Project title:	Enhancing crop quality and diminishing water use in bedding plants
Project number:	PO 017
Project leader:	Dr Ian Dodd, Lancaster University; Dr Martin McAinsh, Lancaster University
Report:	Final Report, September 2015
Previous report:	N/A
Key staff:	Richard Boyle, Lancaster University
Location of project:	Lancaster Environment Centre, Lancaster University
Industry Representative:	Sarah Fairhurst
Date project commenced:	01/07/2013
Date project completed (or expected completion date):	30/10/2015

DISCLAIMER

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

AUTHENTICATION

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

[Name]

[Position]

[Organisation]

Signature .... Date ....

[Name]

[Position]

[Organisation]

Signature .... Date ....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature .... Date ....

[Name]

[Position]

[Organisation]

Signature .... Date ....

CONTENTS

GROWER SUMMARY

Headline

- Plant morphology and water use can be manipulated through reducing irrigation volumes and frequency (deficit irrigation), and by altering light quality via LEDs

Background

Bedding plant producers aim to ensure plants are at the appropriate developmental stage and of a high quality prior to sending to market, all within a strict production schedule. Reducing irrigation volumes and frequency tailored to plants water requirements (deficit irrigation) can either delay, or accelerate crop development, but knowledge of these management regimes with regard to the impact on plant quality are limited to a few species.

Many growers make use of supplementary lighting (e.g. high pressure sodium lamps), particularly during the winter, but the economic costs of this lighting can be substantial. LEDs provide a more energy efficient alternative lighting source. Current research has highlighted that LEDs may prove to be a sustainable and economically more viable approach as a sole lighting source for annual crop production.

Both deficit irrigation and the spectral quality of LED lighting will affect multiple plant physiological processes. These include photosynthesis and transpiration, both of which contribute to the efficiency with which a plant uses water, as well as the biosynthesis of, and/or sensitivity to, endogenous plant hormones that regulate crop growth and quality. Impacts of these different treatments on plant quality (particularly leaf colouration and plant compactness) have received relatively little attention, despite their commercial significance.

Summary

This project focused on improving resource use efficiency in bedding plants using sustainable plant management strategies – alternative irrigation and lighting with LEDs.

In the first part of the project, irrigation frequency was identified as a key aspect of irrigation management that may be under-valued by growers. Further, it is critical that growers can utilise approaches that allow them to better adapt their irrigation scheduling to the requirements of the plant. Initial studies identified that altering deficit irrigation frequency can have significant impacts upon leaf gas exchange, leaf water status and water use within a single bedding plant species, *Pelargonium x hortorum*. This highlights the need for growers to monitor and/or revisit the physiological impact of irrigation practices. Further investigation revealed that growers could increase plant quality whilst reducing water inputs by reducing the volume and frequency of irrigation. Subsequent experiments identified that the stress hormone abscisic acid (ABA) as one of the key signals involved in regulating stomata in response to soil drying in *P. hortorum*. This has important implications for growers scheduling irrigation to increase water use efficiency (WUE).

In the second part of this project, *P. hortorum* plants were grown under different proportions of blue and red LED lighting within a closed environment system. It was found that spectrally different light sources at the same intensities had significant effects upon leaf gas exchange, morphology and leaf pigment concentrations, whilst optimal results were achieved with combined blue and red lighting. This has important implications for growers' decisions to change from conventional lighting to LEDs. However, further work is required to establish the optimal spectra of light for production over a broad range of species which is underway for example in AHDB funded projects (currently CP 085, CP 125 and CP 164).

Financial Benefits

Developing techniques to allow more effective application of irrigation on a commercial scale could significantly reduce costs associated with water consumption. Furthermore, if plant growth can be regulated by reducing water inputs ('deficit irrigation'), this may improve the quality of the plant, and reduce the need for growth regulating chemicals. The implementation of LEDs, although requiring an initial high investment by growers, offers savings through reduced energy consumption. Constructing LEDs with species-specific wavelengths may also lead to other benefits including reduced water use, or improved ornamental value of the plants.

Action Points

- Understanding the specific water requirements of the plant will allow for growers to more accurately schedule irrigation strategies, which can deliver benefits including water savings and increased plant quality.
- LEDs present an alternative supplemental light source that offers growers the option to control plant morphology and quality not offered by more conventional lighting methods. This extends to plant water relations and has potential overlap with the deficit irrigation outcomes highlighted above although work is needed to determine benefit in supplementary lighting rather than sole source lighting situation.

SCIENCE SECTION

Introduction

Developing sustainable irrigation practices is a key challenge for agriculture due to the increasing scarcity of water world-wide. An improved understanding of the physiological processes by which plants respond to reduced water availability and how these can be manipulated to improve plant water use is essential (Wilkinson and Hartung, 2009). Irrigation frequency is a key aspect of irrigation scheduling in horticulture, and by reducing the irrigation frequency, growers may prevent excessive water loss. Furthermore, this approach may provide greater control of plant growth (and thus enhanced ornamental value (Cameron et al., 2008)), and also increased Water Use Efficiency (WUE) (Fereres and Soriano, 2007).

Deficit irrigation (applying irrigation at a lower volume than the plants water requirements) is widely used as an alternative to conventional irrigation (Fereres and Soriano, 2007). Although the benefits of this approach are well recognised, effects of varying irrigation frequency are less well understood, partly because many studies confound both irrigation frequency and volume. Container-based plant production allows accurate measurements of Evaporative transpiration (ET), as well as paired measurements of stomatal conductance (gs) and leaf water potential (Ψleaf) at known whole-pot substrate Gravimetric Water Content (GWC or θpot). Stomatal closure is a primary response to water deficit resulting in reduced transpiration and water loss, thereby contributing to plant survival during periods of drought (Ismail et al., 2002). This represents an important target for improving plant responses to reduced water availability, and understanding the mechanisms behind this may allow growers to optimise their irrigation scheduling for optimal production of an ornamental bedding plant species.

Hydraulic and chemical processes have both been implicated in regulating stomatal responses to water deficit (Wilkinson and Davies, 2010), with often conflicting findings. There is evidence of stomata closing in response to a decreased Ψleaf as a regulatory feedback mechanism, and possibly as a consequence of reduced Ψleaf increasing stomatal sensitivity to ABA (Buckley, 2005, Pantin et al., 2013, Tardieu and Davies, 1992). ABA has been widely characterised as a chemical signal involved in stomatal regulation under reduced water availability. ABA is synthesised either locally in the leaves or is transported from the roots, leading to stomatal closure through a series of cascading biochemical events at the cellular level (Kim et al., 2010, Merilo et al., 2014). In many species stomatal closure is correlated with increased xylem ABA concentration ([ABA]xyl) (Wilkinson and Davies, 2002). In some species, although [ABA]xyl increases under water stress, feeding synthetic ABA at these concentrations to detached leaves does not elicit stomatal closure. These data suggest that although ABA can close stomata, in many instances it may not be the sole and/or primary regulator (Loveys et al., 1987, Perez-Alfocea et al., 2011).

Ornamental growers aim to produce high-quality, high-value plants. Although the definition of quality can vary between species, it may include plant compactness, enhanced foliar and floral characteristics (e.g. pigment composition), rooting characteristics and/or enhanced shelf life (Fustec and Beaujard, 2000, Demotes-Mainard et al., 2008, Macfarlane et al., 2005). Historically, growers have manipulated many of these characteristics by applying chemical growth regulators (Morel et al., 2012), but costs and increased awareness of environmental and health effects has reduced this approach (Lutken et al., 2012). The environmental impact of plant production is now a major consideration for consumers (Khachatryan and Choi, 2014). Manipulating irrigation frequency may also increase plant WUE. Ultimately, accurate understanding of irrigation scheduling may allow growers to tailor regimes to match their specific objectives.

The availability of light is essential for the growth and development of plants. Light not only acts as the sole energy source, but also as a signal for plants with regard to their surrounding environment. This includes, for example, B light driving phototropism (Christie, 2007), and the detection of red to far-red as a shade avoidance response (Casal, 2013). Plant leaves typically absorb red and blue light, with high absorbance and low reflectance (Park et al., 2013), and as such, different combinations of these wavebands have been primarily used in horticultural production systems (Fan et al., 2013). Specific spectra of light regulate many physiological processes. B light regulates stomatal opening, photo-protection and chloroplast migration (Shimazaki et al., 2007), whilst R light is used in assimilate transport and development of the photosynthetic apparatus (Jeong et al., 2012). Manipulating the ratio of B:R can have both positive and negative effects on plant development depending on the species, the specific spectra used, and the desired response (Islam et al., 2012, Heo et al., 2003, Currey and Lopez, 2013).

