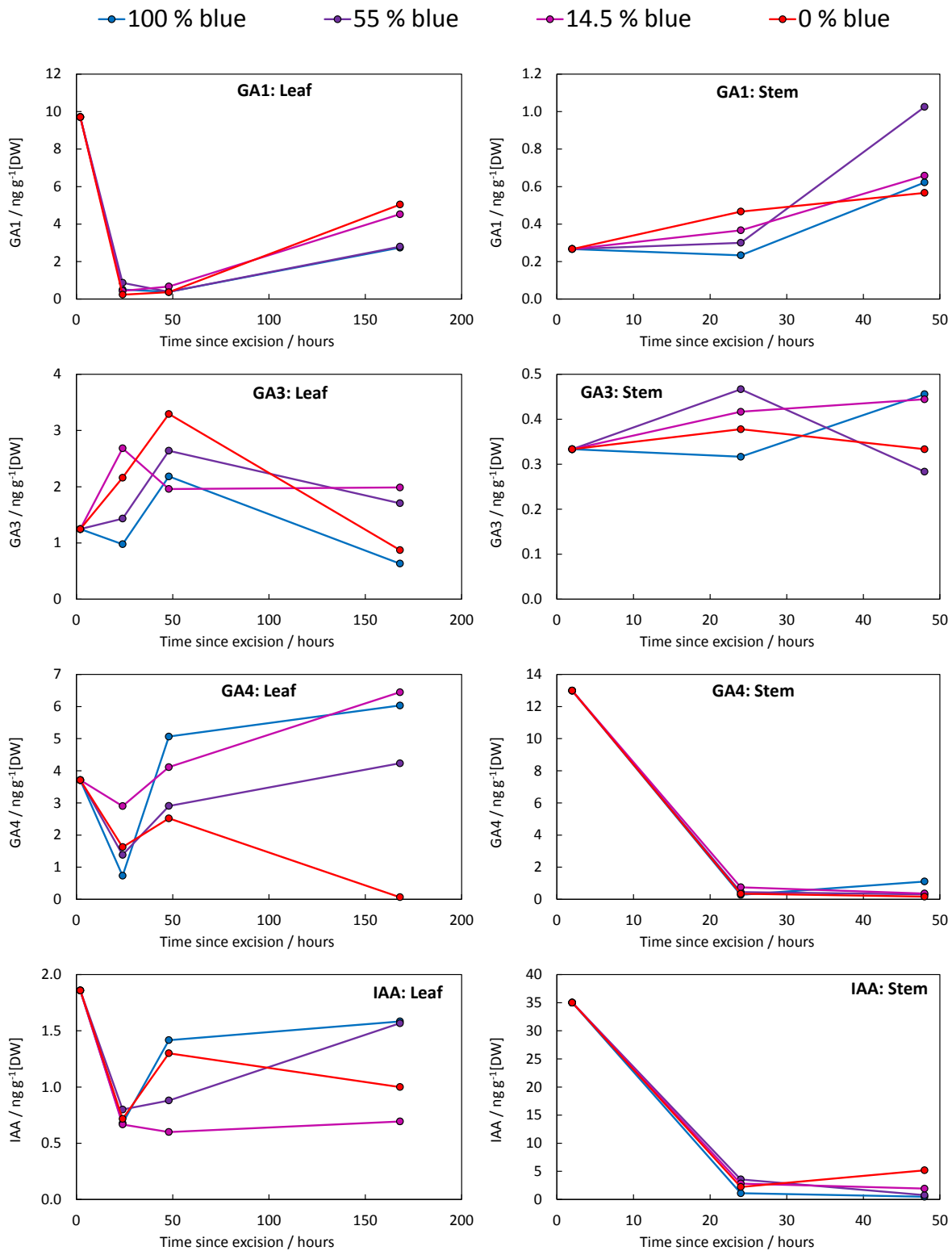

Figure 5.32. Changes in the concentration of three types of Gibberellin (GA1, GA2, GA4) and Auxin (IAA) through time since excision in the leaves (left hand graphs) and the bottom 3cm on the stem (right hand graphs).

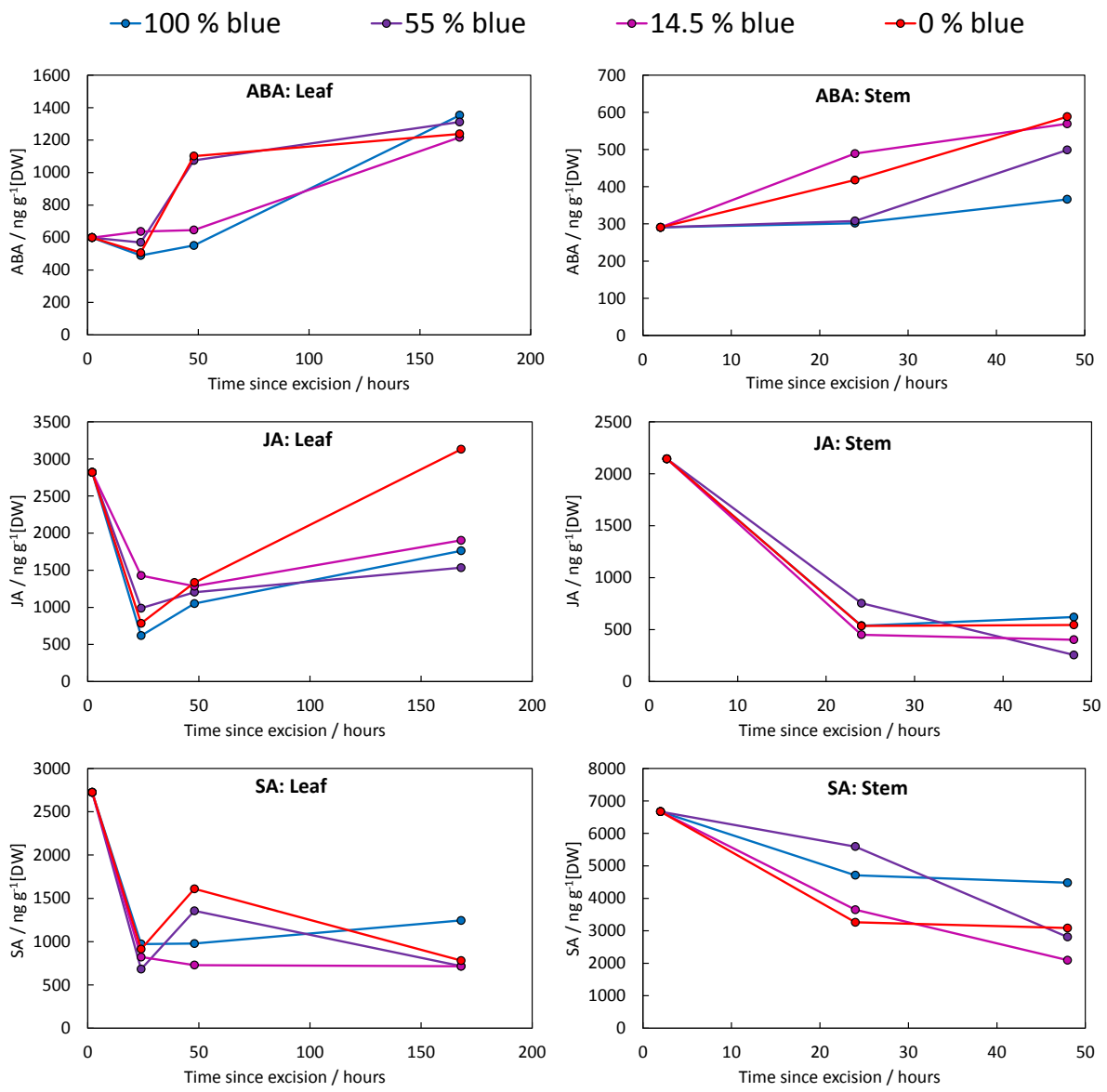

Figure 5.33. Changes in the concentration of three hormones (ABA, JA, SA) through time since excision in the leaves (left hand graphs) and the bottom 3cm on the stem (right hand graphs).

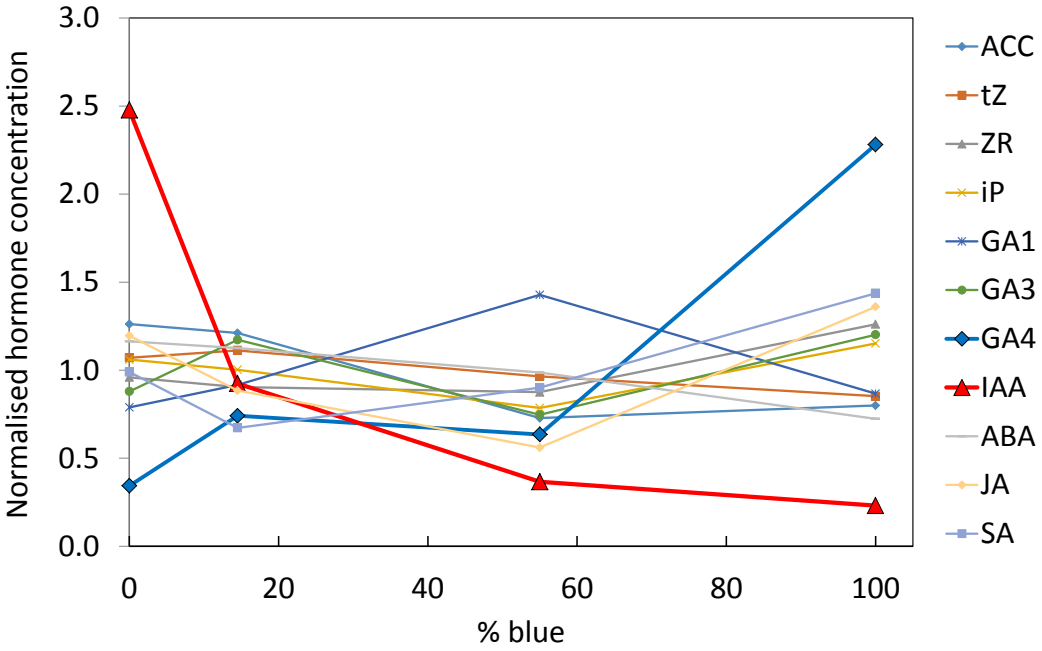

Figure 5.34. The influence of blue light percentage on normalised hormone concentration from stem samples 48h after excision. Values for each hormone measurement were normalised to the average concentration of that hormone.

5.4. Discussion

5.4.1. Cutting water loss and survival

In whole plants, stomatal aperture is controlled by three factors: light, water availability, and root/shoot signalling mediated by hormones such as ABA and ethylene. As these factors fluctuate, stomata open and close to meet the needs of the plants under the prevailing conditions. Light (blue-light in particular) causes stomata to open to improve access to the CO_2 required for photosynthesis. In well-hydrated leaves, stomata open freely in response to light. Under drought conditions, roots are able to detect the onset of water deficits before any hydraulic limitation occurs. Roots produce chemical signals that travel to the shoot and reduce stomatal conductance (Schachtman and Goodger 2010). ABA, xylem pH, cytokinins, an ethylene precursor, and malate have all been identified as having roles in drought signalling (Peleg & Blumwald 2011). As drought stress develops, water becomes physically limiting and hydraulic stress (leaf wilting) begins. Hydraulic stress causes ABA synthesis in the leaves (Christmann et al 2007) and stomatal closure.

When cuttings are collected, not only is their water supply removed but the root/shoot signalling that can reduce stomatal conductance is also removed. Until cuttings have acclimated to their new state (potentially via hydraulic stress induction of ABA synthesis), hydraulic restriction on stomatal aperture may be the only factor limiting further water loss, thus leaving them prone to dehydration. When leaves are excised from a plant, and presumably when cuttings are collected, they exhibit a transient increase in stomatal conductance lasting up to 30 mins before subsequent closure (Powels et al 2006) which further increases their susceptibility to dehydration immediately after collection. While stomatal closure limits further dehydration, over the longer term (days to weeks) reduced access to CO_2 will limit photosynthesis resulting in reduced carbohydrate reserves. If cutting resources are exhausted the cutting will die.

In our experiments, cutting dehydration was found to be a major factor influencing cutting survival and blue light was found to be a major factor driving dehydration (Figure 5.35). In general, cutting survival decreased as blue light intensity, presumably due to a combined effect of dehydration driven by stomatal opening and the subsequent depletion of carbohydrate reserves. There was considerable variability in blue light tolerance between the species examined. Survival of some species (iberis, clematis, and santolina cuttings from motherstock plants exposed to supplemental light treatments) was largely independent of blue percentage and, even in the 66% blue treatment, iberis survival was only slightly reduced (higher blue percentages were not examined for iberis). For several species survival was greatest under 100% red light (lavender, thyme, elagnus, and santolina from

unlit motherstock), and survival decreased as the blue light percentage increased. Eleagnus was observed to be relatively tolerant to up to 33% blue light but at higher percentages survival decreased rapidly. This drop in survival coincided with leaf shedding. This is consistent with the idea that hydraulic stress causes ABA synthesis, as ABA causes leaf excision. Once these cuttings had lost their leaves their carbohydrate reserves would rapidly be depleted with little chance of replenishment, leading to death. Cutting survival in photinia and rhododendron increased as blue light percentage increased from 0% to 11% (photinia) and 0% to 33% (rhododendron). At higher blue percentages, survival decreased rapidly, especially in the case of rhododendron. This provides evidence that for these cuttings post excision photosynthate contributed to ongoing health and the small amount of blue light aided photosynthetic performance via improved access to CO_2 and possibly by preventing red light syndrome (Trouwborst *et al* 2016). As photinia and rhododendron can take months to root, post-excision photosynthesis may have greater importance than in more rapidly rooting species. It is clear that reducing the amount of blue light, or even excluding the blue light altogether, during the early stages of rooting can greatly improve cutting survival, regardless of the underlying factors responsible for differences between species.

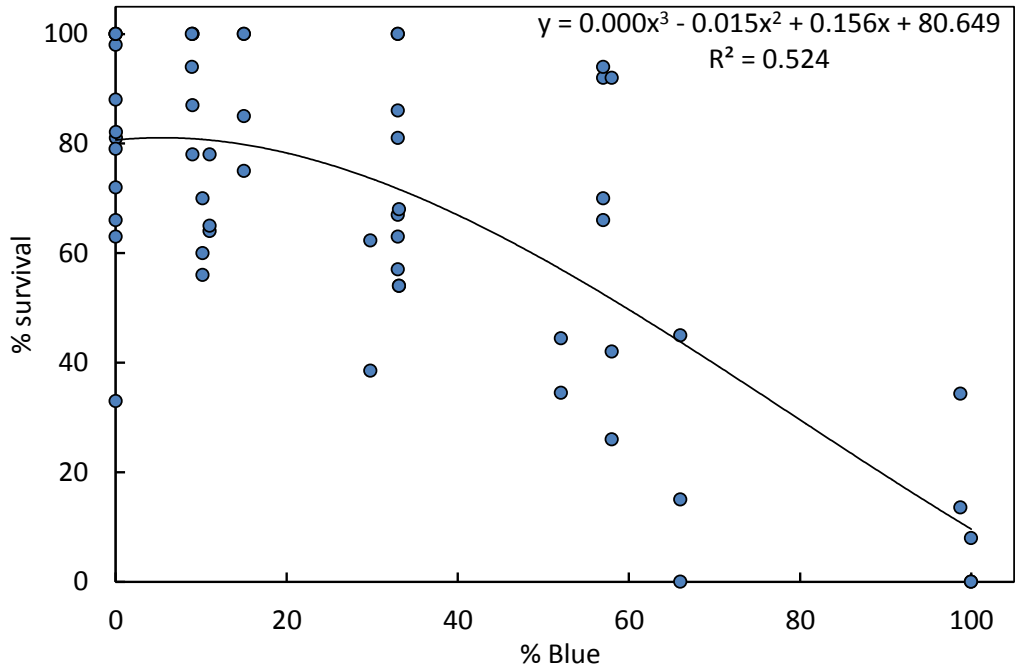

Figure 5.35. The relationship between post-excision blue light percentage (% blue) and the percentage of cuttings surviving. The data are combined from the experiments performed on eight species (photinia, rhododendron, eleagnus, santolina, iberis, clematis, lavender, and thyme).

In these trials, far-red light was found to decrease survival in five species (photinia, rhododendron, eleagnus, clematis-node cuttings, and santolina: Figure 5.31). Far-red responses mediated by phytochromes have been shown to enhance blue light induced stomatal opening (Holmes and Klein 1985). Far-red light has also been shown to cause prolonged increases in stomata opening in *Phaseolus vulgaris* when supplied in a background of white light, though far-red alone induces little stomatal opening (Holmes, Sager & Klein 1986). These prolonged increases in stomatal conductance were independent of phytochrome effects and were thought to be caused by photosynthesis driven by far-red light. If far-red was increasing stomatal conductance in the species examined in this trial, this may have cause increased dehydration. However, further experimentation will be required to confirm that increased dehydration was the cause of far-red reduced cutting survival.

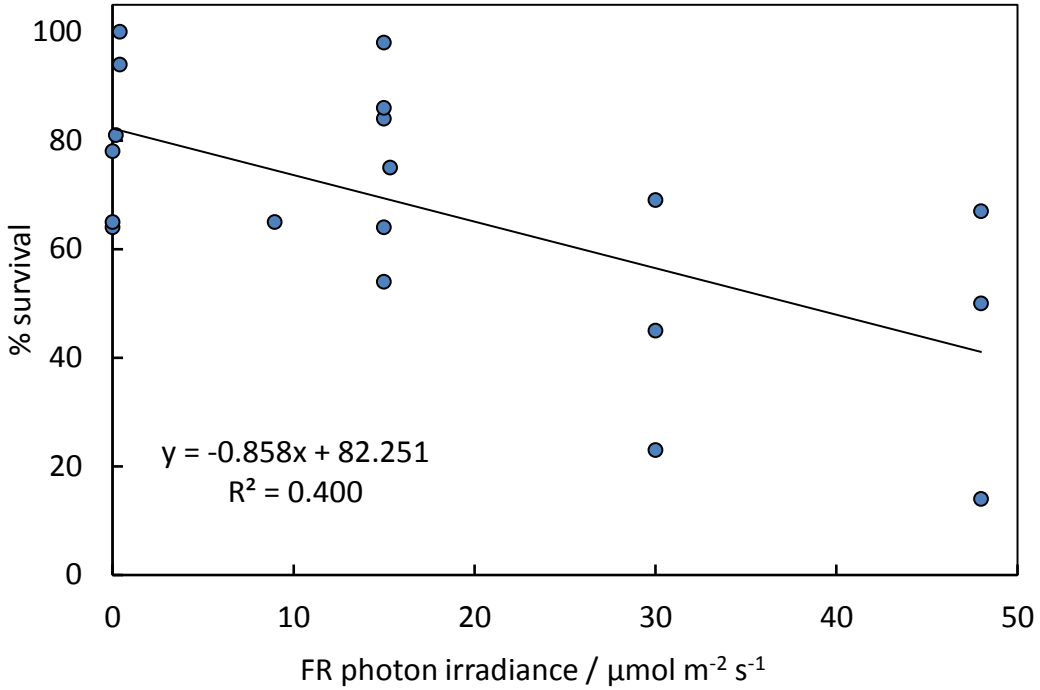

Figure 5.31. The relationship between post-excision far-red (FR) photon irradiance and the percentage of cuttings surviving. The data are combined from the experiments performed on photinia, eleagnus rhododendron, clematis (node cuttings) and santolina.

5.4.2. Cutting rooting and light quality

In order to ascertain how light quality influenced rooting we calculated the survival-corrected rooting percentage with the aim of separating some of the effects of dehydration and its effects on survival from the data. The variability in survival-corrected rooting percentage (under the 100% red light treatment rooting rates ranged between 100% and 9%) was greater than the variability in survival between the species examined and pre-treatment

effects (Figure 5.32). This reflects the range in the abilities of different species to form adventitious roots, which are driven by multiple factors including age and history of the cutting material, hormonal state of the cuttings (tip cuttings are expected to contain more auxin than nodal cuttings due to the presence of an apical meristem), and quantity of resources (carbohydrates) retained in the cutting. Some of these factors will be influenced by the state of dehydration in the cuttings and so the survival-corrected rooting values are not completely independent of their water status. Despite this variability, light quality was still found to have an important influence on root initiation and development. In 100% blue light conditions, rooting percentage did not exceed 50% in any of the species examined. This is consistent with the findings of Fuernkranz et al. (1990), where blue light was found to inhibit rooting, but in contrast with the findings of Alvarenga et al., (2015), where blue light provided the best conditions for rooting *Achillea*. In this trial, 100% red light was found to result in efficient rooting of most species. Monochromatic red light has also be found to result in good rooting in *Ficus* (Gabryszewska and Rudnicki, 1997), grapes (Poudel et al.,2008) lavender, rhododendron, and chrysanthemum (Christiaens et al 2014). Combined red: blue treatments improved rooting in rhododendron (33% blue) and photinia (15% blue) in this trial. The optimal red: blue ratios for rooting reported in the literature are diverse and probably represent differences in species and variety responses to light quality and the different experimental conditions (Christiaens et al 2016).