Light can impact upon many aspects of plant growth through manipulating the photosynthetic apparatus, but has also been shown to regulate other processes including flowering and leaf development (O' Carrigan et al., 2014). For instance, when rose (Rosa x hybrida) plants were exposed to high B (20%B:80%R), there was an increase in photosynthesis and stomatal conductance, and an 18% reduction in stem length compared to a low B (5%) source (Terfa et al., 2013), with similar results found in pelargoniums (Appelgren, 1991) and soybeans (Dougher and Bugbee, 2004). In poinsettias a combination of B and R LEDs (20% B:80% R) decreased growth by 20-34% and decreased leaf and bract area, without reducing bract colour or post-production performance (Islam et al., 2012). Different combinations of B and R at different photoperiods and intensities were used to grow Cyclamen persicum (Heo et al., 2003). While sole B and R individually reduced the flowering response, B and B-R decreased the peduncle length, and R and B-R delayed the blooming period of flowering by 20 days (double that of the control plants). Hence, by tailoring the spectra of lighting, the grower may therefore be able to tightly control plant production to meet their specific demands. Additional benefits may be found if manipulating light spectra can replace chemical control of plant morphology (Abidi et al., 2013).

The opportunity to carry out research investigating the effects of light quality on plant growth and physiology has become more feasible in recent years as LEDs have become more readily available (Park et al., 2013). LEDs are considered more energy efficient, convenient and effective when compared to traditional light sources, particularly if the spectra of lighting can be tailored towards the species of interest (Liu et al., 2011, Stutte et al., 2009). There is a desire for LEDs to replace conventional lighting on a commercial scale, but this may require further research to understand the optimal spectra for individual species of interest. Controlling the spectrum of light can modify plant morphology, gas exchange and water use.

P. hortorum is an annual bedding plant species, popular for its attractive ornamental characteristics (both flowers and foliage). A previous study highlighted that leaf architecture of *Pelargonium zonale* could be modified via localized irradiance with different spectra of light (Fukuda et al., 2008), whilst an in vitro study of *P. hortorum* showed that stem elongation could be promoted with R, and inhibited with B (Applegren, 1991). Therefore the effect of different combinations of B and R LEDs as a sole light source on the morphology and physiology of containerised *P. hortorum* were investigated. The specific aims of this research were to determine and understand the optimal B:R combination to improve plant quality (assessed through the compactness of the foliage and an increase in anthocyanic banding on the leaves), and WUE via alterations in plant gas exchange.

The aim of the first study was to assess the impact of limiting irrigation frequency on the production of *Pelargonium x hortorum*. This species was selected as a representative bedding plant in which soil water deficits are commonly applied by the industry as a management tool, and because it can tolerate prolonged periods of severe soil drying. Two irrigation frequencies were examined, including a frequent (irrigating plants daily at 50 % Evaporative Transpiration) and infrequent (delayed deficit irrigation) deficit irrigation programme, both of which provided the same volume of water to plants over the treatment period (Fig 1). The effect of these different irrigation frequencies on leaf gas exchange and leaf water status, and how this impacted upon plant growth, quality (plant compactness and leaf pigment composition), and WUE was investigated. The role of leaf water status, ABA and other xylem-borne antitranspirants in stomatal closure of *P. hortorum* subject to different types of soil drying (adapted from the different deficit irrigation frequency treatments) was also assessed.

Figure. 1. Methodology for applying and monitoring soil water deficits. a) Evapotranspiration measured daily with balance, and b) measurements of soil water status at the point of sampling with a Theta Probe.

In the second study, the effect of implementing LEDs as sole light sources on leaf gas exchange, leaf pigment composition and morphology of *Pelargonium* x *hortorum* were investigated at the LED4Crops facility. White LEDs were used as a conventional light source, whilst different combinations of blue (B) and red (R) LED lights were examined (at 100%, 66%, 33% and 16% B).

Materials and Methods

Irrigation frequency for ornamental bedding plant production

Plant culture

P. hortorum Bulls Eye (zonal geranium) seeds were germinated in individual 13 cm x 11.3 cm (1.05 dm^3) pots (Pöppelman TEKU®, Germany) containing a peat-based substrate (Levington M3), for which a moisture release curve has previously been published (Dodd et al., 2010). Plants were grown at 24 °C, and at a photosynthetically active radiation (PAR) of 330±4.3 µmol m-2 s-1 (mean data from the duration of the study). Experiments were carried out in a naturally lit glasshouse at the Lancaster Environment Centre, with high-pressure sodium lamps (Osram Plantastar 600W) supplying supplementary lighting for a 14 h day photoperiod (0600 h-2000 h) when ambient PAR was less than 500 µmol m-2 s-1. The daily maximum temperature in the greenhouse was 24 °C with a night temperature of 17 °C, and the average daily relative humidity was 35.6±0.9 %. Environmental conditions in the centre of the glasshouse were recorded using a Hortimax growing solutions Ektron II (Pijnacker, The Netherlands).

Irrigation Treatments

Prior to imposing different watering regimes, individual plants were weighed using a balance with a 0.1 g resolution (Scout Pro Portable balance, Ohaus, Switzerland). All plants were initially irrigated daily to well-watered (WW) conditions (watered until drainage was visible from the bottom of the pot, and then left to freely drain overnight), which was used as a reference value. To calculate daily ET, pots were weighed at 0800 h each day, accounting for any irrigation supplied in the previous 24 h. During the experimental period, plants were subject to three irrigation treatments; maintained at WW conditions, or subject to deficit irrigation at two irrigation frequencies. The two deficit irrigation treatments were infrequent (IDI; withholding water with regular re-watering events) or frequent deficit irrigation (FDI; daily irrigation at 50% of WW plants ET). After 4 days of withholding water, plants subject to IDI received the same cumulative irrigation volume as applied to plants under FDI over the same period. Irrigation regimes were applied at week 5 for both treatments, with a 24 days experimental period. Plants under IDI were subject to 6 cycles of drying and re-watering.

An additional set of experiments investigated the physiological response of the irrigation treatments described above, but over one drying cycle. As such, one group of plants was irrigated at 50 % ET from week 7 (adapted from FDI), whilst an additional group of plants were maintained at WW conditions until week 9, at which point irrigation was withheld (adapted from IDI). Irrigation regimes were applied 7 and 9 weeks after germination for the different irrigation treatments to ensure that sampling was carried out on plants of the same chronological age, just prior to flowering, but also to ensure sampling was carried out at similar soil moisture availability.

Physiological measurements

Measurements of stomatal conductance (gs) were made between 1100 h and 1300 h on the youngest, fully expanded abaxial side of one leaf per plant using a porometer (Model AP4, Delta-T Devices, Cambridge, UK). Leaf water potential (Ψleaf) was determined immediately after sampling for gs on the same leaf as described previously (Scholander et al., 1965), using a pressure chamber (Model 3000F01 Plant Water Status Console; Soil Moisture Equipment Corp. Santa Barbara, CA, USA). After measuring Ψleaf, sap samples from each leaf were collected. Xylem abscisic acid [ABA]xyl was determined by radioimmunoassay with the MAC252 monoclonal antibody (Quarrie et al., 1988). Measurements of gs, Ψleaf and [ABA]xyl were carried out every 4 days from the beginning of the treatment period until the final day of Cycle 6.

Plant biomass and morphology

Canopy volume was measured at the end of each drying cycle to assess the overall compactness of each plant, which was measured as the total height, width and breadth of the plant. After physiological measurements, plant material was harvested and shoot fresh weight (FW), which was separated into leaf and stem, was measured. Leaf number was recorded, and leaf area mas measured using a leaf area machine (LI-3100C Area Meter, LI-COR Inc., Lincoln, NE, USA). Plant material was dried in an oven at 80°C until a constant mass to obtain dry weight (DW). Volumetric water content (θpot) was obtained gravimetrically by weighing the soil FW, and then re-weighing after oven drying. WUE was determined by the volume of water applied to produce the shoot dry biomass of each plant.

Statistics

Differences between soil water deficit regimes, and treatments on each day were evaluated by one-way analysis of variance (ANOVA) at $p<0.05$ using SPSS Statistics 20 (IBM). When ANOVA was significant, means were discriminated using Tukey’s multiple comparison test. Where values were not normally distributed according to a Shapiro-Wilk test, data was Log transformed and re-tested. If values were again found not to be normally distributed, a non-parametric Kruskal-Wallace test was used to determine if significant differences occurred between treatments and days. Analysis of Covariance (ANCOVA) was used to determine whether irrigation regime affected relationships between soil and plant variables. Altered sensitivity of the y-variable to the x-variable is indicated by a significant interaction term. Where significant, regressions were fitted in Sigmaplot 8 (Systat Software Inc.).

A role for LEDs in ornamental plant production

Plant culture

P. hortorum Bulls Eye (zonal geranium) seeds were germinated in individual pots containing a peat based substrate (Levington M2) at 21.7±0.5°C, and PAR of 200 µmol m^{-2} s^{-1}. Plants were irrigated daily. Experiments were carried out in the LED 4 CROPS applied research and knowledge transfer facility at Stockbridge Technology Centre (Fig. 2). LEDs provided a sole light source for a 16h day photoperiod (0600 h-2000 h). Plants were subject to either a white LED light (control) or different combinations of Blue (B) and Red (R) light; 100% B, 66% B, 33% B or 16% B.

Figure 2. Experimental set up using different proportions of blue (B) light at Stockbridge Technology Centre

Physiological measurements

g_s, photosynthesis (*A*) and transpiration (*E*) were measured by infra-red gas analysis (6400 XT LI-COR Portable Photosynthesis System, LI-COR Inc., Lincoln, NE, USA). Measurements were carried out on the youngest, fully expanded abaxial side of per plant. Cuvette conditions were set to match environmental conditions within the research facility. Xylem sap were collected from individual leaves, and $[ABA]_{xyl}$ were determined by radioimmunoassay (Quarrie et al., 1988). Morphological traits of stomata on both the adaxial and abaxial leaf epidermis were determined by creating leaf imprints using super glue on glass microscope slides (Sampson, 1961). The youngest, fully expanded leaf was destructively sampled from three plants per light treatment. All measurements were made at a magnification of x10, and ten rectangular fields of view were photographed and used for counting per sample (Savvides et al., 2012).

Leaf pigment analysis

Leaf anthocyanin concentrations were determined spectrophotometrically. Frozen leaf tissue (15-20 mg) was ground with 600 µl of 99%Methanol:1%HCl , and then incubated overnight at 4°C with gentle shaking. 400 µl of Milli-Q water was added followed by chloroform extraction. 300 µl of the supernatant was added to 500 µl 99%Methanol:1%HCl & Milli-Q water (60:40 v/v), and the absorbance was then measured at using a spectrophotometer. Anthocyanin absorbance (A_{530}-0.25*A_{657}) was used to calculate concentration of anthocyanin per gram FW (Jeong et al., 2010).

Leaf chlorophyll (*a* and *b*) and carotenoid concentrations were determined spectrophotometrically. Frozen leaf tissue was extracted in 2 ml DMSO for 2 h, and measured with a spectrophotometer at 750 nm, 665 nm, 649 nm and 480 nm. If absorbance values were >1, samples were diluted. Leaf pigment concentrations were calculated using the following equations (Sumanta et al., 2014) –

Chl *a* (µg ml^{-1}) = (12.47*A_{665})-(3.62*A_{649})

Chl *b* (µg ml^{-1}) = (25.06*A_{649})-(6.5*A_{665})

Carotenoids (µg ml^{-1}) = ((1000*A_{480})-(1.29*Chl *a*)-(53.78*Chl *b*))/220

Biomass & growth parameters

At the end of the experimental period, leaf area, petiole length and leaf angle from the stem were all measured on the youngest, fully expanded leaf. Plant volume was used to assess the overall compactness of each plant, which was measured as the height, width and breadth. Stem architecture was assessed by measuring internode number and internode length. Shoot FW, which was separated into leaf and stem, and root FW was measured. Leaf number was recorded, and leaf area mas measured using a leaf area machine (LI-3100C Area Meter, LI-COR Inc., Lincoln, NE, USA). Plant material was dried in an oven at 80˚C until a steady state was achieved. Samples were then re-weighed for plant DW.

Statistics

Differences between light treatments were evaluated by one-way analysis of variance (ANOVA) at $p<0.05$ using SPSS Statistics 20 (IBM). When ANOVA was significant, means were discriminated using *Tukey's* multiple comparison test. Where values were not normally distributed according to a Shapiro-Wilk test, data was Log transformed and re-tested. If values were again found not to be normally distributed, a non-parametric Kruskal-Wallis test was used to determine if significant differences occurred between treatments and days. All graphs were created in Sigmaplot 8 (Systat Software Inc.).

Results and Discussion

Irrigation frequency for ornamental bedding plant production

Both deficit irrigation treatment groups received 50% of WW plants ET demand (cumulatively at the same volume), but at different irrigation frequencies (Fig.3 a & b). Delaying the irrigation frequency resulted in IDI plants showing a series of increases in ET after re-watering (typically within 24 h), followed by a decrease over the subsequent 24 h (Fig. 3c). Plants under FDI showed a more stable rate of ET over the experimental period, albeit lower than WW plants. The peaks of ET under IDI suggest rapid (within 1-2 days) partial recovery of gas exchange upon re-watering.

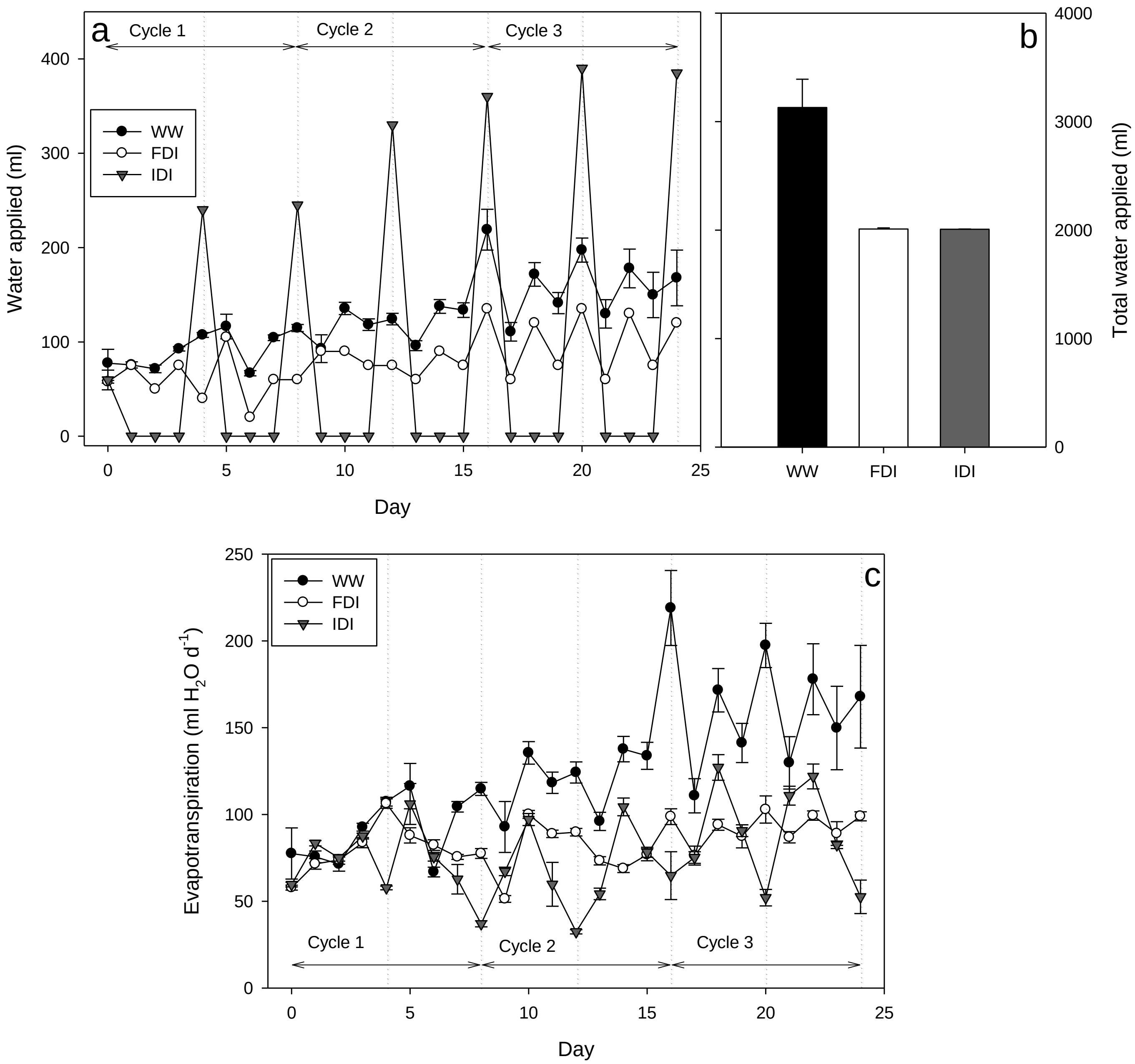

Figure 3. a) Volume of water applied per treatment per day; b) total volume of water applied to each treatment over the entire experimental period; c) daily evapotranspiration for *P. hortorum* plants subject to well watered conditions, frequent or infrequent deficit irrigation. Bars represent means ± SEM (n=13). Vertical lines indicate each re-watering event for the IDI treatment, and each cycle is indicated by horizontal lines.