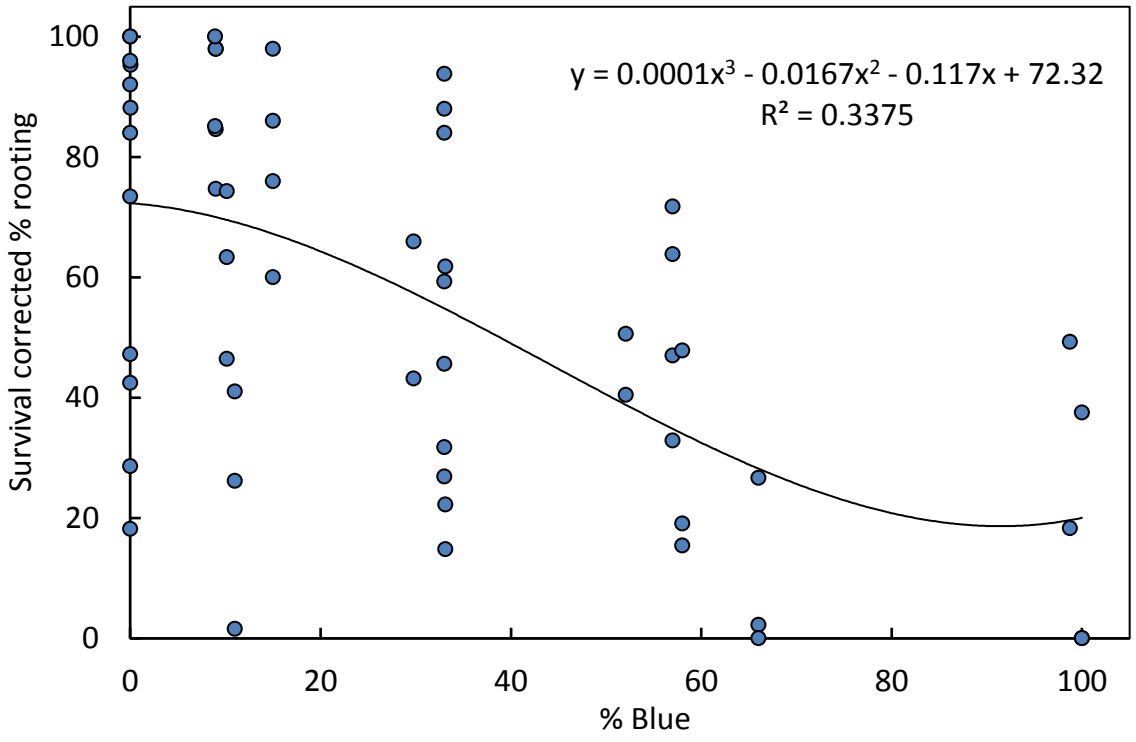

Figure 5.32. The relationship between post-excision blue light percentage (% blue) and the survival corrected percentage of cuttings that rooted. The data are combined from the experiments performed on eight species (photinia, rhododendron, eleagnus, santolina, iberis, clematis, lavender, and thyme).

In these trials, far-red was found to have a negative impact on rooting in the species examined. This is in contrast to the results examining far-red in the rooting of chrysanthemum reported in section two and by Kurilčik et al., (2008). It is possible that the far-red treatments failed to promote adventitious rooting because the far-red intensities were too high. It is also possible that, in these species, the addition of far-red is disrupting hormone synthesis and transport. Further research will be required to understand the underling factors leading to far-red reduced survival and rooting.

5.4.3. Hormones and light.

Following excision, several processes are initiated that lead to initiation and development of roots. Immediately after excision the concentration of several hormones increases rapidly. Once released, hormones are transported to the cut end of the stem where they influence gene expression. The hormones activate several gene families that are important for rooting, including those associated with carbohydrate metabolism (Druege et al. 2016). In the tomato work reported in this study, several hormones were at high concentrations at the first sampling point. While this indicates that our first sampling point was not representative of the pre-collection hormonal status of tomato plants it does demonstrate that tomato plants respond similarly to other species examined. The concentrations of JA and IAA were also found to be elevated at the first sampling point and both have been implicated in the early stages of root initiation (Druege et al. 2016).

Light quality is known to influence the synthesis and transport of plant hormones (Friml 2003) and light quality is thus expected to have a significant impact on the speed and ability of cuttings to root via hormonal as well as physiological effects. At the 48 hour time point, the light quality was observed to impact the concentration of several hormones. Auxin in particular was found at higher concentrations under low blue light environments, coinciding with the light treatments that resulted in the best rooting. Polar auxin transport is important for root initiation and chemical inhibitions of auxin transport reduce root formation (Christiaens et al (2014). More detailed studies will be required to refine our understanding of the complex interactions linking light quality, hormonal status, and adventitious rooting in different species, but these results provide confidence that these methods will be able to elucidate those interactions.

5.4.4. Internal cutting resources

Carbohydrates are important for root development as they are the building blocks for root growth. The post-excision concentration spike of auxin has been shown to activate expression of carbohydrate metabolism genes in petunia cuttings, beginning the conversion of starch reserves to sugars. A positive correlation between root formation and carbohydrate concentration has been reported in petunia (Ahkami et al 2013) and *Pisum sativum* (Veierskov, Stummann and Henningsen, 1982). In addition, *Protea cynaroides* produced more root mass when cuttings contained higher starch concentrations (Wu 2006). Several studies have also reported the benefits of treating cuttings with sugars (sucrose) for improving strike rates (Calamar & de Klerk 2002, de Souza et al 2012, Vanajalatha, Sharma, Singh & Mishra 2015). However, carbohydrates have not always been found to improve rooting and in *Pinus sylvestis* carbohydrate concentration was observed to reduce the formation of adventitious roots (Hansen Stromquist & Ericsson, 1978).

There are two major factors that influence the endogenous carbohydrate concentration in cuttings: 1) the amount of reserves held in the cutting at the time of excision, and 2) the ability of a cutting to photosynthesize after excision. The reserves held within a cutting will differ between species and will also differ due to the environment encountered by the plant from which the cuttings were collected. In these trials, santolina cuttings rooted more successfully and rapidly when the mother stock plants were exposed to supplemental lighting through the winter months. Iberis stock plants that were provided with supplemental lighting rooted more rapidly than those from unlit mother stock plants. It is possible that the supplemental light treatments increased the reserves held in these cuttings, boosting rooting. In our santolina trials, day extension lighting treatments resulted in some etiolation of the cutting material and these cuttings performed poorly in rooting trials. It may be the case that plants under these conditions used their internal reserves to power the etiolated growth, subsequently reducing rooting. These data are in contrast with studies that have reported the benefits of inducing etiolation in woody cuttings (Eucalyptus – Hoad and Leakey 1996; fourteen woody species - Maynard & Bassuk 1985, Protea - Wu 2006). Etiolation has been found to reduce lignification (Reid 1923) and differentiation (Gardner, 1936) while increasing the concentration of phenolic compounds that promote rooting (Wu 2006). Further research is required to identify which species can benefit from pre-excision far-red light treatments and which do not.

If cuttings are able to photosynthesize after excision this will help maintain carbohydrate concentrations and this may be especially important in species that take longer to root. To photosynthesize efficiently, plants must open their stomata. In high blue

light treatments the blue light will initial prompt stomatal opening, causing the symptoms of dehydration. Once dehydrated, stomata would close to minimise further dehydration and would remain closed until the cuttings have fully rehydrated. The closed stomata would then reduce the ability to photosynthesize and over time carbohydrate reserves would be depleted. In the cuttings propagated under low-blue conditions, cuttings remained hydrated and stomata would still be able to open (stomata do open under 100% red light but to a lesser extent than in the presence of blue light), enabling ongoing photosynthesis to occur.

Our results add to the growing body of research indicating that light quality has strong influences on rooting cuttings. While identification of an ideal light treatment for rooting all species is unlikely, these results demonstrate that substantial improvements can potentially be made via spectral manipulation. We have also made the first steps in linking the interactions between light quality, hormonal status, and development of roots.

5.5. Key Findings

- There are three key factors to consider when designing light treatments for optimised rooting of cuttings: 1) preventing post excision dehydration, 2) changes in hormonal status, and 3) internal cutting resources.
- The light environment provided to cuttings during rooting has a strong influence on strike rates.
- Cutting survival and strike rates are greatest under light environments with low blue percentages and no far-red light, potentially due to a reduction in cutting dehydration.
- Small amounts of blue light (11%) promoted rooting in some species and may be associated with improved carbon fixation.
- Far-red light reduced survival but may promote root development in some species. Further experimentation would be required to understand this process.
- Red light environments cause higher auxin concentrations in tomato cuttings after 48 hours. This is thought to be a major factor contributing to improved rooting under red light.

Section 6: Insect light responses: light quality and its influence on invertebrate pests and biocontrol agents

6.1. 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 (hereafter taken to include mites for the purpose of this report) and biological control organisms. Nonetheless, production of high-quality plants requires an understanding of pest and beneficial insect species' responses to light conditions, to enable efficient monitoring and control of populations before economic damage can be caused to crops.

Sensitivity to light mediates many behavioural and physiological responses in insects (Shimoda & Honda, 2013). Light controls, for example, insect circadian rhythms, but also modulates innate and learned behaviours via visual cues perceived through compound eyes and ocelli (light sensor organs found in many invertebrates). There is a great diversity in insect visual systems, and spectral sensitivities differ between species. Many invertebrates have trichromatic vision with photoreceptor spectral sensitivity peaking in the UV, blue and green wavelengths (Shimoda & Honda, 2013). This is true of, for example, honeybees (Menzel & Blakers, 1976), though other hymenoptera have been shown to have red receptors as well (Peitsch *et al*., 1992). The thrip *Caliothrips phaseoli*, on the other hand, is only able to perceive UV wavelengths (Mazza *et al*., 2009). In addition to colour, insects also respond to light polarisation, intensity, and to areas of contrast. Within species, the sexes may also respond in differing ways. For example, male and female western flower thrips (*Frankliniella occidentalis*) have comparable visual sensitivities, but exhibit differing swarming behaviours resulting in males being more likely to gather on flowers than females of the species (Matteson *et al*., 1992).

Insect sensitivity to light affects behaviours and decisions such as orientation, flight initiation and landing, dispersal, host location, and feeding rates. The impact of light *per se* on insect activity has been shown repeatedly, and though much work has been reported on the influence of light intensity, the impact of light spectra is less well studied and more poorly understood. This, however, is likely to play an important role in LED-based crop production facilities, where both green and UV wavelengths are typically absent. The above in mind, we designed a series of experiments to investigate light effects on pest and beneficial insect responses, with a view to identify whole system responses that could inform monitoring and control systems for crop protection. For the purposes of this report, the experiments have been broadly classed into three aspects: 1) monitoring pests under

LED lit environments, 2) pest responses to the different light environments, and 3) the responses of beneficial insect to the LED lit environment.

1) Pest monitoring

Early identification of pest presence is essential for successful control, and insect populations are often monitored using coloured sticky traps as they are low-cost and easy to use. Sticky traps are commonly yellow or blue, with different species having preferences for different colours. Sticky traps are only effective if they attract the targeted pest species, thus allowing identification prior to the development of significant problems. In most cases, the spectral sensitivity of insect vision is not known and so designing insect traps is largely trial and error. Trap effectiveness has been evaluated in naturally illuminated systems (with supplemental HPS lighting). The benefits of adding green LEDs to traps has also been assessed and found to enhance capture of certain, but not all, insect species (AHDB CP088; Nakamoto & Kuba, 2004). In enclosed structures illuminated with red and blue LEDs, colour perception is greatly altered. Any species that show preferences for yellow sticky traps or increased attraction to green LED-enhanced traps are expected to be less likely to be caught on traps under red: blue illumination. The experiments reported in this work package outline the sticky trap pest monitoring program performed in the LED4CROPS facility. This monitoring assessed the effectiveness of different coloured sticky traps under LED lighting and initiated the first steps in improving sticky trap effectiveness.

2) Pest responses to different light environments

In addition to the direct effects expected on visually-based pest insect orientation, dispersal and host location responses, and potential impact on insect physiology (e.g. modulation of insect circadian rhythm), the altered light conditions in enclosed LED-productions systems are also likely to elicit indirect effects on pest populations as well. Such effects would be mediated through changes to plant chemistry, particularly given the pivotal role UV-B wavelengths, along with red:far-red wavelength ratios, are known to play a role in modulating or upregulating plant defence chemistry. The degree to which different light regimes affect different pest insects is, therefore, likely to be dependent on the degree to which the target organism relies upon vision and host plant chemistry in host plant location, as well as the degree to which the plant responds to varying light treatments, 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, through both physical means (e.g. changes to leaf morphology such as thickening or curling) and chemical means (e.g. higher levels of defensive compounds in plant tissue).

Whilst it would not be possible to fully understand the mechanisms behind such responses within the current study, a series of experiments were planned with the aim of providing data to demonstrate whole-organism responses of pest insects 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.

3) Responses of beneficial insects to different light environments

Currently, biological control is the primary method used to manage pest populations in the enclosed LED facility at STC. Effective deployment of biological control in enclosed LED-lit systems presents a challenge, given that the absence of UV and green light in enclosed systems is likely to affect many beneficial insects that rely on vision. The absence of non-red: blue wavelengths has potential to impact upon beneficial insect activity and ability to orientate and disperse within LED units for such species, subsequently affecting how they locate and navigate towards target pests. In addition, changes to plant chemical responses to pest attack, which in many cases provide chemical signals for biocontrol organisms, may be modulated by plant light responses, adding an additional level of complexity to the plant-pest-beneficial insect interaction. It is therefore essential to understand the responses and control potential of existing biological control organisms under different red: blue wavelengths to inform pest management strategies under enclosed LED production systems. A series of experiments were designed to investigate the biocontrol potential of several commonly used beneficial insect species. Parasitoid wasps and predatory mites were selected as pest natural enemies for investigation, based on varying life histories and visual acuities.

6.2. INSECT MONITORING

6.2.1. Methods

Qualitative assessment of insect species presence in LED facility

The insect species present in the LED4CROPS facility were qualitatively assessed to determine which species were present, and which of these were attracted to the sticky traps used. No quantitative assessments of insect populations were made. Assessments were based only on those species that were passively introduced into the facility on soil and plants brought in. Insects were not deliberately introduced or released into the facility, and pest species were actively discouraged through altered management, introduction of biocontrol agents and, when necessary, spraying of plant material.

Insect detection and sticky trap attractiveness

Standard yellow and blue sticky traps were placed side by side, flat, on an upturned tray to keep them out of the irrigation solution, at the centre of each LED bench between the experimental plants. Traps were placed flat rather than hung to ensure even illumination of the traps; when hung vertically, the traps were illuminated unevenly in terms of both colour and light intensity which could be expected to influence the results. The numbers of insects on each trap were counted at regular intervals. The numbers of species of insect identified on each trap were recorded. After each count, fresh traps were placed out. Traps were located on light benches illuminated with red: blue mixtures of light as well as a red: white mix that acted as the control in this experiment.

Improving sticky trap effectiveness

In order to examine if sticky trap effectiveness could be enhanced in red: blue light conditions, the use of fluorescent coloured card to make sticky traps that appeared different colours even when viewed under the red: blue light mixtures (Figure 6.1) was assessed. The fluorescent pigments contained in the card function by absorbing blue light and re-emitting (‘fluorescing’) the energy as light with a longer wavelength (yellow, green, or pink).

The spectrum of the light emitted from the fluorescent card was determined by measuring the reflectance spectrum of the traps when illuminated with blue light only. Reflectance spectra were measured using a Jaz spectroradiometer (Ocean Optics Inc., Florida, USA). The sensor head was mounted 5cm above the trap at 45°, such that the sensor did not shade the trap.

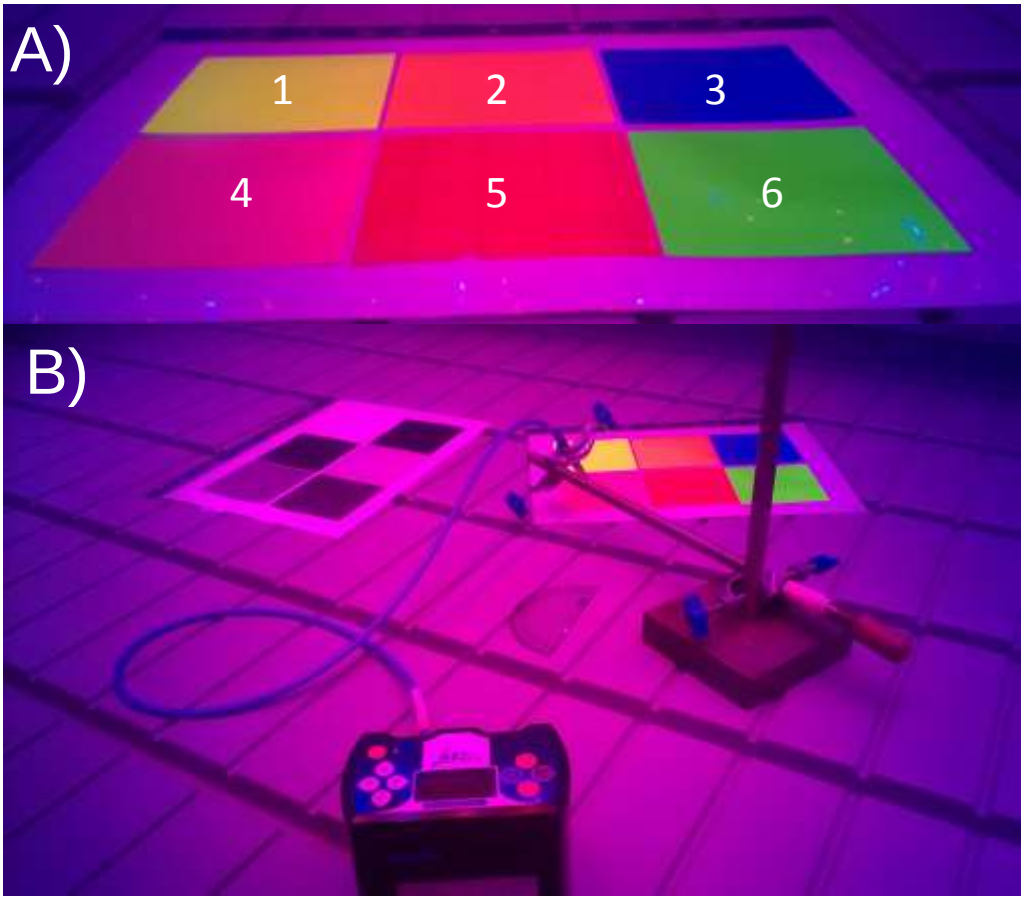

Figure 6.1. A) Coloured sticky traps viewed under red: blue light, where: 1 = Fluorescent yellow card; 2 = Fluorescent orange card; 3 = Standard blue sticky trap, 4 = Fluorescent pink card; 5 = Standard yellow sticky trap; 6 = Fluorescent green card. **B)** Set-up for measurement of reflectance spectra of the different coloured traps.

6.2.2. Results

Qualitative assessment of insect species presence in LED facility

Several pest species were observed in the facility over the course of this study, including shore flies (*Scatella stagnalis*), fungus gnats (*Bradysia spp*.), aphids (e.g. *Myzus persicae*), onion thrips (*Thrips tabaci*), two-spotted spider mites (*Tetranychus urticae*), owl midges (family *Psychodidae*), and leafhoppers (family *Cicadellidae*). Total numbers are summarised in Table 6.1.

Fungus gnats and shore flies were the most numerous species, and numbers correlated with the transient changes in the area of exposed soil within the facility. Once introduced into the facility, fungus gnat and shore fly populations could be sustained by algal growth occurring on the trays or on rock wool growing media. Adjustments to soil water content through changes in management of the irrigation system were partially successful in reducing numbers, though they did not eradicate these species. Treatment of

the soil with nematodes (Nemasys® [*Steinernema feltiae*], BASF UK plc., Cheadle, UK) was successful at reducing fungus gnat populations but did not influence shore fly numbers.

Aphid and thrip populations were observed occasionally within the facility. When observed, these species were typically found to have high numbers, but with very localised spatial distributions. Thrip populations were also more persistent in the facility than those of aphids. Leafhopper nymphs were observed on the *elaeagnus* cuttings during the propagation trials. No adults were observed or trapped before crop damage would have occurred.