Stomatal closure of *P. hortorum* is a well characterised response to soil drying, which is tightly regulated to limit water loss (Alvarez et al., 2013, Sanchez-Blanco et al., 2009). Stomatal closure occurred as soil moisture decreased under both deficit treatments (Fig. 4), but g_s decreased earlier under IDI. This is likely due to the initially quicker depletion of soil moisture and length of time irrigation was with-held under IDI. Leaf water status can provide a valuable indicator of plant stress, as well as having a role in stomatal regulation (Buckley, 2005). Although initially all treatments showed similar Ψ_{leaf}, by Day 10 plants subject to IDI began to show reductions in Ψ_{leaf}, and by Day 12 was lower than FDI plants, which was maintained over the rest of the sampling period (albeit not significantly different from WW plants). This was in contrast to FDI, where the slower imposition of soil drying, along with regular re-watering and the more gradual reductions in g_s may have acted to maintain a more positive Ψ_{leaf} (Fig. 4).

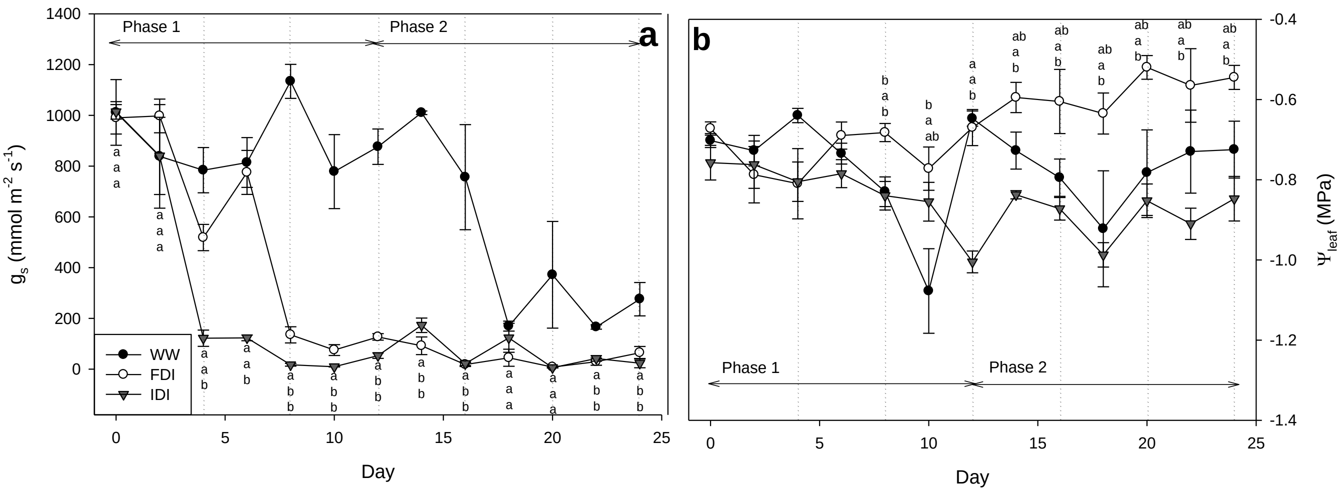

Figure 4. a) Stomatal conductance (g_s); and b) leaf water potential (Ψ_{leaf}) every two days (n=4) of *P. hortorum* plants subject to well-watered (WW) conditions, frequent (FDI) or infrequent (IDI) deficit irrigation. Data are means ± SEM (n=4). Vertical lines indicate each re-watering event for the IDI treatment, and each Phase is indicated by horizontal lines. Different letters indicate significant differences between irrigation treatments on each day according to a one-way ANOVA ($p<0.05$).

Significant differences in growth and biomass between WW plants and those under the two deficit irrigation treatments were detected by Day 12 (Fig. 5), and by Day 24, IDI plants also had significantly smaller biomass than FDI plants. This implies that prolonged periods of withholding irrigation eventually decreased plant growth. Smaller, compact plants are favourable for their aesthetic value, and because they are more suitable for transport (Cameron et al., 2008). Regulating growth via irrigation frequency may reduce the use of chemical growth retardants. Delaying irrigation frequency also had no negative effect on foliar quality (as indicated by leaf anthocyanin concentration; Fig. 6a). Thus ornamental value can be increased by altering (deficit) irrigation frequency.