Table 6.1. Total numbers of each insect species observed on sticky traps under the red:blue light treatments during between May and August 2014, and whether plant damage occurred prior to identification on traps. NP = not present during this period or under the red:blue treatments. NO = pest never observed on sticky traps.

Species	Number caught between May and August 2014	Plant damage observed before presence on traps
Shore fly	2715	No
Fungus gnat	5071	No
Owl midge	102	No
Onion thrip	92	Yes
Leafhopper	NP	Yes
White fly	NP	No
Aphids	NO	Yes

Insect detection on sticky traps

Both fungus gnats and shore flies were regularly observed on the sticky traps, and variations in numbers were consistent with changes in observed populations (Figure 6.2). The presence of owl midges on traps was the first indication of their presence in the facility, possibly due to the difficulty of distinguishing between flying insects under the red: blue light conditions (where human vision is compromised). Onion thrips were observed on the sticky traps but in low numbers and only in locations in the facility where populations were very high and significant plant damage had already occurred. Plant damage was the first sign of these pests. Whitefly were observed on the sticky traps but these pests were only observed in the facility on plants grown under the white light treatments, so no information regarding trap effectiveness under the red: blue LEDs was gathered. Leafhopper adults were observed on the sticky traps, but this was long after nymphs had been observed and damage to plants had occurred.

Sticky trap attractiveness and insect colour preferences

Under the control white light (red: white LED light mix), fungus gnats were five times more likely to be caught by a yellow trap than a blue trap (Figure 6.3). This is similar to the colour preferences observed in natural light environments. Under red: blue light conditions, the fungus gnats were three times more likely to land on a yellow sticky trap than a blue trap, indicating a reduction in attraction towards yellow traps. Under control white light, shore flies were 30% more likely to land on blue traps compared to yellow traps, though no preference for either colour was observed under red:blue light.

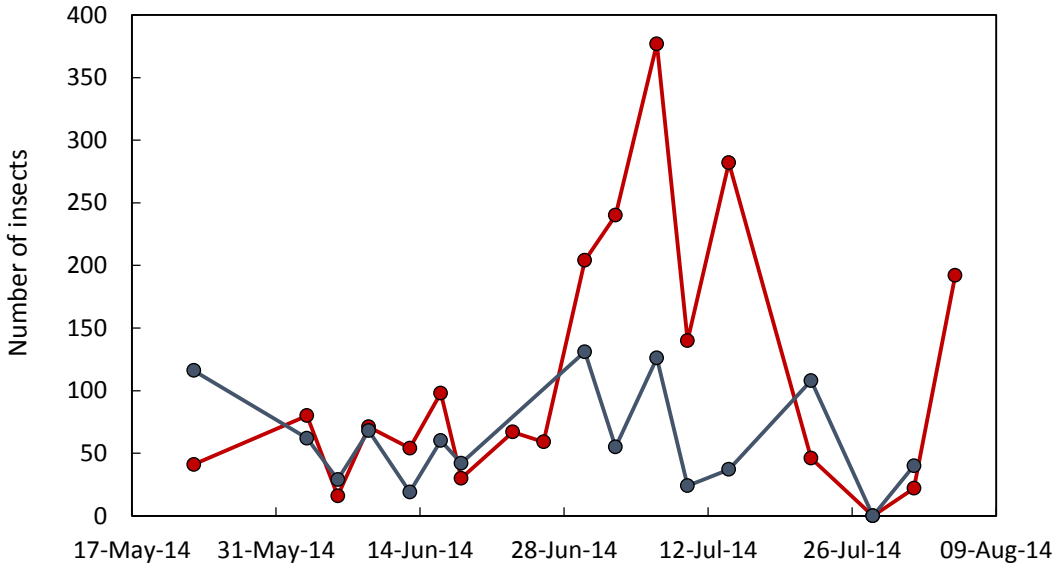

Figure 6.2. Numbers of shore fly (blue) and fungus gnats (red) observed on sticky traps in the LED4CROPS research facility between May and August 2014.

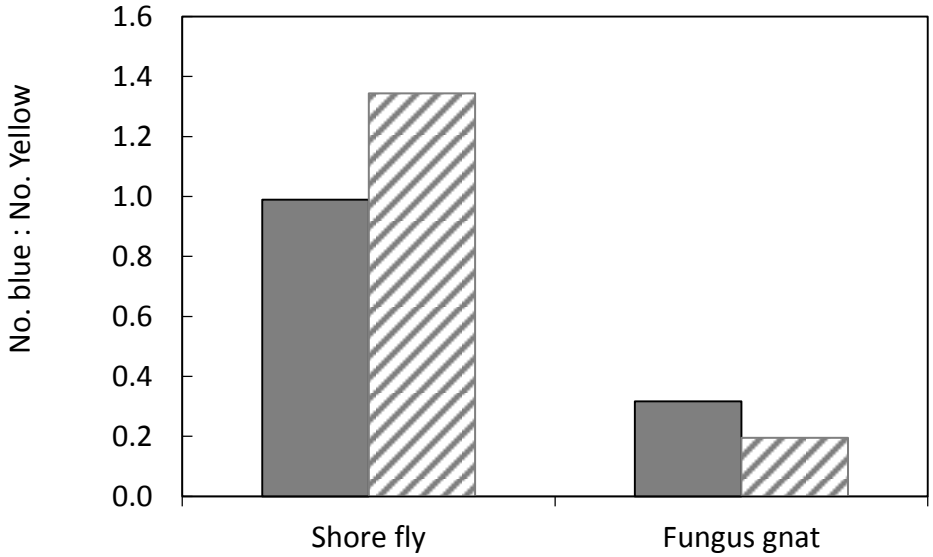

Figure 6.3. Insect colour preference (standard yellow *vs*. standard blue sticky traps) for sticky traps illuminated with red: white (hatched bars) compared with traps illuminated with a 11% blue : 89% red (solid bars) LED lights.

Thrips were observed to have a preference for blue sticky traps (especially under the 100% blue light treatments), with up to five times more insects landing on blue rather than yellow traps. The overall number of insects caught was low, however, so caution must be employed with this dataset and results are not shown. The preferences of other insects were also difficult to determine due to low trap counts.

Closer examination of the trap data for fungus gnat and shore flies (the two species where sufficient numbers of insects were observed to perform this analysis) was able to show alterations for the blue/yellow count ratios (Figure 6.5) as the blue percentage of the LED light changed. For fungus gnats, the greatest preference for yellow was observed under 33% blue light and the least preference occurred under 100% blue light. For the shore flies, a slight increase in preference for blue traps was observed as the blue light percentage increased.

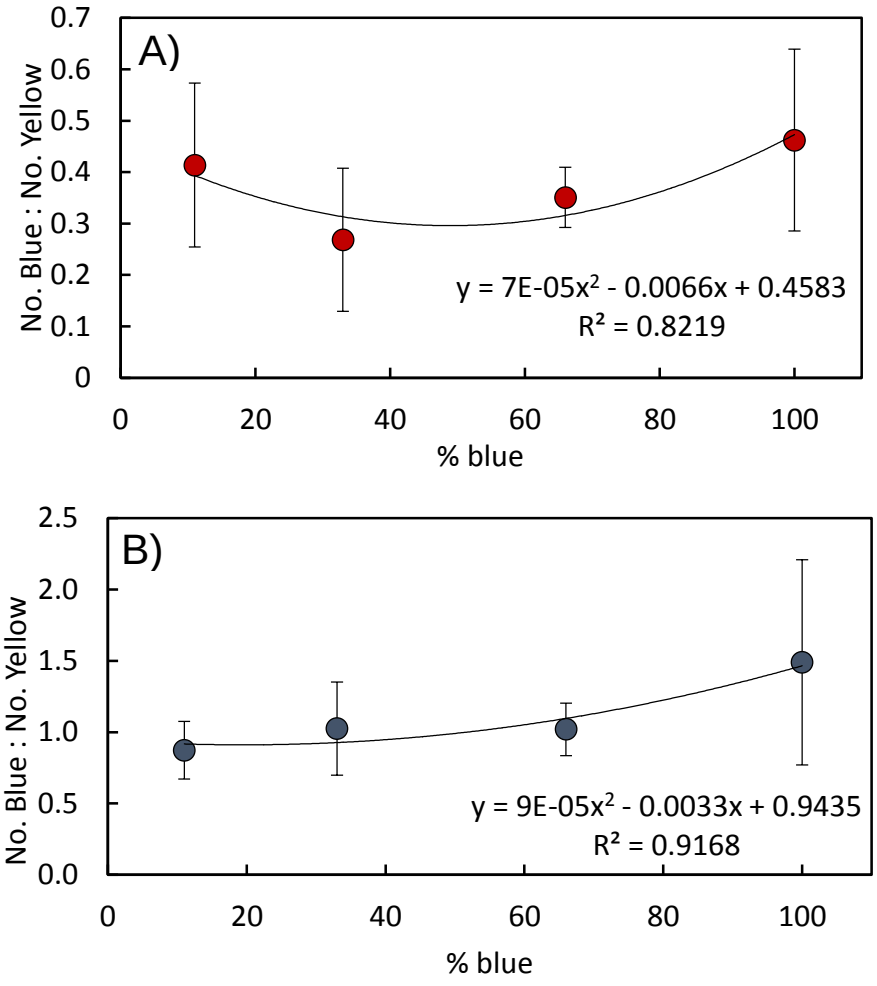

Figure 6.4. The influence of red: blue ratio of the illuminating light on the colour preference (yellow versus blue sticky traps) of **A)** fungus gnats and **B)** shore flies. Data are expressed as the ratio of the number of insects caught on blue traps divided by the number of insects caught on yellow traps.

Fluorescent card attractiveness

As many insects are sensitive to green light, which is not present under the red: blue light treatments, the reflectance spectra of several fluorescent cards to identify colours that may improve sticky trap effectiveness were examined (Figure 6.5). Under 100% blue light, the standard blue traps reflected the greatest amount of light in the blue region and reflected no other colours of light. The standard yellow sticky traps reflected the least amount of blue light and reflected no light in the rest of the spectrum. The fluorescent yellow and green traps reflected an intermediate amount of blue light and fluoresced in the green region of the spectrum, with an emission peak near 525nm. The emission band of the yellow fluorescent trap extended further into the red region than the green and this is the basis for the different colours of the traps. The orange and pink sticky traps fluoresced at a longer wavelength than the yellow and green traps (peak emission near 610nm). The orange trap emitted more fluorescent red light than the pink trap, while the pink trap reflected more blue light (the orange trap reflected the same amount of blue light as the fluorescent yellow trap). These reflection spectra indicate that the fluorescent yellow and green traps should increase insect trapping efficiency if the insects are sensitive to green light.

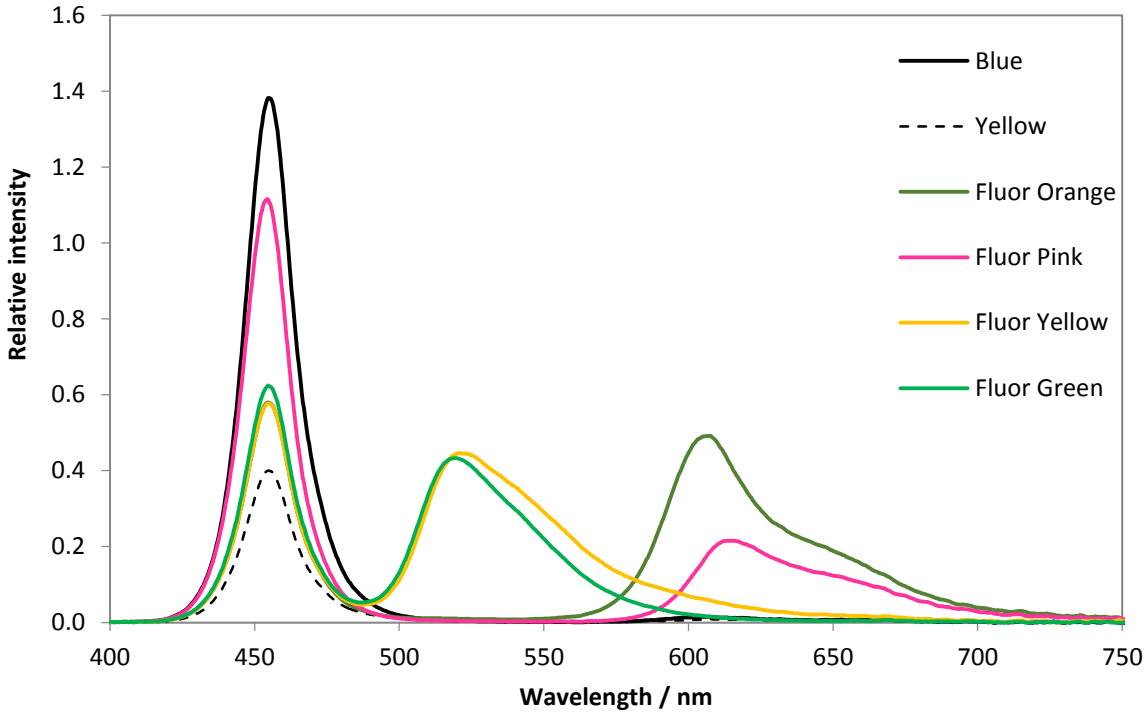

Figure 6.5. Reflectance spectra of the six colours of sticky trap used in the insect colour preference experiments when illuminated with only blue LEDs.

Shore flies were found to exhibit different preferences for the different coloured traps, with fluorescent yellow, green and orange traps attracting more insects than standard yellow traps. Fluorescent pink traps attracted fewer insects than standard yellow but more than standard blue (Figure 6.6). For fungus gnats, a similar, though more pronounced, response was observed. Yellow and green fluorescent traps were twice as likely to trap fungus gnats as standard yellow traps. Pink trap effectiveness was similar to that of the standard blue traps, with about half the number of insects trapped as on the standard yellow traps. When total numbers of insects caught was expressed *vs*. the amount of green light measured in the reflectance spectrum, a positive correlation was observed for both shore flies and fungus gnats (Figure 6.7). When the colour preference relative to the standard yellow sticky trap was plotted versus green reflectance, the effect of colour was found to be greater for the fungus gnats than for shore flies (Figure 6.7B).

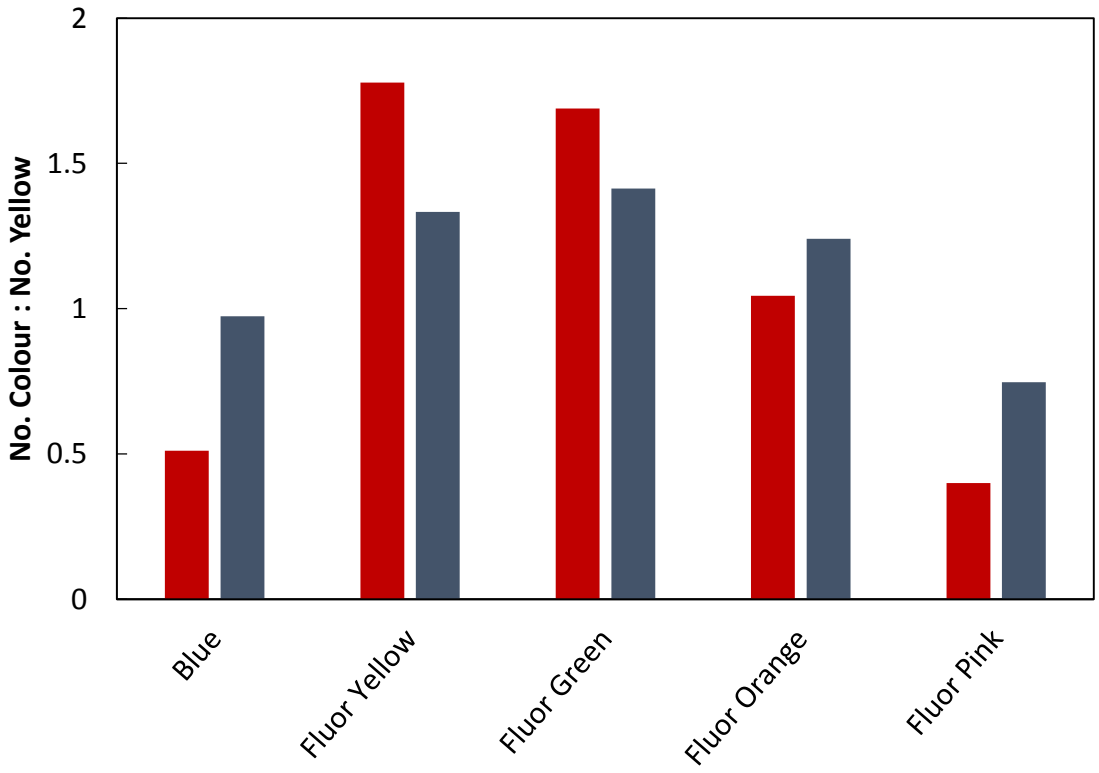

Figure 6.6. The colour preference of shore flies (blue) and fungus gnats (red) for standard blue and four fluorescent coloured sticky traps in comparison to standard yellow sticky traps when illuminated with red:blue light mixtures.

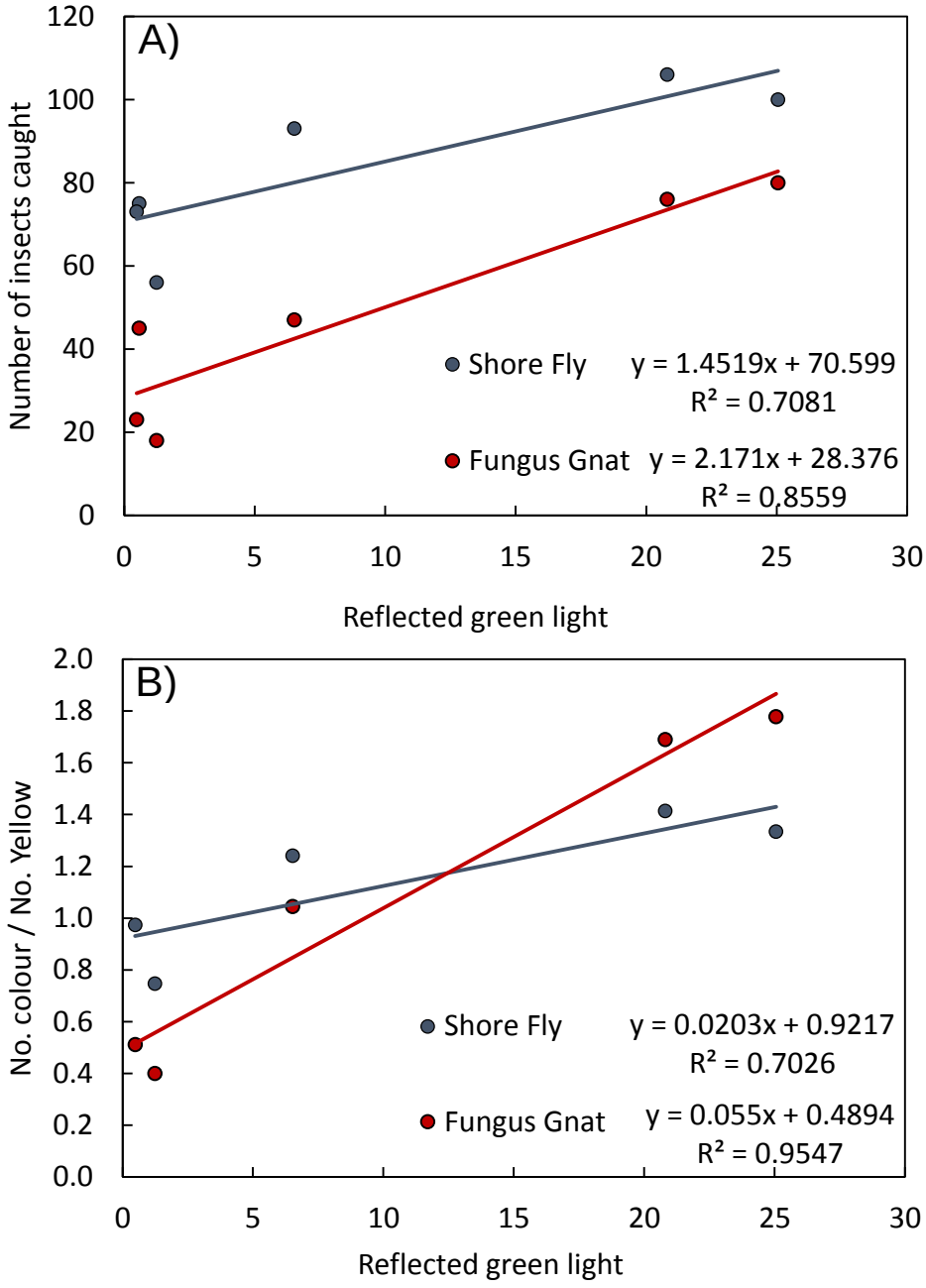

Figure 6.7. The influence of the amount of green light reflected from the different coloured sticky traps on **A)** the total number of insects caught and **B)** the relative preference for the five colours of sticky traps compared with a standard yellow trap.

6.2.3. Discussion

Insect monitoring using sticky traps under red:blue light was effective for some species but not all. Fungus gnats and shore flies were found to land on standard yellow and blue sticky traps regularly, and trap counts closely tracked changes in observed populations. For other species such as aphids and thrips, sticky traps were less effective. No aphids were observed on sticky traps and thrips were only observed when pest numbers were high enough to have already caused significant damage to plants. The most likely reason for poor trap efficiency under the LED light treatments in these species is reduced insect flight within the LED facility. Aphid and thrip populations attained high numbers at specific locations, but their spread within the facility was limited. Aphids were not observed to spread from their initial infestation area. Aphid populations are typically composed of a majority of non-winged individuals, which would not fly. Winged form production, however, is triggered by both crowding and certain light condition triggers. It may be that the altered light conditions under LEDs inhibited the production of these forms despite localised crowding. Thrips were observed to spread between light racks but at a lower frequency than expected based on their population sizes. Thrip flight may have been repressed under LED lighting, or the threshold level for triggering of flight behaviours could have been altered. Broadly generalised, if the insects remain on the plants, the traps will be unable to attract the insects. Investigation into the mechanisms behind such responses was beyond the scope of this work package, however, and any such speculation must be circumspect. There is also the possibility, given that thrips are known to be attracted to blue *per se*, that the blue LEDs themselves proved a greater attractant to thrips than the traps they were illuminating, even where these traps were blue in colour, reducing trap catches as a result. This is again speculative, however, with no supporting evidence of such behaviour in thrips observed within the LED facility.

Even though the standard yellow and blue traps caught certain insect species under the red: blue light mixtures, insect colour preference was altered. If insects are less able to distinguish between colours under the red: blue lights then it is expected that the traps will be less attractive and, therefore, less effective at providing an early warning system. Use of fluorescent materials that appear yellow or green under the red: blue lights improved the relative trap attractiveness. The improvement in trap attractiveness is likely to result from the traps appearing brighter to insect vision systems, with brighter traps more effective at trapping insects (Bowden, 1982).

6.2.4. Conclusions

Monitoring insect pest populations in enclosed LED-production facilities will require modification of existing techniques and an understanding of insect responses to the light conditions. Colour perception and preference of insects is altered under different red:blue light treatments, which in turn alters sticky trap effectiveness. The use of fluorescent sticky traps may help limit such changes, and improve their effectiveness as a monitoring technique in LED facilities.

6.3. PEST RESPONSES TO LIGHT QUALITY: APHIDS.

6.3.1. Aphid Methods

All experiments investigating aphid responses to light quality were conducted on five light treatments, as described in Table 6.2.

Table 6.2. Specification of the light treatments used in aphid light response trials. All irradiances indicated in μmol m^{-2} s^{-1}.

Light treatment	White Control	100 % B : 0% R	66 % B : 34% R	33% B : 67% R	0% B: 100% R
PAR (400-700nm)	200	145	200	200	200
Blue (400-500 nm)	12	145	132	66	0
Green (500-600 nm)	15	0	0	0	0
Red (600-700 nm)	173	0	68	134	200
% blue	*6*	*100*	*66*	*33*	*0*

Aphid performance indicators

Fifty plants in individual pots were grown under five different LED light treatments, such that there were ten plants per treatment. Plants were watered throughout by the automated system in the facility.

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

One adult *Myzus persicae* 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 photoperiod) was caged on each of the plants with a clip cage at the start of each experimental trial. Each cage was left undisturbed for 24hrs 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.

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.

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 assumed to be zero.

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 rm 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 assumed to be zero.

Plant chemical analyses: Plant foliar material was collected from the lettuce plants 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.

Aphid population growth

Six verbena plants were reared under each light treatment until ten weeks of age in the LED facility. Stems were then trimmed to 15cm length. A segment of verbena leaf with approximately twenty melon aphids (*Aphis gossypii*), taken from stock cultures maintained at 20°C under a 16L:8D hr photoperiod in a CT room, was then placed on a leaf midway up a stem to artificially infest each trimmed plant. A bread bag was then placed around each plant and secured around the pot. Plants were then placed under white light in the CT room and were left undisturbed for ten days to allow aphids to establish. The segment of cut leaf was then removed and the numbers of aphids assessed before plants were returned to their designated light treatment in the LED facility. Plants were left undisturbed for a further ten days under LED light treatment. They were then destructively sampled and aphid numbers counted to give an assessment of population increase.

Statistical analyses

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 & 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). Aphid population growth effects were analysed in Minitab (v.17) by one-way ANOVA and post-hoc Tukey's HSD tests.

6.3.2. Aphid Results

Aphid performance on lettuce

Pre-reproductive period (*D*): The number of pre-reproductive days was found to be affected by light regime ($F_{4,8}$ = 2.79, P < 0.05; Figure 6.8). A post-hoc Tukey's HSD Test indicated aphids reared under 66% blue light were found to have a greater number of pre-reproductive days than under the white control, 100% blue and 100% red light treatments.

Mortality: Aphid pre-reproductive percentage mortality was found to differ by treatment ($F_{4,8}$ = 5.14, P < 0.05; Figure 6.9) and by trial ($F_{2,8}$ = 11.70, P < 0.01); where mortality was increased under 66% blue light when compared with a white light control, and where mortality was lower in trial 3 than that in the other trials. An interaction between treatment and trial was not supported. A post-hoc Tukey's HSD Test also indicated a pairwise difference between mortality under 66% blue light and 100% blue light, with mortality under the former being greater than that of the latter.

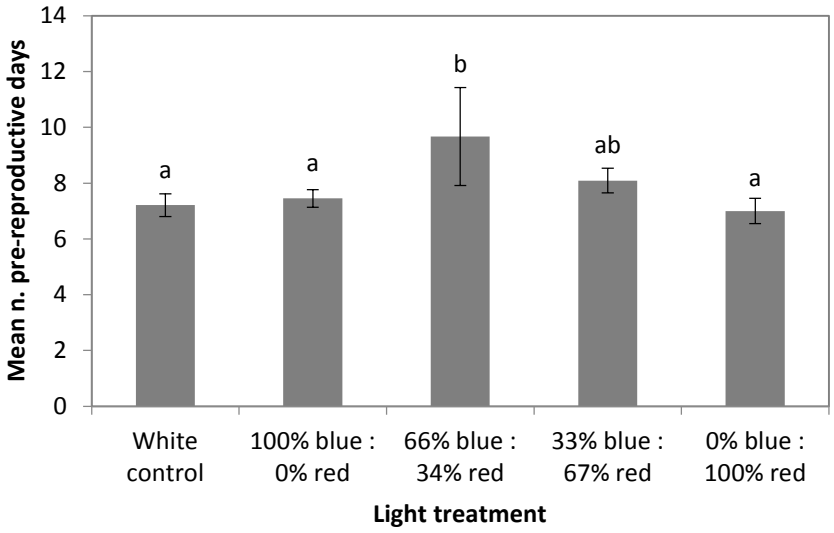

Figure 6.8. Mean number of pre-reproductive days of *Myzus persicae* on lettuce under LED lighting. Means are displayed ± SEs, where n = 30. Means not sharing a common letter are significantly different (P < 0.05) as indicated by a Tukey's HSD Test.

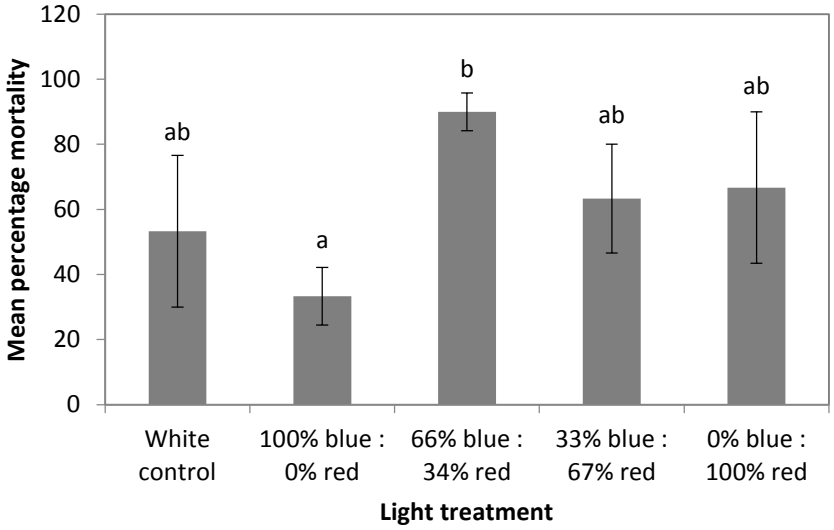

Figure 6.9. Mean pre-reproductive percentage mortality of *Myzus persicae* on lettuce under LED lighting. Means are displayed ± SEs, where n = 30. Means not sharing a common letter are significantly different (P < 0.05) as indicated by a Tukey's HSD Test.

Seven-day fecundity: Seven-day fecundity was not statistically affected by light regime ($F_{4,8}$ = 1.25, P > 0.05; Figure 6.10), though appeared generally reduced under some light treatments, particularly 66% blue.

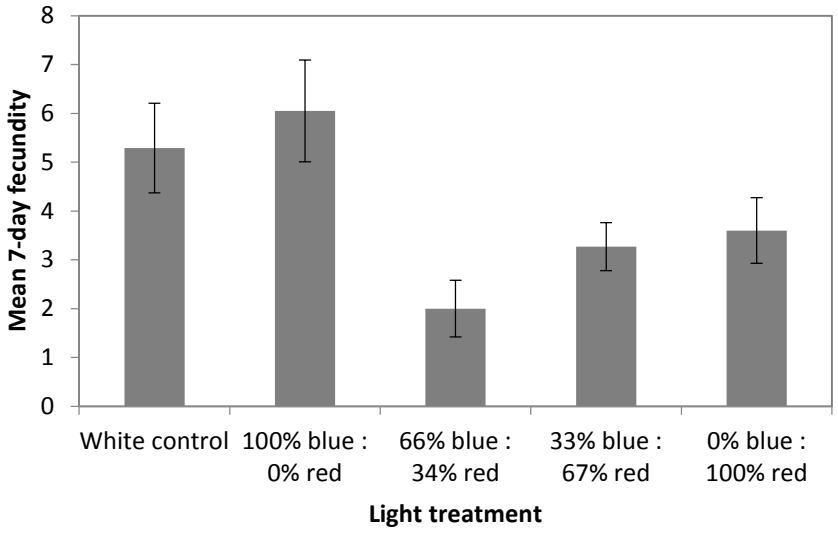

Figure 6.10. Mean seven-day fecundity of *Myzus persicae* on lettuce under LED lighting. Means are displayed ± SEs, where n = 30. Means not sharing a common letter are significantly different (P < 0.05) as indicated by a Tukey's HSD Test.

Intrinsic rate of increase (r_m): Intrinsic rate of increase was not statistically affected by light regime ($F_{4,8}$ = 1.96, P > 0.05; Figure 6.11), though appeared generally lower under 66% blue light.

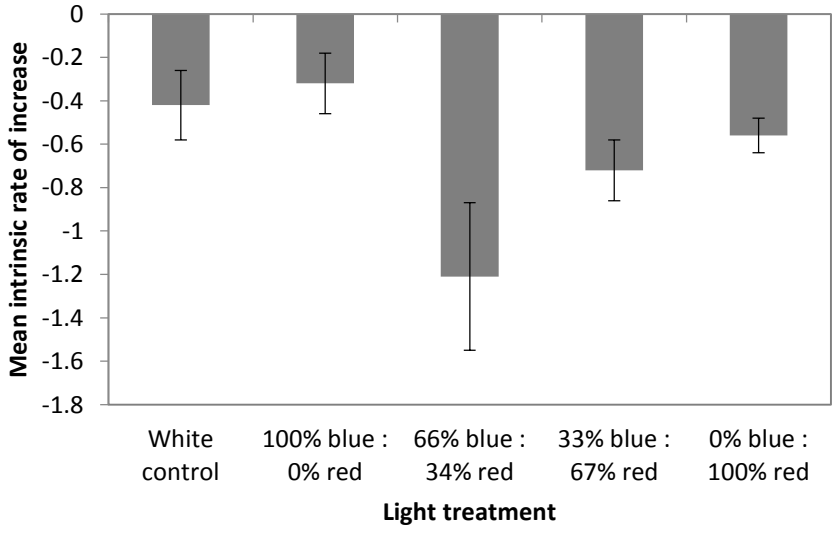

Figure 6.11. Mean instrinsic rate of increase (r_m) of *Myzus persicae* on lettuce under LED lighting. Means are displayed ± SEs, where n = 30. Means not sharing a common letter are significantly different (P < 0.05) as indicated by a Tukey's HSD Test.

Lettuce foliar chemistry effects on aphid performance

Following macro- and micro-nutrient analysis, only mean percentage nitrogen content of lettuce used in this trial was found to differ statistically by light treatment ($F_{4,10}$ = 5.16, P < 0.05), where plants under 100% blue, 33% blue and 66% blue light were found to have higher levels of nitrogen than plants under the white control by post-hoc testing. These changes were not found, however, to affect the number of pre-reproductive days ($F_{1,4}$ = 1.21, P > 0.05), seven-day fecundity ($F_{1,4}$ = 0.32, P > 0.05) or intrinsic rate of increase ($F_{1,4}$ = 1.22, P > 0.05) when interacting with light treatment.

While no statistically significant difference in mean manganese (mg/kg) content was found by light treatment, levels of this micro-nutrient were found to influence aphid performance. An overall response of aphid pre-reproductive time to an interaction between the light treatment and mean manganese content was found ($F_{1,4}$ = 6.25, P = 0.05), where the number of pre-reproductive days of aphids on plants under 66% blue light was lower than that of those under the white control. Seven-day fecundity also responded to an interaction between the light treatment and mean manganese content ($F_{1,4}$ = 11.31, P < 0.05), where fecundity of aphids on plants under 100% blue, 100% red and 33% blue light was reduced in comparison to those under the white control. Finally, the intrinsic rate of increase was also found to be affected by an interaction between the light treatment and mean manganese content ($F_{1,4}$ = 65.11, P < 0.001). Aphid performance for this indicator on plants under 100% blue, 100% red and 33% blue was reduced relative to the performance of aphids on plants under the white control, while it was marginally improved under 66% blue light.

Aphid performance on verbena

Pre-reproductive period (*D*): The number of pre-reproductive days was found to be affected by light regime ($F_{4,8}$ = 2.87, P < 0.05; Figure 6.12). Post-hoc analysis indicated aphids reared under 66% blue light were found to have more pre-reproductive days than those 100% blue light treatment.

Mortality: Aphid pre-reproductive percentage mortality was not found to differ by treatment ($F_{4,8}$ = 0.63, P < 0.05; Figure 6.13), though was relatively variable with lowest mean mortality under the white light treatment.

Seven-day fecundity: Seven-day fecundity was found to be affected by light regime ($F_{4,8}$ = 3.14, P < 0.05; Figure 6.14). Aphids reared on verbena under 100% blue light were shown to have higher fecundity by post-hoc analysis, when compared with aphids under the white light control.

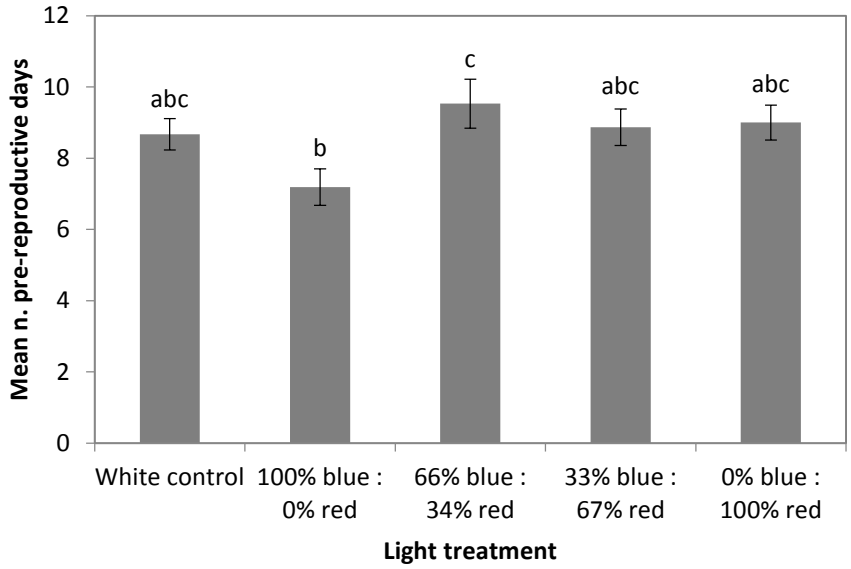

Figure 6.12. Mean number of pre-reproductive days of *Myzus persicae* on verbena under LED lighting. Means are displayed ± SEs, where n = 20. Means not sharing a common letter are significantly different (P < 0.05) as indicated by a Tukey's HSD Test.

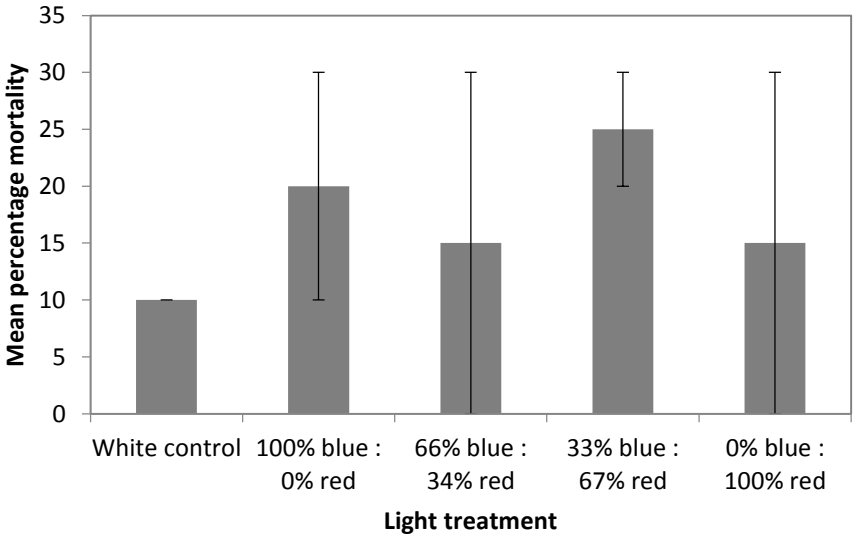

Figure 6.13. Mean pre-reproductive percentage mortality of *Myzus persicae* on lettuce under LED lighting. Means are displayed ± SEs, where n = 20. Means not sharing a common letter are significantly different (P < 0.05) as indicated by a Tukey's HSD Test.

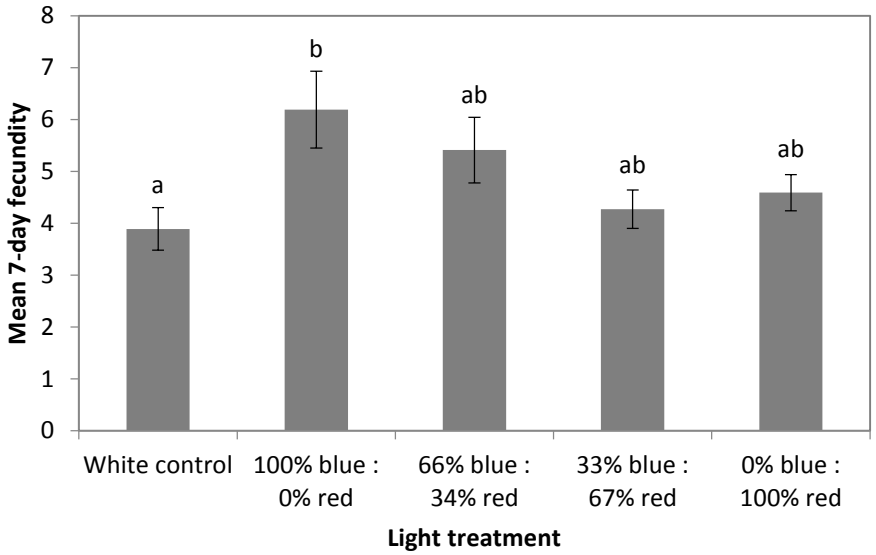

Figure 6.14. Mean seven-day fecundity of *Myzus persicae* on verbena under LED lighting. Means are displayed ± SEs, where n = 20. Means not sharing a common letter are significantly different (P < 0.05) as indicated by a Tukey's HSD Test.