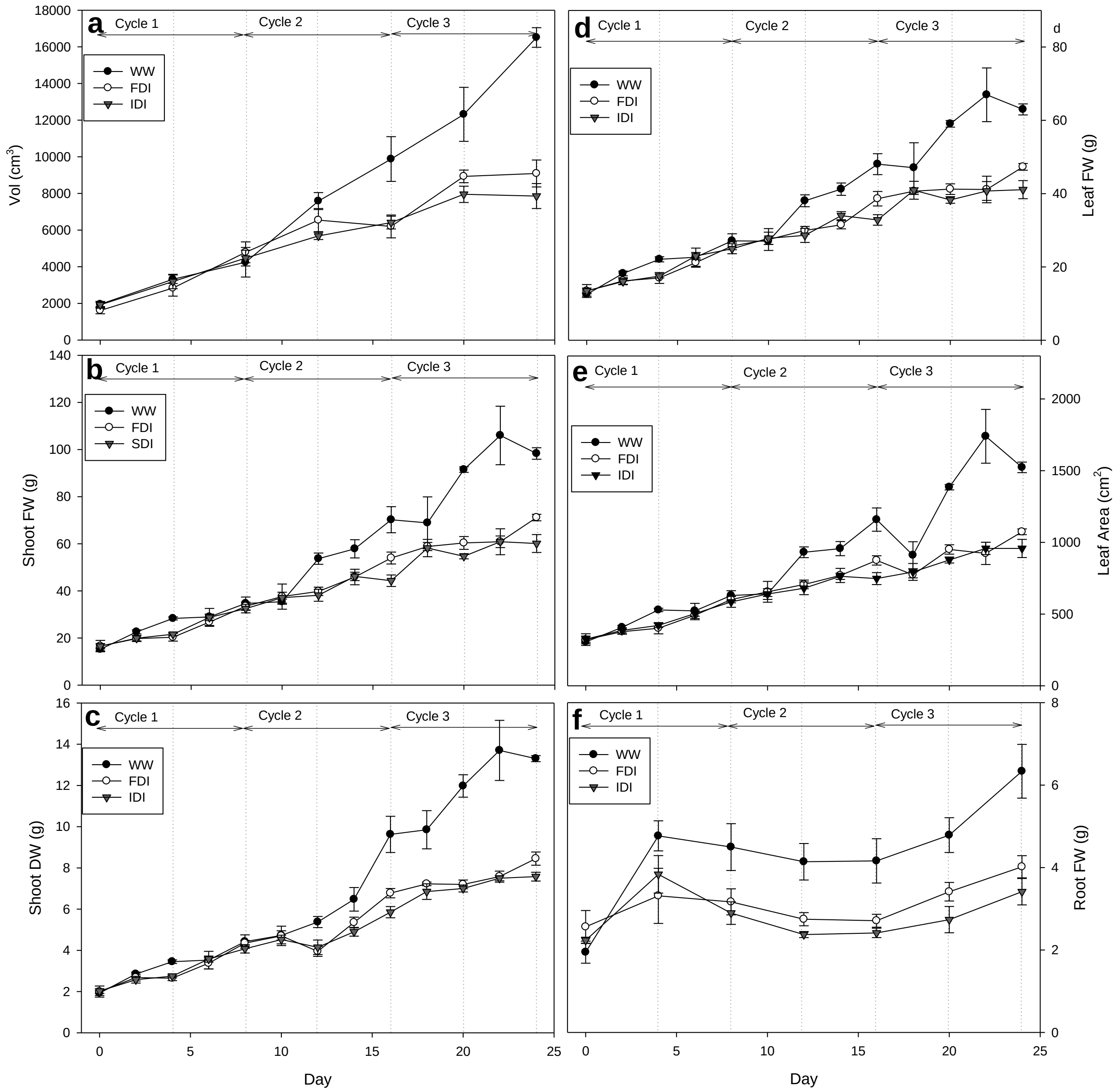

Figure 5. a) Canopy volume; b) shoot fresh weight (FW); c) shoot dry weight (DW); d) leaf FW; e) leaf area; f) root FW every two-four days of *P. hortorum* plants subject to well-watered conditions, frequent (FDI) or infrequent (IDI) deficit irrigation. Bars represent means ± SEM (n=4-5). Different letters indicate significant differences between irrigation treatments on each day according to a one-way ANOVA ($p<0.05$). Vertical lines indicate each re-watering event for the IDI treatment, and each cycle is indicated by horizontal lines.

Over the experimental period, two distinct periods of plant WUE were observed. Initially, both IDI and FDI plants had higher WUE than WW plants (Fig. 6b). However, after Day 12 there was a large reduction in WUE in both IDI and FDI treatments. Thus applying 50 % ET (either frequently or infrequently) over a longer period of time does limit WUE by decreasing biomass, but over the short-term it can significantly increase water productivity. While this is important for a broader understanding of the effects of irrigation frequency, biomass alone has less importance than the overall quality of an ornamental species. Therefore, it is clear from this study that irrigation volume can be decreased whilst maintaining foliar quality and increasing canopy compaction, thereby increasing the ornamental value of the plant, reducing irrigation costs, and ultimately increasing the sustainability of irrigation practices. If this irrigation strategy is used on a commercial scale, there may be significant environmental and economic benefits for the growers.

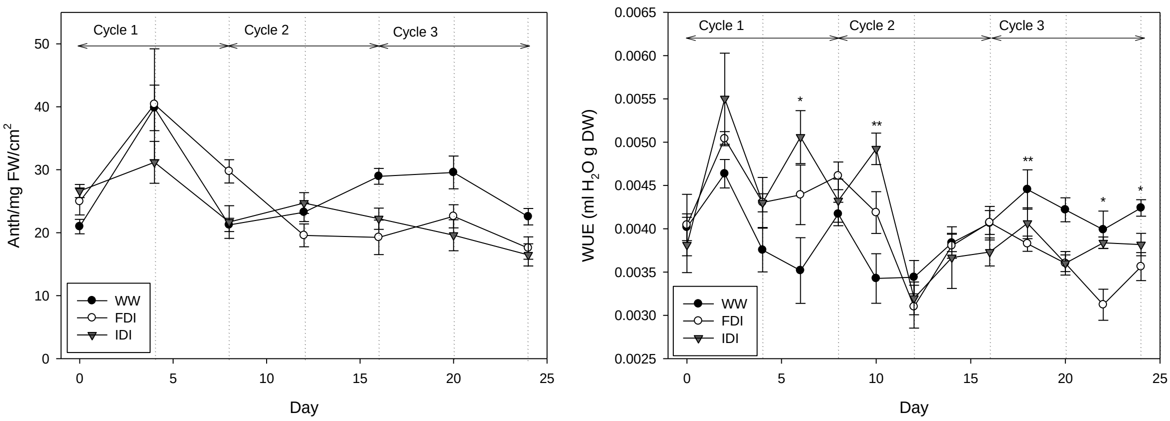

Figure 6. a) Leaf anthocyanin concentration every four days; b) water use efficiency (WUE) every two days of *P. hortorum* plants subject to well-watered conditions, frequent (FDI) or infrequent (IDI) deficit irrigation. Bars represent means ± SEM (n=4). Different letters indicate significant differences between irrigation treatments on each day according to a one-way ANOVA ($p<0.05$). Vertical lines indicate each re-watering event for the IDI treatment, and each cycle is indicated by horizontal lines.

To further understand the physiological responses, the different deficit irrigation frequency treatments were adapted to allow measurements during a single drying period. Withholding irrigation significantly reduced substrate Gravimetric Water Content (GWC) within 24 hours, whilst irrigating at a fraction of crop ET resulted in a more constant GWC over the sampling period (Fig. 7).

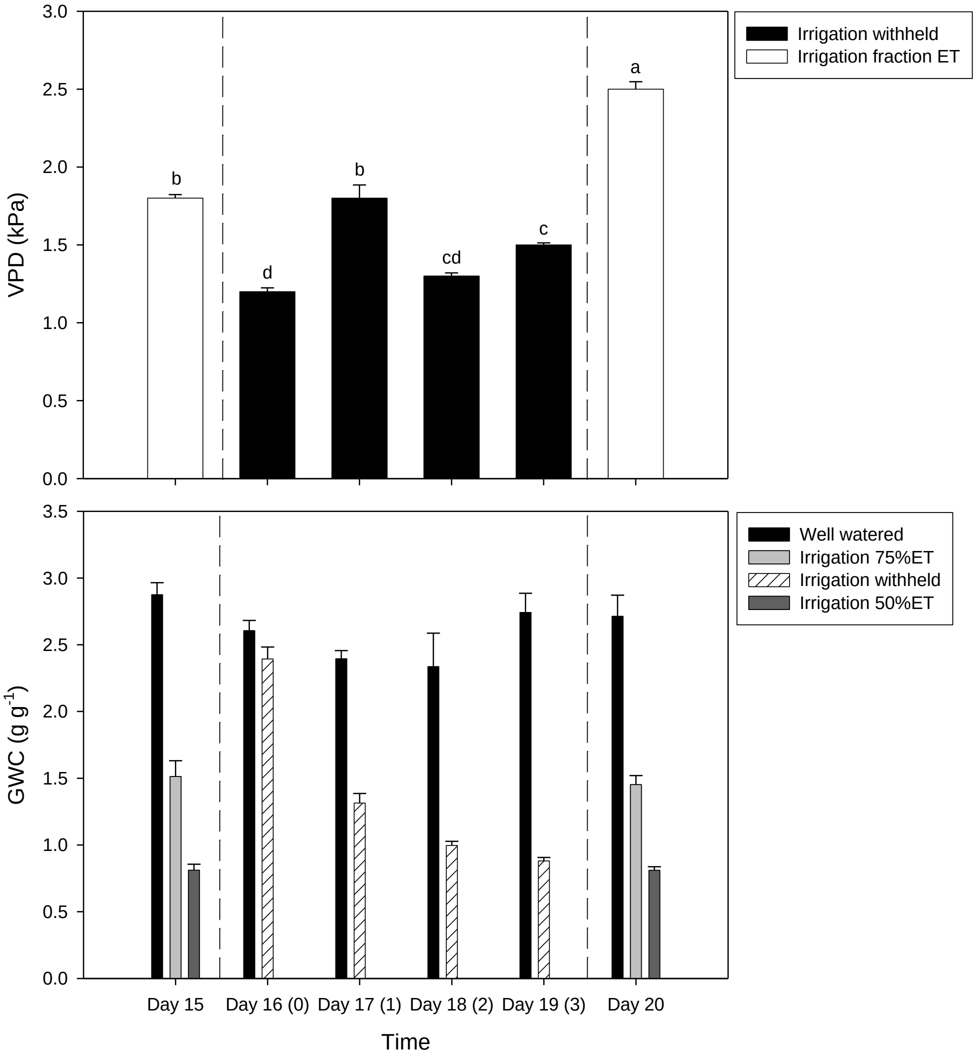

Figure 7. GWC (g g^{-1}) of *P. hortorum* subject to water being withheld (Days 16-19) or applied at either 75% or 50% of evapotranspiration (ET;Days 15 & 20). n = 5. Bars represent SE of mean. Different letters within a panel indicate significant differences according to a one-way ANOVA (p<0.05).