Intrinsic rate of increase (r_m): An effect of light treatment on the intrinsic rate of increase was observed ($F_{4,8}$ = 2.45, P < 0.05; Figure 4.15), where aphids under 100% blue and 66% blue light treatments were found to perform better than those under white light treatments.

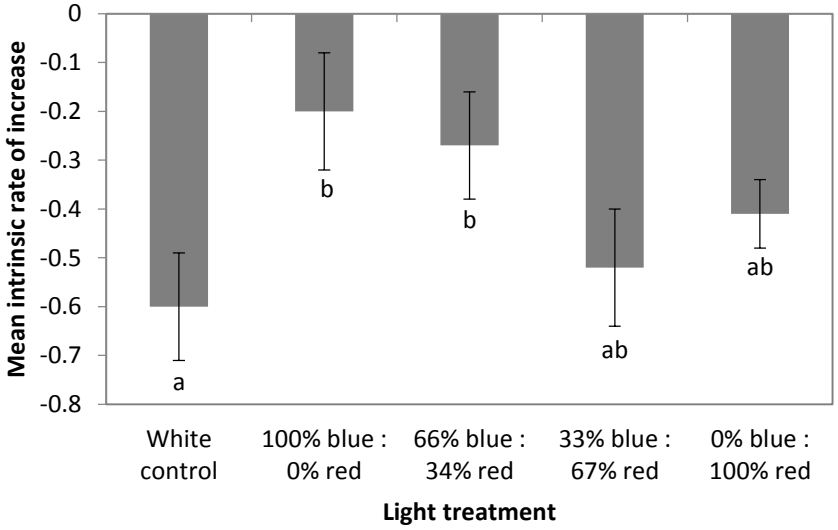

Figure 6.15. Mean instrinsic rate of increase (r_m) of *Myzus persicae* on verbena under LED lighting. Means are displayed ± SEs, where n = 20. Means not sharing a common letter are significantly different (P < 0.05) as indicated by a Tukey's HSD Test.

Verbena foliar chemistry effects on aphid performance

No statistically significant difference was observed in the levels of macro- and micro-nutrients tested under the different light treatments in these trials on verbena, nor was there an influence of the interaction between light treatment and the levels of these nutrients on aphid performance.

Aphid population growth on Verbena

For the overall effect of treatment on aphid reproduction rate, a P-value approaching significance was obtained ($P = 0.095$), with Tukey's Tests able to discriminate between means at $P < 0.05$. Though statistically this result should be interpreted with caution, and would benefit from confirmation by repeating the experiment, reduced reproduction under 100% red vs the white light treatment appeared generally evident (see Figure 6.16), as well as statistically supported.

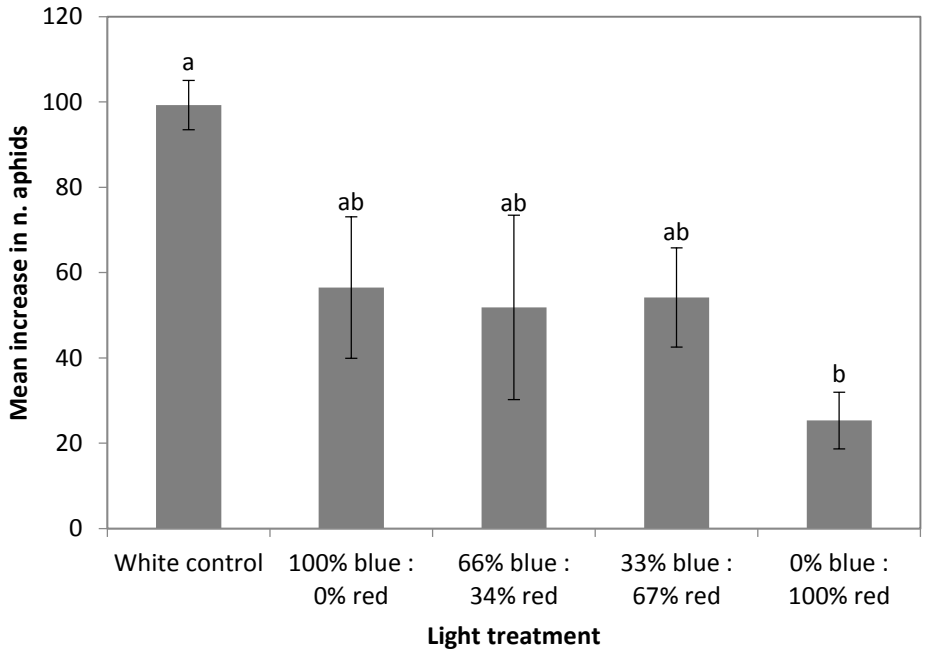

Figure 6.16. Mean net increase in number of *Aphis gossypii* on verbena, 10 days after being placed under LED lighting. Means are displayed ± SEs, where n = 6. Means not sharing a common letter are significantly different ($P < 0.05$) as indicated by a Tukey's HSD Test.

6.3.3. Discussion

Overall, the results support an effect of red: blue light treatment ratios on aphid performance. Reproductive performance and potential of individual aphids showed a trend

towards being reduced by red: blue treatments *vs* white light, though the nature of the responses tended to differ by aphid species and host plant. *Myzus persicae* tended to respond negatively to 66% blue, and occasionally 33% blue, light treatments when on lettuce, but when on verbena 66% blue light and 100% blue light tended to improve reproductive performance. True in all cases, however, was the fact that under enclosed LED-lit conditions, the instrinsic rate of increase of the aphids was negative. This indicates that the number of deaths in aphid populations would be greater than the number of births, and implies that population growth should be negative in such facilities. The assessment of population growth in this work package, where the mean increase in number of aphids was found to be reduced under red: blue light treatments, supports that population growth should be reduced, or at least limited, under red: blue LED-lighting. It deserves note, however, that pests may quickly adapt to new conditions, and as such any limitations to aphid performance induced by red: blue wavelengths under LED-based production systems may be increasingly overcome from one generation to the next.

Furthermore, the extent of any influence of light treatment on aphid performance would be mediated by the host suitability. In the individual reproductive performance investigations, it should be noted that the verbena used was a wild-type, which could be expected to display improved resistance to aphids *vs* a cultivated type. Additionally, verbena is not known to be a preferred host of *M. persicae*. It is likely that these factors affected the extent to which the aphids were negatively affected by treatment when 'forced' to feed upon this plant. In contrast, melon aphids were observed to naturally infest the same verbena variety (D. George, pers.comm.), which would explain why populations of this aphid species were shown to increase on verbena, albeit at a slow rate, despite *M. persicae* intrinsic rates of increase having been consistently negative. Certainly, the use of a wild-type provides explanations as to some of the large variance observed in results, which could have masked some of the effects of light on performance by reducing statistical power, though trends are still discernible. The specific response of the lettuce plants that could result in the decreased aphid performance remain 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.

Macro- and micro-nutrient levels of the lettuce and verbena plants used in this series of experiments did not show statistical differences, with the exception of nitrogen in lettuce. Aphid reproductive performance has previously been shown to be influenced by nitrogen

levels in the host plant, with higher nitrogen contents resulting in higher fecundity, so it could have been expected that a corresponding effect on aphid performance should have been observed, at least in lettuce. The lack of interaction between the altered nitrogen levels and light treatment on aphid performance, however, could suggest that any such influence was either: (a) not a main driver of treatment effects, and aphid response was primarily influenced by changes to plant chemistry or morphology resulting from light treatment, or (b) that any response to nitrogen levels was masked by aphid responses to plant chemistry or morphology under the light treatments. *Myzus persicae* on lettuce, however, did appear to respond to an interactive effect of manganese and light treatment. It is plausible that a similar response was not observed on verbena due to the influence of the plants being a wild-type, and that this provided sufficient difficulty for the aphids in terms of reproductive potential that any effects of plant nutrient levels were masked. Manganese is thought to be one of the single-most important micro-nutrients in enhancing plant oil production (Nandi & Chatterjee, 1991), and it has been shown that treatment of a plant with manganese can improve the levels of defensive chemistry (Ghannadnia *et al*., 2014), particularly of monoterpenes, levels of which are known to mediate insect herbivore performance.

The mechanisms underpinning the observed aphid responses remain to be defined, however, the results in this series of experiments suggest that both direct and indirect plant-mediated effects on aphid behaviour would play a role in mediating aphid numbers in enclosed LED facilities. The individual performance parameter experiments, and the plant chemistry findings, suggest the importance of host-plant effects on performance. In the population growth experiment of melon aphids, however, plants were initially cultured under natural white light. While this may make direct comparison between the experiments difficult, the results could reflect the influence of light treatment, rather than changes to plant chemistry or morphology, more directly in terms of aphid response, as plants would have had less time to be affected by the light treatments themselves. For example, the 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 this could not be performed due to the effects on the plants while in the LED facility.

6.3.4. Conclusions

The findings of this series of aphid-focused trials indicate a complex, species- and host-dependent response of aphid pests to different red: blue light treatment ratios. The evidence suggests, however, that alterations to the light ratios and regimes could be used to help provide some control of pest aphid populations and population growth.

6.4. PEST RESPONSES TO LIGHT QUALITY: SPIDER MITES

6.4.1. Spider mite Methods

Individual Cucumber plants (*Cucumis sativus* var. Carmen) were sown into individual pots of growing medium (Levington M2 potting and bedding compost). Pots were then placed under the specified light treatments (see Table 6.3). Plants were left to grow under the designated light treatments until they reached the two true leaf stage. They were split for use into two staggered runs to start on different days, with three plants in each treatment being infested on each day. At point of infestation, a vial containing twenty adult two-spotted spider mite (*Tetranychus* urticae) was positioned at the base of each plant. Each plant was then enclosed individually by fixing a bread bag over the plant and attached to the pot base. Infested plants were left undisturbed under the light treatments for a further fourteen days. The plants were removed from light treatments and individual leaves were studied under microscopy, with the number of adult and juvenile spider mites recorded.

Table 6.3. Specification of the light treatments used in spider mite population trials. All irradiances indicated in μmol m^{-2} s^{-1}.

Light treatment	White Control	100 % B : 0% R	66 % B : 34% R	33% B : 67% R	0% B: 100% R
PAR (400-700nm)	200	145	200	200	200
Blue (400-500 nm)	12	145	132	66	0
Green (500-600 nm)	15	0	0	0	0
Red (600-700 nm)	173	0	68	134	200
% blue	*6*	*100*	*66*	*33*	*0*

Statistical analyses

Statistical analyses were performed using the software package GraphPad Prism (v.6). Data were analysed by ANOVA as assumptions for parametric testing were met (homoscedasticity and normality). Post-hoc Tukey's HSD tests were conducted where significant differences in main effects were observed.

6.4.2. Results

Two-way ANOVA of the data revealed no significant differences between start dates (Adults: $F_{1,20} = 1.195$, $P = 0.287$; Juveniles: $F_{1,20} = 1.308$, $P = 0.266$; Total spider mite: $F_{1,20} = 1.485$, $P = 0.237$), indicating no impact of start date on results obtained. This supported the analysis of data as one cohesive set.

Analysis using one-way ANOVA indicated an effect of light treatment on spider mite numbers (Adult: $F = 6.444$, $P < 0.001$; Juvenile: $F = 2.929$, $P < 0.05$; Total spider mite: $F = 4.075$, $P < 0.05$; Figure 6.17). Spider mite population growth was consistently lower under the white light control, and the 33% blue: 67% red treatment than the 100% blue or 100% red treatments, this often being statistically significant according to Tukey's tests.

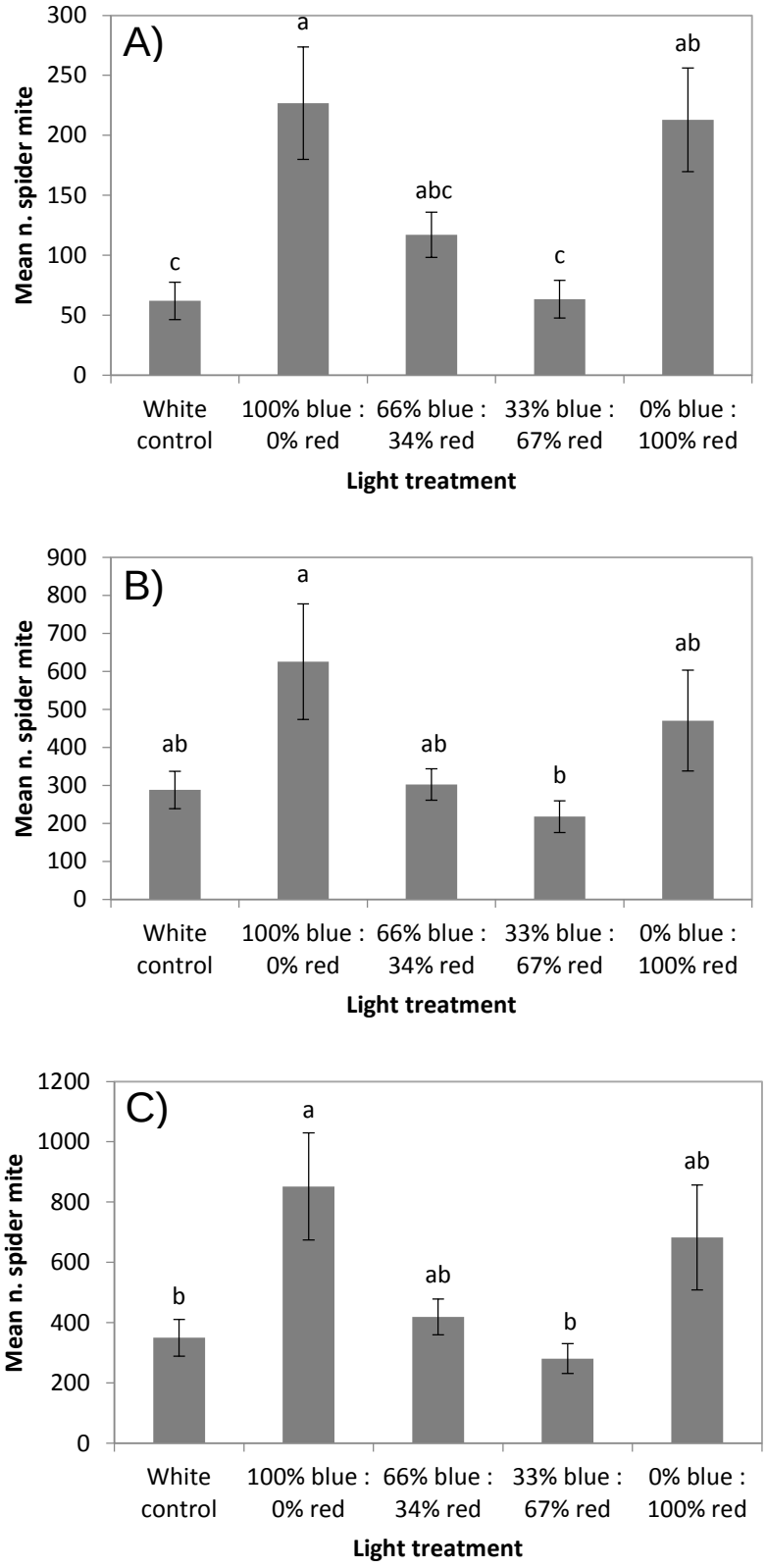

Figure 6.17. Mean number of two-spotted spider mite (*Tetranychus urticae*) **A)** adults, **B)** junveniles, and **C)** total numbers on cucumber under LED lighting 14 days post-infestation. Means are displayed ± SEs, where n = 6. Means not sharing a common letter are significantly different ($P < 0.05$) as indicated by a Tukey's HSD Test.

6.4.3. Discussion

This experiment supported a pest-suppressive effect under the white light control, relative to certain red: blue combinations of light wavelengths, with population growth slowest under the white light. There appeared to be a trend towards greater population growth with increasing percentage of blue light, though significantly higher rates were also observed under 100% red light. The precise reasons behind this trend are unclear as a more detailed investigation into the mechanisms at play was not within the scope of this project, though several hypotheses may be drawn. While spider mites have been shown to display phototaxis, and are believed to be sensitive to UV and green light wavelengths, it is possible that, given the absence of these wavelengths in the facility, individuals may be primarily responding to plant morphology and chemistry that are rendered more favourable for their population growth in the absence of white light. Alternatively, the red: blue light treatment wavelength ratios may be triggering physiological responses directly in the spider mite, for example by triggering behaviours (such as feeding or mating) promoted by the presence of certain light wavelengths.

6.4.4. Conclusions

Results support an effect of red: blue light wavelengths on two-spotted spider mite population growth. Although this was increased relative to a white light control, the findings of this study suggest potential for suppression of populations of this species of pest through modified light treatment.

6.5. BIOCONTROL RESPONSES TO LIGHT QUALITY : PARASITOID WASPS

6.5.1. Methods

Wasp flight activity

Aphidius matricariae and *A. colemani* transport containers were placed in separate insect cages in a controlled temperature room maintained at 20°C, with a 16L:8D photoperiod for one week. Each cage was supplied with a feeding station comprised of three cotton wool balls saturated with (a) water, (b) 50% sugar solution and (c) 50% honey solution, changed every other day, to sustain the wasps until use in experiments. Twenty-four hours before use, containers were removed from the cage, to ensure that all used insects would be at least 24 hours old at point of use and to allow feeding and mating to take place before the start of each trial. Staggered trials for each wasp were carried out in the enclosed LED facility, such that there were eleven replicates per light treatment for *A. matricariae* (two trials with four replicates, and one with three, per light treatment), and eight replicates per light treatment for *A. colemani* (two trials with four replicates per light treatment). As no plants were needed for this experiment, a true dark control was included.

Flight chambers were constructed from modified Bugdorm-44545 insect cages (MegaView Science, Taiwan, reduced in height to approximately 40cm), and were positioned under each of six light treatments (see Table 6.4) on aluminium benches used as production racks within the LED unit at STC. Each was surrounded by a black plastic ‘wrap’ to ensure that there would be no accidental light interference from neighbouring benches; light could only enter from the top of the chamber, which was left uncovered. Furthermore, throughout all experiments, curtains between racks were kept drawn to exclude lateral light interference. Non-attractant sticky traps were secured suspended in the centre of each flight chamber. These were constructed from clear plastic 9cm diameter Petri dishes, coated on one side with a thin layer of clear non-drying insect glue (Tanglefoot, USA).

Wasps were collected from culture cages using a pooter, and were transferred to flight chambers in the LED facility within 30 mins. Twenty wasps were released per flight chamber. Chambers were then closed and the ‘wrap’ secured around the chamber. These were then left undisturbed for 24 hours, after which sticky traps were removed and the number of wasps recovered in the trap over the course of the experiment were counted.

Table 6.4. Specification of the light treatments used in wasp flight activity trials. All irradiances indicated in μmol m^{-2} s^{-1}.

Light treatment	White Control	100 % B : 0% R	57 % B : 43% R	13% B : 87% R	0% B: 100% R
PAR (400-700nm)	110	60	65	55	54
Blue (400-500 nm)	8	59	37	7	0
Green (500-600 nm)	19	0	0	0	0
Red (600-700 nm)	82	0	28	48	54
% blue	*7*	*100*	*57*	*13*	*0*

Extended wasp flight towards plant material

Aphidius matricariae and *A. colemani* were kept in the same manner and under the same conditions as for the flight activity experiments in a CT room. Extended flight and host location trials were conducted in a series of staggered trials were carried out in the enclosed LED facility, such that there were eight replicates per light treatment for *A. matricariae* (four trials of two replicates per light treatment), and four replicates per light treatment for *A. colemani* (two trials with two replicates per light treatment). Light treatment details are indicated in Table 6.5. Though live plants were used in this experiment, the short duration still permitted a dark control to be included.

Table 6.5. Specification of the light treatments used in extended wasp flight and host location trials. All irradiances indicated in μmol m^{-2} s^{-1}.