Stomatal closure of *P. hortorum* under both deficit irrigation treatments was strongly associated with decreased GWC (Fig. 8), with a similar relationship between the two irrigation treatments (no significant treatment x GWC interaction). Consequently, there was a difference between irrigation treatments in the response of Ψ_{leaf} to GWC, such that Ψ_{leaf} decreased when irrigation was withheld, but no significant relationship was found in plants irrigated at a fraction of crop ET (significant treatment x GWC interaction; Fig. 9). These results are consistent with findings from the longer term irrigation frequency experiments, but the lack of a consistent trend for Ψ_{leaf} suggests it isn't the key regulator of g_s.

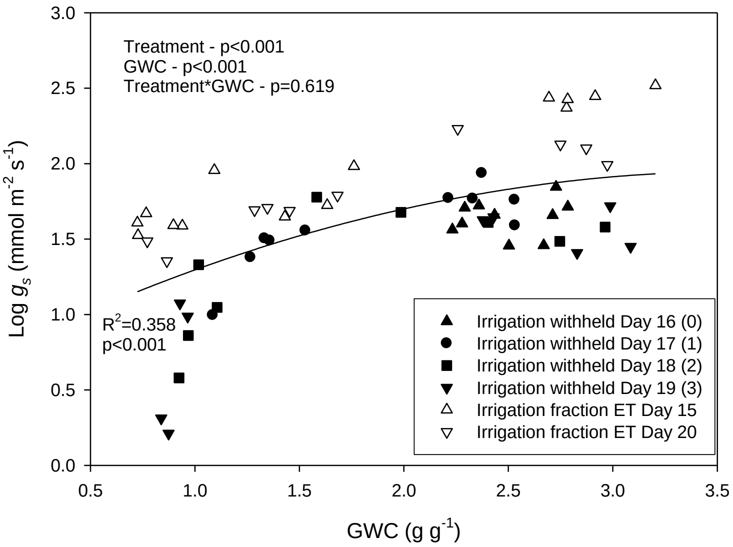

Figure 8. Log stomatal conductance (g_s) of *P. hortorum* in drying substrate under different soil water deficit regimes. Closed symbols show data from plants subject to irrigation being withheld (n= 38); open symbols show data from plants where irrigation was applied at a fraction of plant evapotranspiration (ET; either 75% or 50%) (n= 28). Data points are individual samples, single regression lines fitted and p value is reported. P values from ANCOVA also reported.

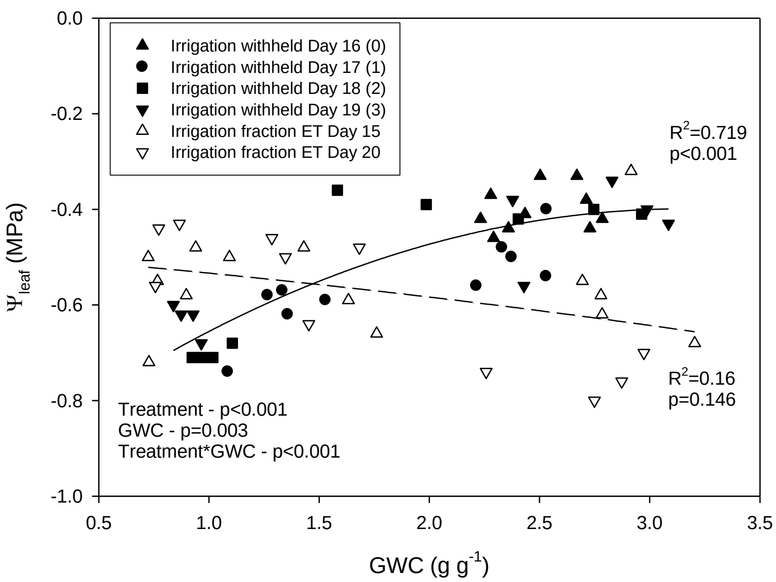

Figure 9. Leaf water potential (ψ_{leaf}) of *P. hortorum* in drying soil under different water deficit regimes. Closed symbols show data from plants subject to irrigation being withheld (n= 38); open symbols show data from plants where irrigation was applied at a fraction of plant evapotranspiration (ET; either 75% or 50%) (n= 28). Data points are individual samples, regressions lines fitted and P values reported. ANCOVA between the two treatments is reported.

Further analysis showed that $[ABA]_{xyl}$ increased significantly with decreased GWC under both deficit irrigation treatments (Fig. 10). However, when irrigation was applied at a fraction of crop ET, there was an attenuated $[ABA]_{xyl}$ response compared to when irrigation is withheld (significant treatment x GWC interaction). Stomatal conductance declined similarly with increasing $[ABA]_{xyl}$ (Fig. 11) under both water deficits (no significant treatment x ABA interaction). These findings suggest that ABA has an important role in regulating stomata under soil drying, and is likely involved in the underlying physiological mechanisms that regulate growth in *P. hortorum*.

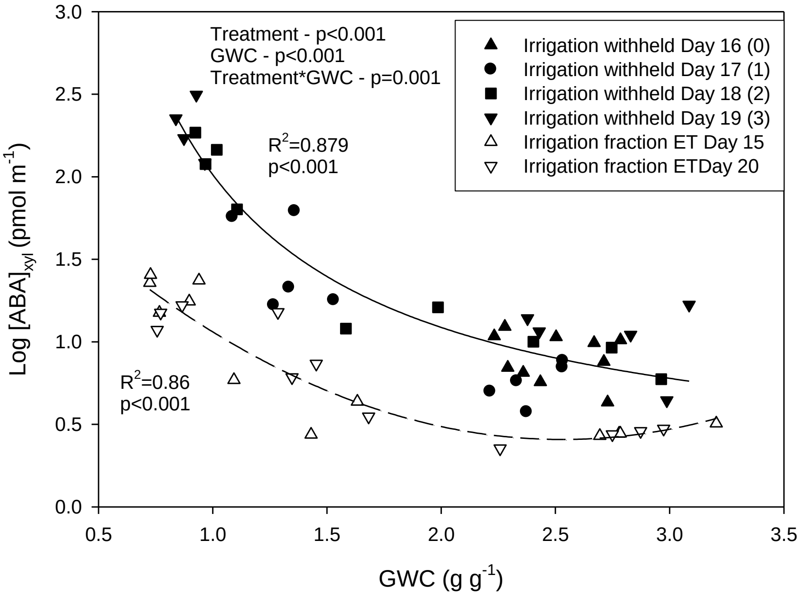

Figure 10. Log abscisic acid ($[ABA]_{xyl}$) of *P. hortorum* in drying soil under different soil water deficit regimes. Closed symbols show data from plants subject to irrigation being withheld (n= 38); open symbols show data from plants where irrigation was applied at a fraction of plant evapotranspiration (ET; either 75% or 50%) (n= 23). Data points are individual samples, regressions lines fitted and P values reported. ANCOVA between the two treatments is reported.

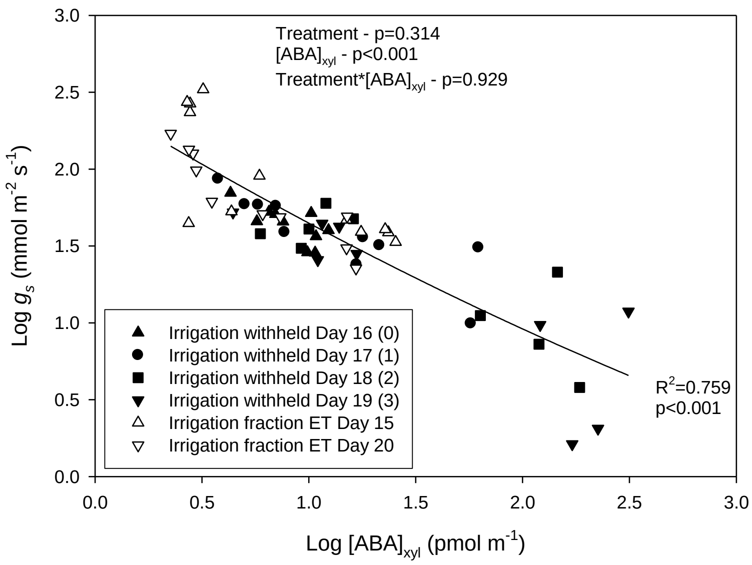

Figure 11. Log stomatal conductance *(g_s)* of *P.hortorum* in response to log xylem sap abscisic acid ($[ABA]_{xyl}$) under different water deficit regimes. Closed symbols show data from plants subject to irrigation being withheld (n= 38); open symbols show data from plants where irrigation was applied at a fraction of plant evapotranspiration (ET; either 75% or 50%) (n= 23). Data points are individual samples, a single regression line is fitted and P value reported. ANCOVA between the two treatments is reported.