Light treatment	White Control	100 % B : 0% R	54 % B : 46% R	14% B : 86% R	0% B: 100% R
PAR (400-700nm)	177	108	109	119	113
Blue (400-500 nm)	14	107	59	17	0
Green (500-600 nm)	33	1	0	0	0
Red (600-700 nm)	126	0	50	101	113
% blue	*8*	*100*	*54*	*14*	*0*

Flight ‘arenas’ were constructed from the benches in the LED facility (Figure 6.18). Each of the 1.24m x 2.10m x 0.44m (w x l x h) aluminium benches (two benches per light treatment) was enclosed with a black polythene cover to form the outer boundary of the arena. A modified Bugdorm-44545 insect cage (MegaView Science, Taiwan, reduced in height to approximately 40cm), was positioned at the far end of each bench containing plant

material. A 9-inch fan (DF9010, iGENIX) was placed behind the cage and run at the lowest setting, to provide airflow and ensure that plant volatiles would reach the release area at the opposite end of each bench arena. Seven-week-old chinese cabbage plants were used in cages and damaged by means of piercing fifty holes into two leaves with a needle, to replicate the effect of insect feeding damage to foliage. One plant was placed into each of the cages, and a non-attractant sticky trap was then secured to the centre of the front of each insect cage. These were constructed from clear acetate sheets (Inkjet Transparency Film KF26074, Q-Connect; 442cm^2 area per trap), coated on one side with a thin layer of clear non-drying insect glue (Tanglefoot, USA). At each release site, a cotton-wool ball dipped in 50% sugar-solution was provided for sustenance of the wasps at time of release. Throughout all experiments, curtains between racks were kept drawn to ensure that there was no light interference.

Wasps were collected from culture cages using a pooter, and were transferred to flight chambers in the LED facility within 30 mins. Forty *A. matricariae* and 100 *A. colemani* wasps were released per arena per trial. Arenas were then closed by securing the polythene cover around the chamber. These were then left undisturbed for 24 hours, after which sticky traps were removed and the number of wasps recovered in the trap over the course of the experiment were counted.

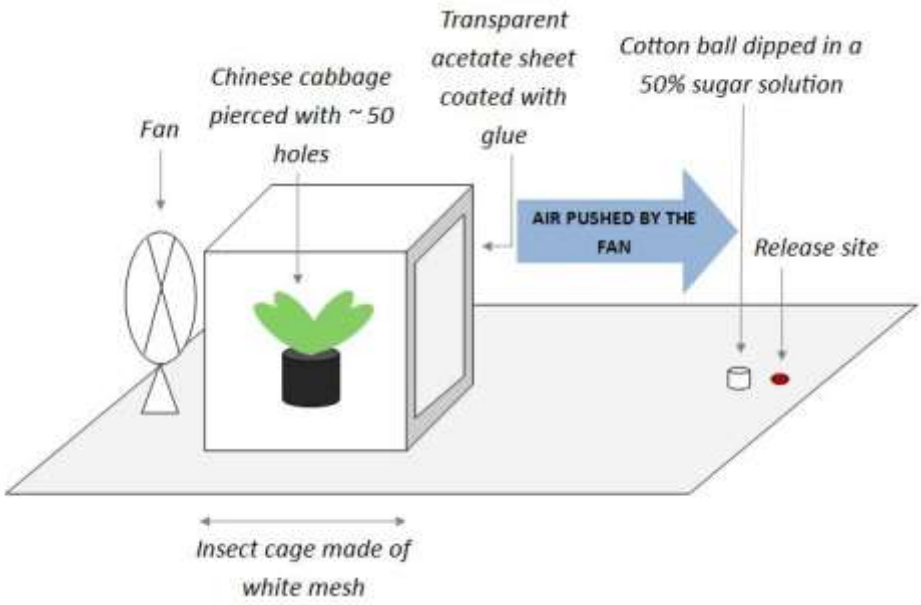

Figure 6.18. Diagram of the layout of each flight 'arena' in the enclosed LED facility.

Wasp parasitisation rates and reproductive potential

Chinese cabbages grown under standard greenhouse conditions were each infested with twenty adult *M. persicae* (from a stock culture maintained in a CT room at 20°C, with a 16L:8D photoperiod). Each infested plant was then surrounded by a bread bag, secured to the pot. They were left undisturbed for 48hrs under the white light conditions of the CT room to allow the aphids to establish (establishment having previously been shown to be unpredictable under red:blue treatments in the LED facility), before being moved into the enclosed LED facility and being placed under each of five light treatments (Table 6.6). The infested plants were distributed across two trials, with six plants per light treatment in the first and seven plants per light treatment in the second, giving a total of thirteen replicates per light treatment.

Aphidius matricariae wasps were obtained from a biocontrol company, with packing containers placed in insect cages in the CT room with a feeding station comprised of three cotton wool balls saturated with (a) water, (b) 50% sugar solution and (c) 50% honey solution, changed every other day, to sustain the wasps until use in experiments. Two female wasps were introduced to each infested plant. There were 24-36 hours old in the first trial, but freshly emerged in the second (<24 hours old). Therefore, in the second trial, a male wasp was also released along with the two females to ensure mating and therefore parasitisation could take place. In the first trial, wasps would have had the opportunity to mate before the start of the trial. The bread bag was re-secured to the pot immediately after release to ensure the wasps did not escape. These were left undisturbed under the LED light treatments for 24 hours, after which the wasps were removed and the plants returned to the CT room to allow for mummies to develop for ten days. After this time, the plants were destructively sampled, and the numbers of aphids and mummies assessed. Mummies were gently moved into a vented Petri dish, secured with Parafilm®, and were left undisturbed for a further ten days in the CT room. The number of emerged wasps was then counted.

Table 6.6. Specification of the light treatments used in wasp reproduction rate trials. All irradiances indicated in μmol m^{-2} s^{-1}.

Light treatment	White Control	100 % B : 0% R	66 % B : 34% R	33% B : 67% R	0% B: 100% R
PAR (400-700nm)	200	145	200	200	200
Blue (400-500 nm)	12	145	132	66	0
Green (500-600 nm)	15	0	0	0	0
Red (600-700 nm)	173	0	68	134	200
% blue	*6*	*100*	*66*	*33*	*0*

Statistical analyses

Flight activity, extended flight and host location data were analysed by ANOVA (one-way or nested, as appropriate), while wasp parasitisation and reproductive potential were analysed by multi-way ANOVA. Post-hoc Tukey's HSD Tests were conducted if significant differences in main effects were observed. Analyses were carried out using the statistical software, R (version 2.15.1, R Development Core Team (2012)).

6.5.2. Results

Wasp flight activity

A statistically significant difference was observed in flight activity of *A. matricariae* under the different light treatments ($F_{60,65}$ = 8.11, P < 0.001; Figure 6.19). Post-hoc Tukey testing indicated that this species of wasp flew significantly less under the dark control when compared to all other light treatments, but that there was no difference in flight activity under the red:blue treatments and the white light control.

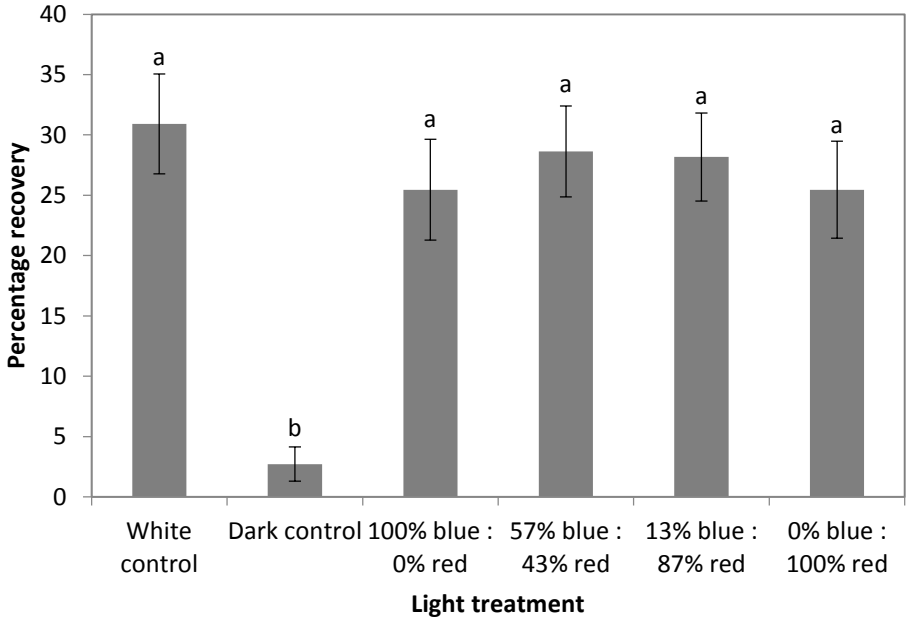

Figure 6.19. Mean percentage recovery of *Aphidius matricariae* under LED lighting 24 hours post-release in a flight chamber. Means are displayed ± SEs, where n = 11. Means not sharing a common letter are significantly different (P<0.05) as indicated by a Tukey's HSD Test.

Similarly, *A. colemani* also showed an effect of light treatment on flight activity ($F_{47,42}$ = 5.6, P < 0.001; Figure 6.20). Post-hoc Tukey testing of data suggested that flight activity was lower under the dark control, but, in contrast to *A. matricariae*, that this did not differ from activity under 100% red light (though activity under 100% red light was notably variable and increased over the control in general terms). Furthermore, no difference in flight activity was shown between any of the lit light treatments, including under 100% red light.

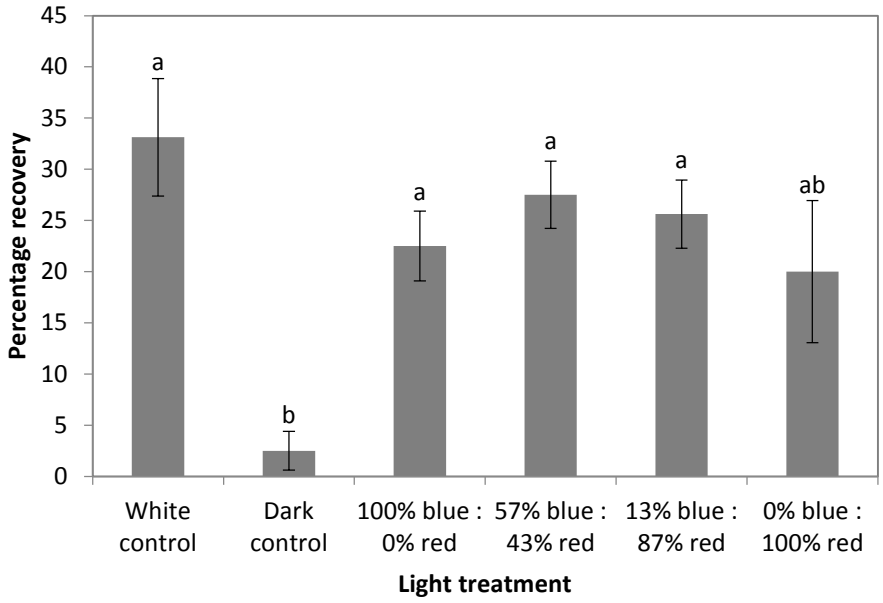

Figure 6.20. Mean percentage recovery of *Aphidius colemani* under LED lighting 24 hours post-release in a flight chamber. Means are displayed ± SEs, where n = 8. Means not sharing a common letter are significantly different (P<0.05) as indicated by a Tukey's HSD Test.

Extended wasp flight towards plant material

A significant effect of light treatment was observed on *A. matricariae* extended flight ($F_{47,42}$ = 11.79, P < 0.001; Figure 6.21). Post-hoc Tukey testing indicated that the ratio of red:blue light mediated the percentage of wasps recovered across the distance, with a significantly higher percentage of wasps recovered under the high-red light treatments. Furthermore, the percentage recovery of wasps under the higher-blue light treatments was not found to differ from those of the controls.

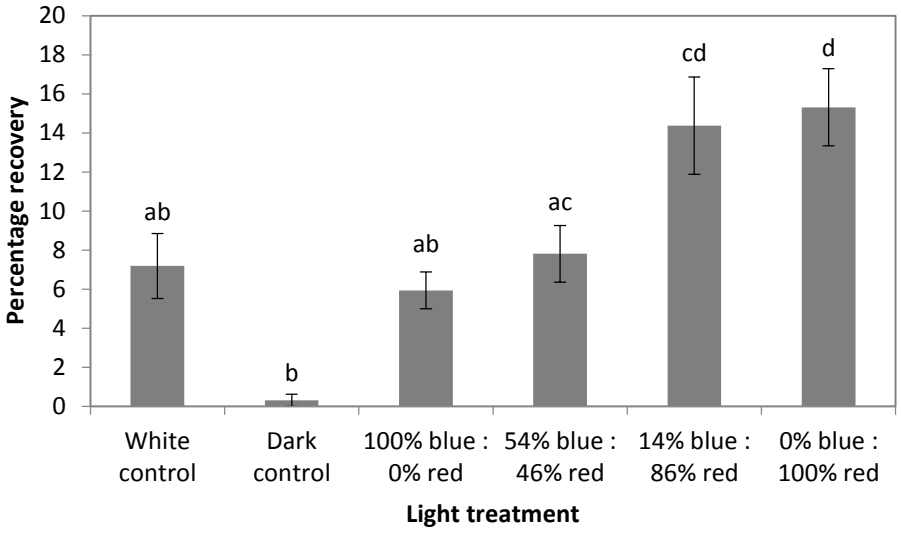

Figure 6.21. Mean percentage recovery of *Aphidius matricariae* under LED lighting 24 hours post-release in a distance flight arena. Means are displayed ± SEs, where n = 11. Means not sharing a common letter are significantly different (P<0.05) as indicated by a Tukey's HSD Test.

Light treatment was also found to have a significant effect on *A. colemani* extended flight ($F_{23,18}$ = 10.03, P < 0.001; Figure 6.22). Post-hoc Tukey testing indicated that a greater percentage of *A. colemani* were recovered under the 100% red light treatment when compared to all other light treatments, the recapture rates for which did not differ from each other.

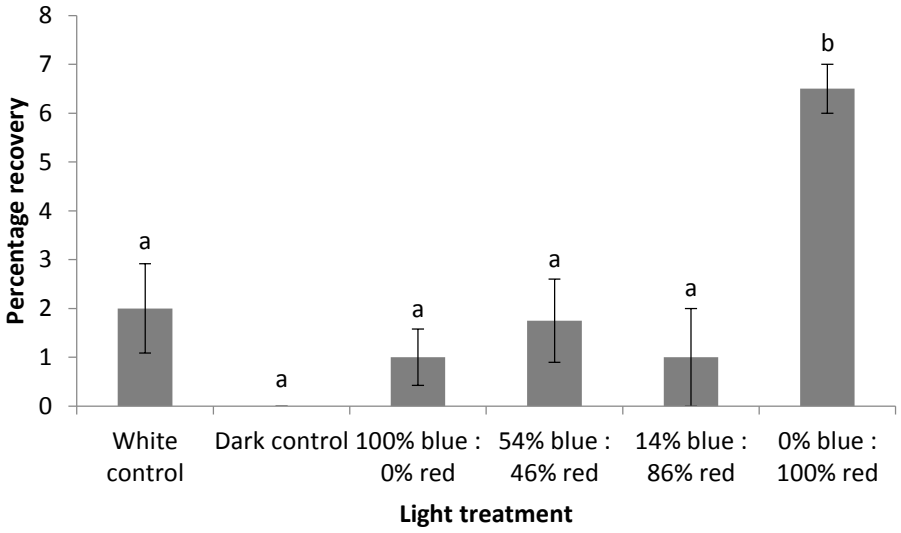

Figure 6.22. Mean percentage recovery of *Aphidius colemani* under LED lighting 24 hours post-release in a distance flight arena. Means are displayed ± SEs, where n = 8. Means not sharing a common letter are significantly different (P<0.05) as indicated by a Tukey's HSD Test.

Wasp reproductive potential

Analysis of the pooled data indicated that any differences could be explained by 'trial', rather than light treatment. Un-pooled, there was no significant difference in either the number of mummies, or the number of mummies from which wasps emerged, detected as a result of differences in light treatment in either trial (Figure 6.23). The mean percentage parasitism and emergence rates are shown in Figure 6.24. Though no statistically significant difference was observed, in both trials the 33% blue treatment had the most successful reproduction, with rates notably greater than those under 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.

6.5.3. Discussion

The results obtained in this suite of experiments indicate that wasp species tested, which are commonly released for biological control of aphid pests, will be active, that they will fly over a relative distance from release site, and that they will successfully parasitise aphid hosts under red: blue LED lighting.

In demonstrating flight activity in *A. matricariae* and *A. colemani* under a range of red: blue light combinations, these experiments support that the species tested could be expected to exhibit flight behaviour in enclosed LED crop production facilities, confirming that lack of flight activity would be unlikely to limit biological control programmes in these systems. Furthermore, flight activity under 100% red light suggests that the red light wavelength triggers the photoreceptors of the tested wasp species. This is likely to occur through one of two potential mechanisms. The first of these assumes that the wasps tested lack red photoreceptors, with a response to red light mediated by the green photoreceptor. These green photoreceptors may be triggered, at the tail end of their sensitivity, by the red light, eliciting a behavioural response. The second potential mechanism would be that the organisms tested possess true red-sensitive photoreceptors (Peitsch et al., 1992), though this would require further investigation.

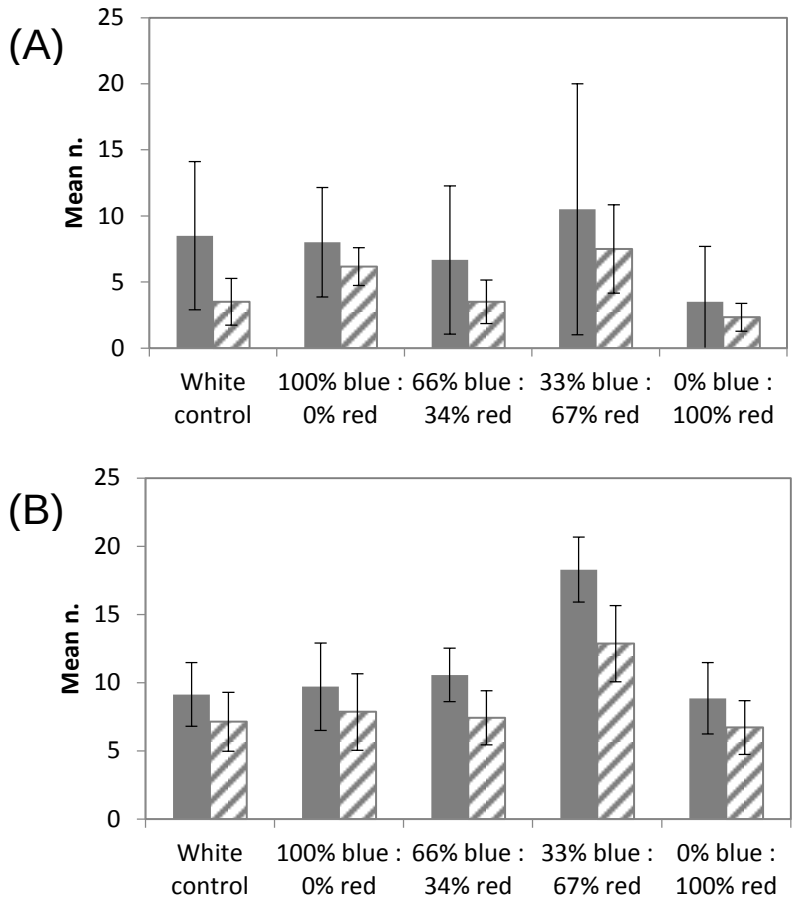

Figure 6.23. Mean number of *Myzus persicae* mummies on Chinese cabbage 10 days after exposure to *Aphidius matricariae* for 24 hours (solid bars), and the number of wasps emerged from those mummies after a further 10 days (hatched bars) under LED lighting, in each of two trials, A) and B). Means are displayed ± SEs, where n = 6 in (a) and n = 7 in (b).

Another potential influence on the extent of activity under different red: blue light treatments is the light intensity as perceived by the wasps, which hinges, in part, on photoreceptor sensitivity. In a white light treatment, for example, the light spectrum composition included 8% blue light wavelength, 19% green light wavelength and 73% red light wavelength. In contrast, the blue light treatment included 99% blue light, 1% green light and 0% red light wavelength. That equates, roughly, to some 27% visible wavelength under the white light treatment, compared to 99% visible wavelength under the 100% blue light treatment, if the assumption is made that the wasp species tested do not possess red photoreceptors. This may manifest, in terms of insect vision, as a perceived difference in light intensity under the different light treatments, and may explain some of the variability observed, given that insect behaviour has been shown to be mediated by light intensity. It is therefore possible that perceived light intensity under LED lights in enclosed production facilities would modulate flight behaviour, or at the very least could theoretically be expected to do so, and that these

responses could be species-specific. Further investigation into photoreceptor sensitivity, or into which photoreceptors a species possess, would help provide clarity by allowing mapping of behavioural responses to physiological sensitivity.