A role for LEDs in ornamental plant production

Plants grown under 66% B showed the highest rate of photosynthesis (*A)*, stomatal conductance (g_s) and transpiration (*E)*, which was significantly higher than plants grown under 100% B or white light (Fig. 12). With the exception of 100% B, *A*, g_s and *E* decreased as the percentage of B decreased. Plants grown under 66% B showed the highest whole plant transpiration rate, consistent with published findings, although this was not significantly different from plants grown under the other light sources (Fig. 12). Intrinsic WUE was highest under 100% B, but not significantly different from other treatments (Table 1). No significant differences in $[ABA]_{xyl}$ were found between plants grown under any of the light sources (Table 1). No significant differences were found in adaxial, abaxial or total stomatal density (SD) between plants grown under any light treatment (Table. 1). The results above highlight that by altering the proportion of B:R, there may be significant changes in the leaf gas exchange. This is an important consideration for growers due to the impact this may have on plant growth and water use.

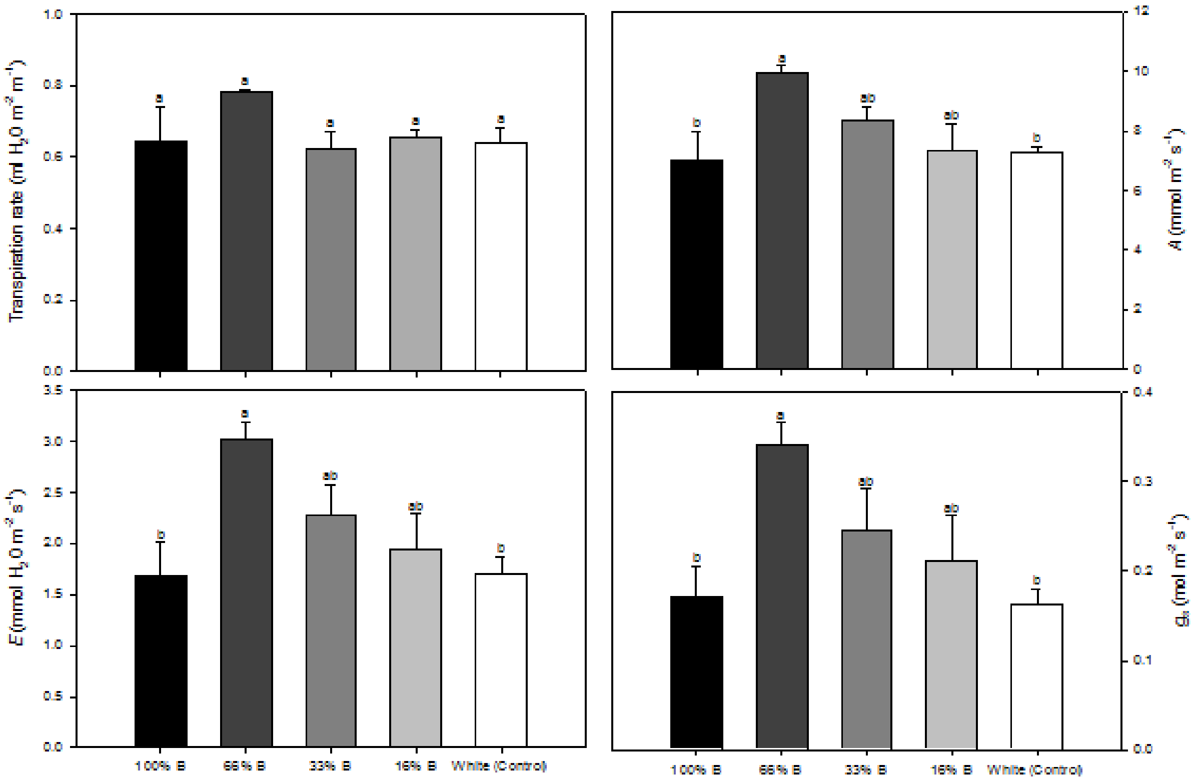

Figure 12. a) Whole plant transpiration rate; and leaf b) photosynthesis (*A*); c) transpiration (*E*); and d) stomatal conductance (g_s) of *P. hortorum* plants grown under different percentages of blue light (B). Different letters indicate significant differences according to a one-way ANOVA ($p<0.05$). Bars represent means ± SEM (n=6).

Table 1. iWUE and $[ABA]_{xyl}$ (n=6), adaxial and abaxial stomatal density (n=10), total leaf chlorophyll, carotenoids and anthocyanin concentration (n=5) of *P. hortorum* plants subject to different percentages of blue light (B). Different letters within a column indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ± SEM.

Light	iWUE (mmol mol m^{-2} s^{-1})	$[ABA]_{xyl}$ (nM)	Adaxial SD (No.)	Abaxial SD (No.)	Total Chl (mg g FW)	Carotenoids (mg g FW)	Anthocyanin (mg g FW)
100%B	56.8±16.6a	4.16±1.12a	13.97±0.86a	5.27±0.92a	0.86±0.09b	0.36±0.02a	3.04±0.54b
66%B	30.0±2.7a	2.37±0.81a	14.83±0.03a	4.43±0.13a	1.35±0.07a	0.42±0.01a	4.66±0.63ab
33%B	40.1±6.7a	6.94±1.20a	13.60±0.32a	5.47±0.09a	1.28±0.10a	0.41±0.03a	6.60±1.02a
16%B	43.4±8.2a	6.08±1.65a	14.40±0.51a	4.63±0.29a	1.27±0.08a	0.99±0.01a	4.32±0.34ab
7%B	46.5±4.3a	6.02±1.43a	13.73±0.69a	5.50±0.72a	1.16±0.06ab	0.78±0.01a	4.38±0.98ab

Leaf chlorophyll concentration of plants grown under 66% B was significantly higher than plants subject to 100% B, whilst no variation in the concentrations of carotenoids was found (Table 1). Leaf anthocyanin concentrations were highest in plants grown under 33% B (Table 1), which was significantly higher than plants grown under 100%B (which showed the lowest leaf anthocyanin concentrations), consistent with visual inspection of the plants (Fig. 13). As the anthocyanic region (the 'zonal band') of the leaf of ornamental value, this highlights that the quality of the plant can be increased by altering the percentage of B.

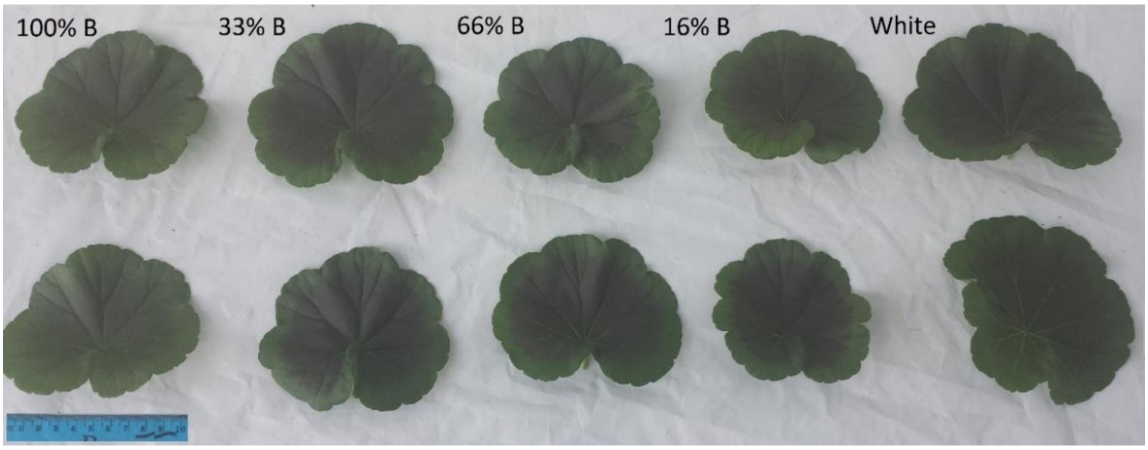

Figure 13. Zonal banding of leaves of plants grown under different percentages of blue light (B). Percentage of B that each plant was grown under is indicated above each leaf.