Although flight activity may not be a limiting factor in achieving full biocontrol potential in LED facilities, these trials suggest an important effect of wasp quality on achieving acceptable levels control. The biggest impact on wasp parasitisation and emergence rates was shown to depend on the particular trial from which the data was collected, and therefore the batch of wasps received, in the wasp reproduction experiment. Indeed, the second batch of *A. matricariae* received for the wasp reproduction experiments showed higher mortality levels in the CT room before use in the second trial (T. Irving, pers.comm.). Variability at the source, or induced during transit and handling, will render achievement of efficient and sufficient biological control more difficult, especially given that the impact of red: blue light mediation of wasp reproductive potential appeared limited in these trials.

Further study of the innate variability of biocontrol potential, prior to use, could be recommended across all biocontrol species; investigation of between and within species differences, between both batches and suppliers, could yield important results to inform improved biocontrol performance on farm across all sectors. Further study to investigate wasp orientation to hosts over greater distances under red: blue light would also be interesting, and potentially inform bespoke release strategies tailored to LED-based production systems, and/or light-based approaches to improve biocontrol performance in any production system where programmable lights are used (e.g. short-term light recipes that could improve plant signalling to biocontrol organisms post-release).

6.5.4. Conclusions

Parasitoid wasps can be expected to both fly and parasitise aphid pests under enclosed LED-light conditions, though there may be species-specific effects of different red: blue light ratios on the levels flight activity. These may affect parasitoid performance, and could theoretically be manipulated to potentially improve it. Performance, however, may be more limited by wasp batch quality than red: blue light treatment.

6.6. BIOCONTROL RESPONSES TO LIGHT QUALITY :PREDATORY MITES

6.6.1. Methods

Primula plants were infested with two-spotted spider mite (*Tetranychus urticae*) by placing a cut section of leaf material from a stock culture containing twenty to thirty individual mites onto a clean, uninfested plant. Each plant was then enclosed individually by fixing a bread bag over the plant, attached to the pot base. The now infested plants were left undisturbed in a controlled temperature room under white light (maintained at 20°C, with a 16L:8D photoperiod) for 72 hours, to allow the spider mite to transfer from the cut leaf segments onto the plant material, and establish. After this period of time had elapsed, ten individual adult *Phytoseiulus* mites were transferred to each plant with a fine-bristled paintbrush, before the bread bags were re-secured and the plants immediately transferred into the LED facility. Plants were placed under light treatments (Table 6.7) and then left undisturbed for a further five days, before being removed from the facility and frozen at -30°C to halt any further predation or spider mite development. Counts were then made under microscopy of total spider mite and *Phytoseiulus* numbers from whole plants.

Statistical analyses

Statistical analyses were performed using the software package Minitab (v.17). Data were analysed by one-way ANOVA if assumptions for parametric testing were met (homoscedasticity and normality). Post-hoc Tukey's HSD tests were conducted where significant differences in main effects were observed. Where data did not meet the assumptions for parametric analysis, a non-parametric Kruskal-Wallis Test was used to analyse data collected, with Mann-Whitney post-hoc testing used to investigate differences between pairs of treatments where significant differences were observed.

Table 6.7. Specification of the light treatments used in spider mite population trials. All irradiances indicated in μmol m^{-2} s^{-1}.

Light treatment	White Control	100 % B : 0% R	66 % B : 34% R	33% B : 67% R	0% B: 100% R
PAR (400-700nm)	200	145	200	200	200
Blue (400-500 nm)	12	145	132	66	0
Green (500-600 nm)	15	0	0	0	0
Red (600-700 nm)	173	0	68	134	200
% blue	*6*	*100*	*66*	*33*	*0*

6.6.2. Results

The total number of spider mite remaining on the infested plants was analysed by one-way ANOVA on untransformed data. No statistically significant difference between treatments was observed ($F_{4,36}$ = 0.53; P = 0.717; Figure 6.24A).

The total number of *Phytoseiulus* did not meet the assumptions for parametric analysis, so a non-parametric Kruskal-Wallis Test, adjusted for ties, was used to analyse data collected. No difference in numbers between treatments was observed (H_4 = 1.13; P = 0.890; Figure 6.24B).

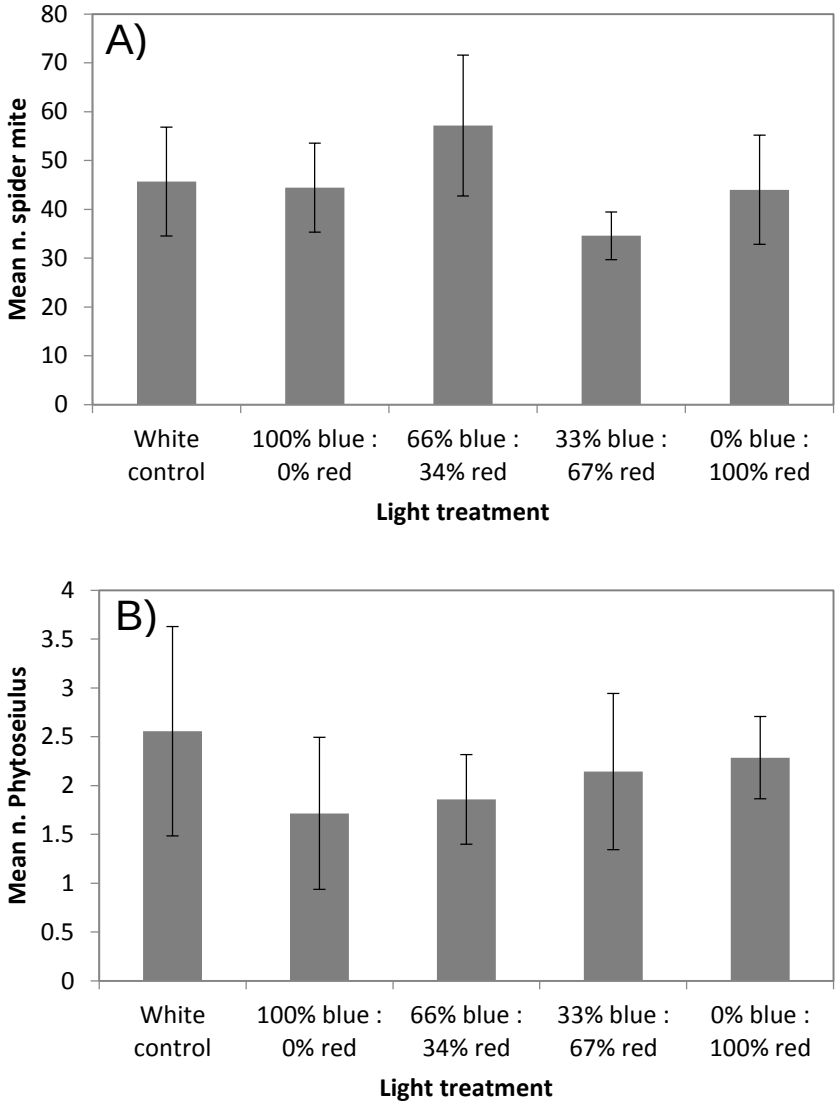

Figure 6.24. Mean number of (A) two-spotted spider mite (*Tetranychus urticae*), and (B) *Phytoseiulus* predatory mites recovered on Primula under LED lighting 5 days post-treatment. Means are displayed ± SEs, where n = 7 for all treatment excluding the white control where n = 9.

6.6.3. Discussion

The results obtained indicate that, under enclosed LED-lighting, there was no effect of red: blue light mixtures on the efficacy of *Phytoseiulus* as a biological control agent of two-spotted spider mite when compared with a white light control. These findings are in line with what might be expected as mites tend to have low visual acuity, with behaviours typically controlled more significantly by chemical and tactile cues. These trials were designed to assess the direct influence of light quality on *Phytoseiulus* and to minimise the effects of light quality on the red spotted spider mites. It should be noted, as previously reported in this work package, that spider mite population growth has been shown to be altered under differing red: blue light treatments. As such these findings should be caveated that assessment over a longer course of time may indicate differences. Further trials would be recommended to assess the need to alter release rates under different lighting regimes.

The primula plants used were reared under glasshouse conditions, and post-spider mite-infestation they were left undisturbed under white light. This was done to ensure that any differences which might be observed in this trial would be as a result of lighting on the *Phytoseiulus*, rather than any mediating impacts caused by altered plant chemistry due to growing conditions (though plant chemistry could have been expected to have begun to be impacted over the course of treatment exposure), and to replicate the usual route of entry of two-spotted spider mite into the enclosed LED facility. It is possible that, where plants are reared under differing red: blue light treatments, *Phytoseiulus* may respond to changes elicited by the light on plant chemistry, thus altering their efficacy as a control agent. This is, however, unlikely, as feedback from the LED unit supports the efficacy of this, and other predatory mite species under varying light treatments.

6.6.4. Conclusions

The results obtained suggest that the efficacy of *Phytoseiulus* as a biological control agent of two-spotted spider mite is not altered under differing red: blue light treatments in enclosed LED facilities, though more detailed experimentation would be required to fully confirm this result.

6.7. GENERAL DISCUSSION

Insect behaviour and development is affected by light, and the nature of such responses are known to be influenced by a variety of factors (such as intensity, combinations of wavelengths, time of exposure, and light direction and contrast to the rest of the environment) (Shimoda & Honda, 2013). These abiotic influences, which also include temperature, will interact with biotic influences, such as plant chemistry and morphology, to further alter and mediate performance of insects at all trophic levels under altered light conditions. Furthermore, yet another level of complexity is added to the system, as the effects of light treatment can be both direct and indirect; for example, in addition to altering plant chemistry or morphology a light treatment could be having a direct influence on an insect by altering, say, its circadian rhythm or by modulating feeding or mating behaviours. Each of these interactions would occur at each level of the trophic system: (a) plant host, (b) pest insect, and its interaction with the plant host, and (c) natural enemy, and its interactions with both the pest insect and plant. The system and responses are therefore highly complex and difficult to disentangle. The results of this project support that the nature of the responses is primarily both host- and species-specific, and present a preliminary investigation into a complex system that will require further research to fully understand.

Direct visual stimuli on insect vision are most likely to affect passive insect monitoring in enclosed LED units. Not only is trap efficacy altered under red: blue light regimes, due to changes in apparent colour, but the ratio of red: blue light can additionally alter the strength of preference towards one colour or another. Although improved sticky trap performance has been shown when using fluorescently coloured traps, or by adding, for example, green LEDs, the benefit of such improvement methods is only as effective if insect movement towards traps is not inhibited by the light regimes. For example, thrips in the enclosed LED facility appeared less inclined towards flight when disturbed (P. Davis, pers.comm.), which would limit the efficacy of passive traps.

The importance of visual stimuli to particular species is likely to mediate responses to light, both in terms of monitoring and population development. Different light treatments and their schedule modulate a variety of insect physiological responses (such as diapause, dormancy, and circadian rhythms), all of which contribute to feeding, mating and migratory behaviours, and these influences are more likely to be important to species relying at least partially on sight, than those with limited visual abilities. For example, mite species show a basic phototacticity (movement towards or away from light), and therefore alterations of their light environment can be expected to have more limited effect on behaviour and population growth than, for example, species reliant on light, or certain wavelengths of light, for orientation, such as bumblebees. Indeed, our results showed improved reproduction by

mites in red: blue light treatments over the white light control (mites typically move away from light), while no effect of light treatment on predatory mite persistence after release. This is not to say, however, that differences would not be seen under red: blue light; the plant hosts used in these particular trials were not reared under enclosed LED conditions, which would likely have altered plant chemistry and morphology. Mite species are more likely to respond to such plant cues, and therefore divergences may be observed where plants are reared consistently under LED lighting. This is supported by the differences observed under different red: blue light treatments in the more detailed aphid performance experiments, which showed differences in performance under the different treatments (even where these were statistically non-significant, trends could be observed).

Aphids show more elaborate direct responses to light, both behavioural and physiological, and are strongly affected by physical and chemical changes to their host plant, frequently mediated by light. It is unsurprising that they showed more complex performance responses, especially given that the aphids were reared on plants grown under different red: blue light regimes from sowing, such that in addition to direct effects of light, indirect effects through established plant-mediated cues was also observed. For example, on lettuce grown under 60% blue: 40% red light, aphids were observed not to settle on leaf material (pers.obs.). Rather, they moved around more than aphids observed under light treatments, and increased movement would limit feeding time. Equally, lettuce plants under this light treatment were observed to be slower growing, and these combined observations support a change to host suitability, either through morphological or chemical changes. Parasitic wasps, like aphids, are also more responsive towards their light environment, when contrasted to predatory mites. In addition to chemical cues, visual cues also play a role on wasp host location behaviours, and this is reflected in the results. Although different red: blue light treatments may not have affected parasitoid performance, they were shown to influence parasitoid activity in a species-specific manner.

Whilst the results obtained in this project indicate that there are species- and host-specific effects of light treatment on insect responses and behaviour under red: blue light regimes, it deserves note that the experimental designs required to respond to this project's questions excluded behavioural responses from being directly observed or tested. In order to achieve project aims, the target species were placed directly on plant material, and were frequently closely enclosed through use of clip cages or bread bags to prevent migration and dispersal into the rest of the facility. There are, however, many examples in the literature where light quality affects insect migration and location on a host plant. This project has shown, in WP3.1, that landing site selection is disrupted under red: blue light treatments. Casual observations of pest species within the facility also suggest that pest

migration and flight are disrupted under the LED light environments. Further research into such behavioural responses under altered red:blue light conditions would provide clarity in explaining the results observed in this project. Such behavioural research, combined with investigations of physiological drivers, would help elucidate the mechanisms driving pest and natural enemy responses, resulting in the ability to develop a more complete and efficient pest management system in enclosed LED production. This could, for the first time, theoretically include manipulation of the light environment as a tool within IPM programmes, which may also be of benefit to other production systems in which LED lighting is deployed (now or in the future), such as LED-supported glasshouses and polytunnels.

6.8. GENERAL CONCLUSIONS

The interactions between light, plants, pests and beneficial insects are highly complex, with responses being both species- and host-specific. Results of this project suggest that manipulation of light wavelengths and light intensity offer potential for pest management. Overall, there appears to be a general reduction in aphid fitness under red: blue light, while spider mite fitness appears increased under the more extreme light mixture ratios. Biocontrol organisms could still be effective under altered red: blue light according to the results obtained, and biocontrol potential could be similar to that observed in greenhouse conditions, at least for some species, but further research will be required to confirm this. Finally, it appears that enclosed LED production will require a ‘back-to-basics’ approach and novel thinking to amend established pest management methodologies, including monitoring and control procedures, whilst keeping in mind the need to balance pest control with maximising plant development criteria.

Knowledge and Technology Transfer

During the course of this three year trial we have received over 120 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. We have also hosted visitors from the US, Australia and Israle.

We have provided several students with the opportunity to contribute to the research that has been performed in this project. This has benefited the project but has also given then the chance to work in the unique facilities at STC and gain experience of plant and insect responses to LED light.

In addition Dr Davis has presented the results from CP125 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.

September 2014

Dr Phillip Davis presented at International conference on vertical farming and urban agriculture (VFUA) held at Nottingham University. ***The challenges of producing plants in vertical farms*.**

October 2014

Dr Phillip Davis presented at the BHTA meeting held at the University of Worcester. ***Herb responses to LED light.***

November 2014

Dr Phillip Davis presented at a Growsave event held at STC. ***LED update***.

Dr Phillip Davis presented at the IPPS/ HDC/ Fargo/GroSouth/WSNSDG Study Day - Innovation in Plant Production held. ***The influence of light and the future of LEDs.***

Dr Philip Davis presented at the HDC PO panel meeting held at STC. ***HDC project CP125 Understanding crop and pest responses to LED lighting to maximise horticultural crop quality and reduce the use of PGRs.***

January 2015

Dr Phillip Davis & Dr Dave George presented at the BPOA Technical Seminar held at The Oxford Belfry Hotel, Milton Common, Thame, Oxfordshire. ***Understanding crop and pest responses to LED lighting (CP 125)***.

June 2015

Dr Phillip Davis presented at AAB/FES Conference: Knowledge exchange: from research to the food supply chain held at Lancaster University: ***Exploiting photobiology in protected cropping.***

Dr Phillip Davis presented to the Harrogate Science group.

Dr Phillip Davis manned a stand at the Great Yorkshire Show that was demonstrating the uses of LEDs in horticulture.

September 2015

Dr Phillip Davis presented at the TGA Conference. ***Lighting the path to a sustainable furture for UK tomato production.***

October 2015

Dr Phillip Davis presented at the South West Growers Show. ***The use of LEDs for improving plant propagation.***

Dr Rhydian Beynon Davies presented at the CGA / PGA meeting. ***Lighting under the spotlight***

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

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

November 2015

Dr Phillip Davis attended the Sainsbury's Farmer Conference.

January 2016

Dr Phillip Davis presented at the AHDB Manipulation of spectrum for Horticulture conference. ***Exploiting plant light reponses for protected horticulture.***

May 2016

Dr Phillip Davis attended Sainsburys R&D corporate breakfast.

Dr Phillip Davis attended the ISHS 8th Symposium on Light in Hort, East Lansing, MI, USA.

June 2016

Dr Rhydian Beynon Davies attened Greentech

Dr Phillip Davis attended an N8 meeting at Manchester University

July 2016

Dr Phillip Davis & Dr Rhydian Beynon Davies attened Fruit Focus

September 2016.

Dr Phillip Davis attended the LpS 2016 conference and present at the Hi LED project workshop. ***Plant light responsed and their manipulation fro horticultural purposes***

October 2017

Dr Phillip Davis presnteat at the CGA. ***An update of LED work at STC.***

February 2017

Dr Phillip Davis gave a presentation at Liverpool University. ***Lighting the future of horticulture.***

May 2017

Dr Davis gave a presentation at the Horticulure Technologies event held at Teagasc, Dublin. ***Lighting the future of horticulture.***

Dr Davis presented at the Horticulture Lighting conference Europe . ***Lighting the future of horticulture.***

Dr Davis presented at the Waitrose Farm Assurance Meeting. ***Lighting the future of horticulture.***

June 2017

Dr Davis gave a presented at CRD. ***Urban Farming***

July 2017

Dr Davis attended an N8 conference at Duram University.

HDC news / AHDB Grower articles

Colour reactions. December 2014/January 2015 issue pages 16-17.

Colour co-ordinated pest monitoring. (Cover picture) December 2015/January 2016 Issue LEDs: recipes to mix for ornamentals. October 2015 issue 217 pg. 16-18.

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.

Coloured judgment, Balanced decision . (Cover picture) December 2016.

AHDB Technical guides – (all guides can be acessed from the AHDB website)

Lighting: The review (author)

Lighting: The principles (author)

Lighting: In practice (contributor)

Peer reviewed articles

Phillip A. Davis & Claire Burns (2016) Photobiology in protected horticulture. Food and Energy Security **5**: 223:238.

STC LED Open days *(follow the links to view resources on the AHDB website)*

30th November 2016 **What does the future hold for the use of LEDs in protected edible plant production?**

1st December 2016 - **What does the future hold for the use of LEDs in ornamental plant production?**

Glossary

Cryptochrome	A photoreceptor that is sensitive to blue and UVA light.
DLI	Daily light integral, the total number of photons received over a meter squared area in 25 hours and has units of mol m^{-2} d^{-1}
HPS	High pressure sodium lighting.
LED	Light emitting diodes.
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.
PGR	Plant growth regulators.
Photomorphogenesis	The processes that causes plant morphology and pigmentation to change following exposure to light. These processes are activated and controlled by several photoreceptors.
Photon irradiance	A measurement of the number of photons incident on a surface, which has units of µmol[photons] m^{-2} s^{-1}.
Photoreceptor	Light-sensitive proteins that initiate light responses.
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

AHDB Horticulture Project CP 088 Final Report: Enhancing the monitoring and trapping of protected crop pests by incorporating LED technology into existing traps.

Ahkami,A.H.,Melzer,M.,Ghaffari,M.R.,Pollmann,S.,Javid,M.G., Shahinnia, F.,etal. (2013). Distribution of indole-3-acetic acid in *Petunia hybrida* shoot tip cuttings and relationship between auxin transport, carbohydrate metabolism and adventitious root formation. *Planta* 238,499–517.doi:10.1007/s00425- 013-1907-z

Alvarenga, I.C.A., Pacheco, F.V., Silva, S.T., Bertolucci, S.K.V., and Pinto, J.E.B.P. (2015). In vitro culture of *Achillea millefolium* L.: quality and intensity of light on growth and production of volatiles. Plant Cell Tissue Organ Cult. *122* (*2*), 299–308 http://dx.doi.org/10.1007/s11240-015-0766-7.

Amaki, W., N. Yamazaki, M. Ichimura, and H. Watanabe. 2011. Effects of light quality on the growth and essential oil content in sweet basil. Acta Hortic. 907:91–94.

Bates, D., Maechler, M. & Bolker, B. (2012) lme4: Linear mixed-effects models using S4 classes. 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.

Brown BA, Headland LR & Jenkins GI (2009) UV-B action spectrum for UVR8-Mediated HY5 transcript accumulation in Arabidopsis. *Photochemistry and Photobology* **85**: 1147-1155.

Calamar A & de Klerk G-J (2002) Effect of sucrose on adventitious root regeneration in apple *Plant Cell, Tissue and Organ Culture* **70:** 207–212.

Christiaens A. Van Labeke MC, Gobin B and Van Huylenbroeck J (2014) Rooting of ornamental cuttings affected by spectral light quality Acta Hortic. 1104. ISHS 2015. DOI 10.17660/ActaHortic.2015.1104.33 XXIX IHC – Proc. Int. Symp. on Ornamental Horticulture in the Global Greenhouse Ed.: R.A. Criley

Christmann A, Weiler E W, Steudle E & Grill E (2007), A hydraulic signal in root-to-shoot signalling of water shortage. *The Plant Journal* **52**: 167–174. doi:10.1111/j.1365-313X.2007.03234.x

Crawley, M.J. (2007) The R Book. 1st ed. John Wiley & Sons Ltd., Chichester, U.K.

de Carbonnel M, **Davis PA**, Rob M, Roelfsema G, Inoue S, Schepens I, Lariguet P, Geisler M, Shimazaki K, Hangarter R, Fankhauser C (2010). The *Arabidopsis* PHYTOCHROME KINASE SUBSTRATE 2 protein is a phototropin signalling element that regulates leaf flattening and leaf positioning. *Plant Physiology* **152**:1391-405.

de Souza Pinto J R,. de Freitas RMO, Araújo PC, Praxedes S.C (2012) propagation of *Spondias* species by cuttings of different lengths treated with sugars, *Genetics and Plant Physiology* **2** : 171–176

Demotes-Mainard S, Péron T, Corot A, Bertheloot J, , et al (2015) Plant responses to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany* **121**: 4-21.

Drüege U, Franken P & Hajirezaei MR (2016) Plant hormone homeostasis, signaling, and function during adventitious root formation in cuttings. *Frontiers in Plant science* **7**:381 doi: 10.3389/fpls.2016.00381

*Emerson, Robert (1957). "Dependence of yield of photosynthesis in long wave red on wavelength and intensity of supplementary light". Science. **125**: 746.*

Folta KM, Koss LL, McMorrow R, Kim HH, Kenitz JD, Wheeler R, Sager JC. Design and fabrication of adjustable red-green-blue LED light arrays for plant research. BMC Plant Biology. 2005 Aug 23;5(1):17

Fox, J. & Weisberg, S. (2011) An R Companion to Applied Regression. 2nd ed. Sage. Thousand Oaks, CA, USA.

Franklin K (2009) Light and temperature signal crosstalk in plant development. *Current Opinion in Plant Biology* **12**: 63-68.

Friml J (2003) Auxin transport — shaping the plant. *Current Opinion in Plant Biology* **6**: 7-12, ISSN 1369-5266,

Fuernkranz, H.A., Nowak, C.A., and Maynard, C.A. (1990). Light effects on in vitro adventitious root formation in axilary shoots of mature *Prunus serotina.* Physiol. Plant. *80* (*3*), 337–341 http://dx.doi.org/10.1034/j.1399-3054.1990.800302.x.

Gabryszewska, E., and Rudnicki, R.M. (1997). The effects of light quality on the growth and development of shoots and roots of Ficus benjamina in vitro. Acta Hortic. 418, 163–168 http://dx.doi.org/10.17660/ActaHortic. 1997.418.22.

Ghannadnia, M., Haddad, R., Zarinkamar, F. & Sharifi, M. (2014) Manganese treatment effects on terpene compounds of *Cuminum cyminum* flowers. Industrial Crops and Products, 53: 65-70.

Hansen J, Stromquist LH & Ericsson A, (1978) Influecne of the irradiance on carbohydrate content and rooting of cutting of pine seedlings (*Pinus sylvestris* L.) *Plant Physiology* 61:975-979.

Hoad SP & Leakey RRB (1996) Effects of pre-severance light quality on the vegetative propagation of Eucalyptus grandis W. Hill ex Maiden. *Trees* **10:** 317-324.

Hogewoning SW, Trouwborst G, HM, Poorter H, van Ieperen W & Harbinson J (2010). Blue light dose–responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *Journal of Experimental Botany* **61**: 3107–3117.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. Biometrical Journal, 50: 346–363.

Huq, E., B. Al-Sady, M. E. Hudson, C. Kim, K. Apel, and P. H. Quail. 2004. PHYTOCHROME-INTERACTING FACTOR 1 is a critical bHLH regulator of chlorophyll biosynthesis. Science 305:1937–1941.

Islam S Z, Honda Y, & Arase S (1998) Light-induced resistance of broad bean against *Botrytis cinerea*. *Journal of Phytopathology* **146**:479-485.

Islam, M. A., G. Kuwar, J. L. Jihong, R. D. Blystad, H. R. Gislerod, J. E. Olsen, et al. 2012. Artificial light from light emitting diodes (LEDs) with a high portion of blue light results in shorter poinsettias compared to high pressure sodium (HPS) lamp. Sci. Hortic. 147:136–143.

Kim HH, Goins GD, Wheeler RM, Sager JC (2004) Green light supplementation for enhanced lettuce growth under red and blue light emitting diodes. *HortScience* **39**:1617–1622.

Kurilčik ***A,*** Dapkūnienė S, Kurilčik G, Duchovskis P, Urbonavičiūtė A, Žilinskaitė S, Žukauskas A (2011) Effect of far-red light on the growth of chrysanthemum plantlets *in vitro.* Scientific works of the institute of horticulture, lithuanian research centre for agriculture and forestry and Lithuanian University of Agriculture. Sodininkystė ir daržininkystė. 30(3–4).

Kurilčik, A., Miklušytė-Canova, R., Dapkūnienė, S., Zilinskaitė, S., Kurilčik, G., Tamulaitis, G., Duchovskis, P., and Zukauskas, A. (2008). In vitro culture of Chrysanthemum plantlets using light-emitting diodes. Open Life Sci. 3, 161–167.

Lau OS & Deng XW (2010) Plant hormone signalling lightens up: integrator of light and hormones. *Current Opinion in Plant Biology* **13**: 571-577.

Li Q. & Kubota C. (2009) Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental Experimental Botany*. **67**:59-64.

Li, H., Z. Xu, and C. Tang. 2010. Effect of light-emitting diodes on growth and morphogenesis of upland cotton (Gossypium hirsutum L.) plantlets in vitro. Plant Cell, Tissue Organ Cult. 103:155–163.

Macdowall, FD (1972). Phototactic action spectrum for whitefly and the question of colour vision. *The Canadian Entomologist*, *104*: 299-307.

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.

Maynard B, & Bassuk N (1985) Etiolation as tool for rooting cuttings of difficult to root woody plants. *Combined Proceedings of the International Plant Propagator's Society* **35**: 488-495.

Mazza, C.A., Izaguirre, M.M., Curiale, J. & Ballaré, C.L. (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.

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

Menzel, R. & Blakers, M. (1976) Colour receptors in the bee eye – morphology and spectral sensitivity. Journal of Comparative Physiology A, 108: 11-33.

Miyashita, Y. T., Y. Kinura, C. Kitaya, C. Kubota, and T. Kozai. 1997. Effects of red light on the growth and morphology of potato plantlets in vitro using light emitting diodes (LEDs) as a light source for micropropagation. Acta Hortic. 418:169–173.

Moon HK, Park S-Y, Kim YW & Kim CS (2006) Growth of Tsuru-rindo (*Tripterospermum japonicum*) cultured in vitro under various source of light-emitting diode (LED) irradiation. *Journal of Plant Biology* **49:** 174-179.

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.

Nandi, R.P. & Chatterjee, S.K. (1991) Improved cultivation and distillation methods, followed by citronella plantations of Darjeeling hills. Indian Perfumer, 35: 80-85.

Nanya K, Ishigami Y, Hikosaka S, Goto E (2012) Effects of blue and red light on stem elongation and flowering of tomato seedlings. Acta *Horticulturae* **956**: 264-266.

Ouzounis T, Fretté X, Rosenqvist E, Ottosen CO (2014) Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanulas. *Journal of Plant Physiology* **171**: 1491–1499.

Peitsch, D., Fietz, A., Hertel, H., Souza, J., Ventura, D.F. & Menzel, R. (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. Journal of Comparative Physiology A, 170: 23-40.

Peleg Z & Blumwald E(2011) Hormone balance and abiotic stress tolerance in crop plants. *Current Opinion in Plant Biology* **14**:290–295

Poudel, P.R., Kataoka, I., and Mochioka, R. (2008). Effect of red- and blue-light-emitting diodes on growth and morphogenesis of grapes. Plant Cell Tissue Organ Cult. 92 (2), 147–153 http://dx.doi.org/10.1007/s11240-007- 9317-1.

Powles JE, Buckley TN, Nicotra AB & Farquhar GD (2006), Dynamics of stomatal water relations following leaf excision. *Plant, Cell & Environment* **29:** 981–992. doi:10.1111/j.1365-3040.2005.01491.x

R Development Core Team (2012) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria.

Sager JC, McFarlane JC *(1997) in Plant growth chamber handbook, Radiation, eds Langhans R.W., Tibbitts T.W. (North Central Regional Research Publication No. 340. Iowa State Univ, Ames, IA), pp 1–29*

Savvides A, Fanourakis D, van Ieperen W (2012) Co-ordination of hydraulic and stomatal conductance across light qualities in cucumber leaves. *Journal of Experimental Botany* **63**: 1135-1143.

Schachtman, DP & Goodger, JQD (2010) Chemical root to shoot signaling under drought. *Trends in Plant Science* **13**: 281-287

Shimoda, M. & Honda, K. (2013) Insect reactions to light and its applications to pest management. Applied Entomology and Zoology, 48: 413-421.

Snowden MC, Cope KR, Bugbee B (2016.) Sensitivity of seven diverse species to blue and green light: Interactions with photon flux. *Plos ONE* 11 (10): e0163121. Doi:10.1371/journal . pone.0163121

Stutte GW, Edney S & Skerritt T (2009) Photoregulation of bioprotectant content of red leaf lettuce with light-emitting diodes. *HortScience* **44**: 79-82.

Tanaka, M., T. Takamura, H. Watanabe, M. Endo, T. Yanagi, and K. Okamoto. 1998. In vitro growth of Cymbidium plantlets cultured under super bright and blue light emitting diodes (LEDs). J. Hortic. Sci. Biotechnol. 73:39–44.

Tripathy, B. C., and C. S. Brown. 1995. Root-shoot interaction in the greening of wheat seedlings grown under red light. Plant Physiol. 107:407–411.

Trouwborst G, Hogewoning SW, van Kooten O, Harbinson J (2016) Plasticity of photosynthesis after the 'red light syndrome' in cucumber. *Environmental and Experimental Botany* **61**: 3107-3117.

van Ieperen W, Savvides A, & Fanourakis D (2012) Red and blue light effects during growth on hydraulic and stomatal conductance in leaves of young cucumber plants. *Acta Horticulturae* **956**: 223-230.

Vanajalatha D K, Sharma G, Singh D & Mishra G (2015) Effect of sucrose and auxins on rooting of karonda cuttings, *Carissa carandas* L. *International Journal of Farm Sciences* **5:** 139-144,

Varjú D (1976) Visual edge fixation and negative phototaxis in the mealworm beetle Tenebrio molitor. *Biological Cybernetics* **25**:17-26.

Veierskov B, Stummann BM & Henningsen KW (1982) Dynamic of extractable carbohydrate in Pisium sativum. II. Cabrohydrate content and photosynthesis of pea cuttings in relation to irradiance and stock plant temperature. *Physiologia Plantarum* **55:**174-178.

Wiegand CL & Richardson AJ (1983) Leaf area, light interception, and yield estimates from spectral components analysis. *American Society of Agronomy* **76**:543-548.

Wilkinson S, Davies WJ (2010) Drought, ozone, ABA and ethylene: new insights from cell to plant to community. *Plant, Cell & Environment* **33**:510-525.

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.

Appendix: Light treatments

The tables provided in this appendix show the average light parameters from the LED light treatments used in the CP125 trials. The Phytochrome Photostationary State (PSS) is a calculated parameter that gives an estimate of the activation state of the phytochrome B, higher values indicate greater activation. PSS was calculated using the methods of Sager (1986).

Table 1. The specifications of the different red: blue light treatments used during the crop growth trials.

Measure	% blue	Blue photon irradiance	Red photon irradiance	PAR photon irradiance	Daily light integral (DLI)	PSS
Units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	
	0	0	200	200	11.52	0.88
	11	22	178	200	11.52	0.87
	16	32	168	200	11.52	0.87
	33	66	134	200	11.52	0.87
	58	116	84	200	11.5	0.85
	65	130	70	200	11.52	0.85
	100	154	0	156	8.99	0.52

Table 2. The specifications of the different red: far-red light treatments used during the crop growth trials.

Measure	Far-red photon irradiance	% blue	PAR photon irradiance	Daily light integral (DLI)	Red:far-red ratio	PSS
Units	µmol m^{-2} s^{-1}	%	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}		
	1.51	10.4	200	11.5	118	0.86
	6	10.4	200	11.5	36	0.80
	10	10.4	200	11.5	18	0.77
	16	10.7	200	11.5	10	0.70
	19	10.3	200	11.5	9	0.68
	43	10.1	200	11.5	4.	0.55

Table 3. The specifications of the different light intensity / daily light integral treatments used during the crop growth trials.

Measure	% blue	PAR photon irradiance	Daily light integral (DLI)	Blue photon irradiance	Red photon irradiance	PSS
Units	%	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	
	11	99	5.7	10.9	88.1	0.88
	11	198	11.4	21.8	176.2	0.87
	11	274	15.8	30.2	243.8	0.87
	11	385	22.2	42.4	342.6	0.87

Table 4. The specifications of eight combined red: blue: far-red light treatments used during the year two crop trials.

Measure	% blue	Far-red photon irradiance	Blue photon irradiance	Red photon irradiance	PAR photon irradiance	DLI	PSS
Units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	
30% blue treatments							
	31.8	1.2	62.6	133.8	197.2	11.36	0.86
	28.2	11.3	57.1	145.0	202.9	11.69	0.74
	29.1	20.3	59.1	143.4	203.3	11.71	0.66
	30.4	35.2	60.3	137.4	198.5	11.43	0.54
60% blue treatments							
	62.3	0.5	126.8	75.1	203.6	11.73	0.85
	57.4	11.0	117.2	85.4	204.2	11.76	0.67
	54.7	18.5	104.2	84.8	190.4	10.97	0.58
	57.6	32.5	113.9	83.6	197.8	11.40	0.46

Table 5. The specifications of the white light used in the year 1 lettuce trials. These light treatments were performed in a container not the LED4CROPS facility.

	PAR photon irradiance	Daily light integral (DLI)	% blue	% green	% red	Far-red photon irradiance	Red:far-red ratio	PSS
Make Model	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	%	%	%	µmol m^{-2} s^{-1}		
Valoya								
NS2	194	11.2	23	40	37	4.46	16.23	0.80
AP673	190	10.9	14	24	62	16.03	7.38	0.71
SolidLite								
DPM	204	11.8	21	39	40	16.09	5.11	0.67
DPA	200	11.5	24	27	49	23.94	4.15	0.62
CWW	202	11.6	31	36	33	11.58	5.75	0.69

Table 6. The combined red: blue: far-red treatments used during the third year crop trials

Measure	% blue	Far-red photon irradiance	Blue photon irradiance	Red photon irradiance	PAR photon irradiance	DLI	PSS
Units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	
6% blue treatments							
	6.2	1.0	10.9	164.2	175.4	10.1	0.87
	5.8	24.5	11.1	181.6	193.1	11.1	0.66
	5.1	45.6	9.6	177.8	187.7	10.8	0.54
15% blue treatments							
	15.0	0.9	29.0	163.1	192.5	11.1	0.87
	15.6	21.7	28.7	154.7	184.0	10.6	0.66
	13.6	44.1	26.1	165.0	191.6	11.0	0.53
30% blue treatments							
	32.6	0.7	59.0	121.4	181.3	10.4	0.86
	37.7	21.4	77.2	126.1	204.5	11.8	0.62
	30.4	39.6	59.7	135.4	196.1	11.3	0.51
60% blue treatments							
	56.6	0.5	106.1	79.8	187.5	10.8	0.85
	56.2	22.7	100.0	76.9	178.1	10.3	0.51
	59.8	43.0	123.6	81.3	206.7	11.9	0.40

Table 7. The low intensity (100 µmol m^{-2} s^{-1}) red: blue light treatments used during crop growth trials.

Measure	% blue	Blue photon irradiance	Red photon irradiance	PAR photon irradiance	Daily light integral (DLI)	PSS
Units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	
	100	100	0	100	5.8	0.52
	59	59	41	101	5.8	0.85
	14	14	86	100	5.8	0.87
	0	0	100	100	5.8	0.87

Table 8. Low intensity (75 µmol m^{-2} s^{-1}) red: blue: far-red light treatments used during a chrysanthemum propagation trial.

Measure	% blue	Blue photon irradiance	Red photon irradiance	Far-red photon irradiance	PAR photon irradiance	Daily light integral (DLI)	PSS
Units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	
Red: blue treatments							
	100	75	0	0	75	4.3	0.52
	59	42	33	0	75	4.3	0.85
	15	11	63	0	75	4.3	0.87
	0	0	75	0	75	4.3	0.87
Red: far-red treatments							
	15	11	63	8	75	4.3	
	15	11	63	16	75	4.3	

Table 9. Far-red light treatments used during the second chrysanthemum propagation trial.

Measure	% blue	Blue photon irradiance	Red photon irradiance	Far-red photon irradiance	PAR photon irradiance	Daily light integral (DLI)	PSS
Units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	
Red: far-red treatments							
	15	11	63	0	75		
	15	11	63	25	75		
	15	11	63	50	75		
	15	11	63	74	75		

Table 10. The combined red: blue and red: far-red treatments used during the HNS propagation trials.

Measure	% blue	Far-red photon irradiance	Blue photon irradiance	Red photon irradiance	PAR photon irradiance	DLI	Red:far-red ratio	PSS
Units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}		
Blue light treatments								
	0	0	0.0	70.0	70	4	NA	0.88
	33	0	23.1	46.9	70	4	NA	0.87
	52	0	36.5	33.1	70	4	NA	0.86
	66	0	46.2	23.8	70	4	NA	0.85
	100	0	70.0	0.0	70	4	NA	0.52
Far-red treatments								
	11	0	7.7	62.3	70	4	>89	
	11	15	7.7	62.3	70	4	4.1	
	11	30	7.7	62.3	70	4	2.08	
	11	48	7.7	62.3	70	4	1.30	

Table 11. The combined red: blue and red: far-red treatments used during the santolina propagation trials.

Measure	% b	Far-red photon irradiance	Blue photon irradiance	Green photon irradiance	Red photon irradiance	PAR photon irradiance	DLI	PSS
Units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	
Red: blue treatments								
	0	0	0	0	82	83	4.8	
	61	0	49	0	31	82	4.7	
	33	0	31	0	62	93	5.4	
	9	0	7	0	72	80	4.6	
Far-red treatment								
	9	15	7	0	74	82	4.7	
White treatment								
	9	2	8	15	63	86	5.0	
Intensity treatments								
	11	0	4	0	32	37	2.1	
	12	0	8	0	57	65	3.8	
	10	0	9	0	75	84	4.8	

Table 12. The combined red: blue and red: far-red treatments used during the iberis and clematis propagation trials.

measure	% b	Far-red photon irradiance	Blue photon irradiance	Green photon irradiance	Red photon irradiance	PAR photon irradiance	DLI	PSS
units	%	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	mol m^{-2} d^{-1}	
Red: blue treatments								
	0	0.32	0.05	0.1	78.19	78.34	4.51	
	57	0.6	40.47	0.34	29.9	71	4.09	
	33	0	22.87	0.33	49.45	72.65	4.18	
	15	0.24	7.18	0.19	65.02	76.82	4.42	
Far-red treatments								
	15	8.96	11.59	0.17	64.3	76.08	4.38	
	15	15.35	11.04	0.16	61.73	72.96	4.2	
White treatment								
	9	1.51	6.95	15.99	64.4	87.36	5.03	
Intensity treatments								
	10.37	0.18	4.41	0.31	37.8	37.8	2.18	
	11	0.43	8.29	0.57	64.61	73.49	4.23	