Plant morphology was significantly affected by the percentage of B. Plants grown under white light were biggest in all morphological parameters measured (Fig. 14, Table 2). Plants grown under 66% B had the lowest biomass accumulation, with the smallest overall volume, shoot, leaf and stem FW and DWs, leaf area and plant height, and thus produced the most compact canopy (Fig. 14, Table 2). Plants grown under 33% B and 16% B were similar in all morphological parameters to 66% B, but had a larger leaf area and thus an increase in canopy size. Thus it is clear that a combination of both B and R is required for optimal plant production, and can increase ornamental value of the plants compared to conventional, white light (Fig. 14, Table 2).

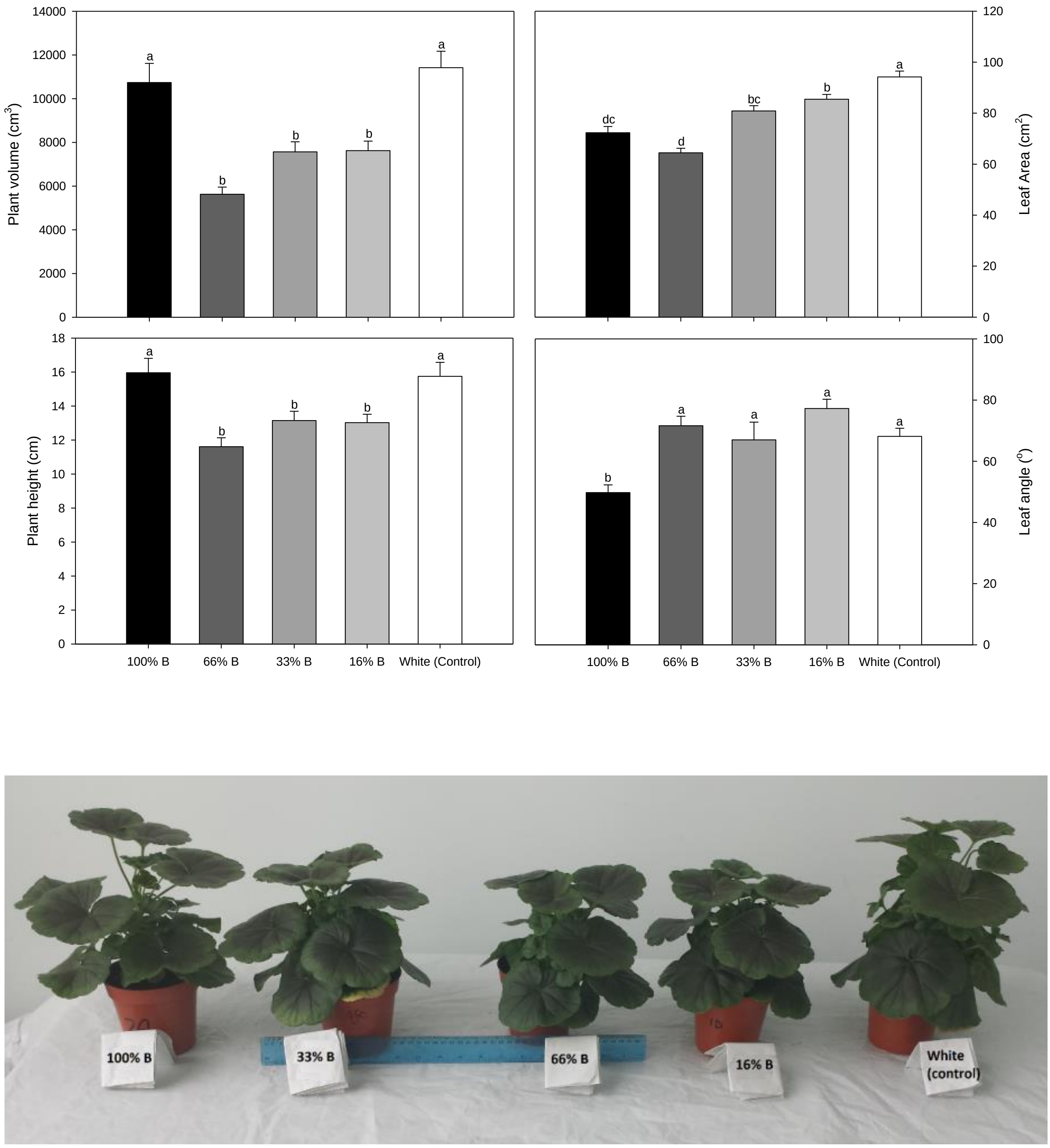

Figure 14. a) Plant canopy volume; b) plant height; c) leaf area; d) leaf angle; and e) morphology of *P. hortorum* plants grown under different percentages of blue light (B). Different letters indicate significant differences according to a one-way ANOVA ($p<0.05$). Bars represent means ± SEM (n=6).

Table 2. Shoot, leaf, stem and root fresh weight (FW) and dry weight (DW) of *P.hortorum* plants subject to different percentages of blue light (B). Different letters within a column indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ± SEM (n=6).

Light	Shoot FW (g)	Shoot DW (g)	Leaf FW (g)	Leaf DW (g)	Stem FW (g)	Stem DW (g)	Root FW (g)	Root DW (g)
100% B	46.5±2.6b	5.1±0.1bc	32.8±2.1b	3.3±0.1bc	13.7±0.6b	1.7±0.1b	4.4±0.1c	0.5±0.03a
66% B	42.1±2.8b	4.4±0.4c	31.4±2.1b	3.0±0.5c	10.8±0.8b	1.4±0.1b	4.8±0.3abc	0.6±0.1a
33% B	53.9±2.9b	6.3±0.3b	39.3±2.2b	4.5±0.2a	14.6±0.9b	1.9±0.1b	4.9±0.1bc	0.6±0.1a
16% B	54.1±31b	6.1±0.3b	39.7±2.3ab	4.3±0.2ab	14.4±0.8b	1.7±0.1b	5.7±0.1ab	0.7±0.1a
White	69.4±4a	8.1±0.4a	48.8±2.7a	5.4±0.3a	20.6±1.5a	2.7±0.2a	5.9±0.3a	0.7±0.1a

Conclusions

These results show that growers can adapt their irrigation scheduling dependent upon whether their aims are to reduce water consumption, improve water productivity, or increase ornamental quality. Less frequent deficit irrigation resulted in a series of peaks and declines in ET, earlier reduction in gs and a lower Ψleaf compared to plants subject to FDI. Neither deficit irrigation treatment diminished foliage quality. IDI and FDI both decreased plant growth compared to WW plants, with IDI plants the smallest by the end of the experiment. This was reflected in WUE, which was higher under both IDI and FDI over the first 10 days, but was lower after Day 10. In addition, [ABA]xyl appears to be the key, long distance antitranspirant regulating stomatal closure in *P. hortorum* in response to soil drying, but this signal was attenuated when soil drying was imposed by daily replacement of a fraction of crop ET. Furthermore, Ψleaf decreased only when irrigation was withheld, suggesting it is unlikely to act as a universal regulator of gs. Indeed, Ψleaf increased with stomatal closure when plants were irrigated with a fraction of crop ET, suggesting that gs regulates leaf water status. This may have important implications for how growers adapt their irrigation scheduling with respect to plant growth and quality, but also the physiological response, which may reduce water use and improve WUE.

This study provides evidence that different percentages of B can significantly affect plant physiology and growth in *P. hortorum*. With the exception of 100% B, leaf gas exchange decreased as the percentage of blue light decreased (indicating that both B and R regulate stomata). This presents an opportunity for growers to regulate plant water use. Further, growth and leaf pigment composition can be tightly regulated by manipulating light quality, with both B and R necessary to produce ornamentally favourable plants. This presents an environmentally favourable approach to increase crop value and potentially reducing the use of chemicals. Ultimately, LEDs present an opportunity to enhance the value of crops in a sustainable manner

Knowledge and Technology Transfer

Boyle, R.K.A., McAinsh, M., and Dodd, I.C. 2015. Increased plant quality and water savings in *Pelargonium* x *hortorum* in response to reduced irrigation frequency. *Acta Horticulturae*. (*Publication, Submitted*).

Boyle, R.K.A., McAinsh, M., and Dodd, I.C. 2015. Stomatal closure of *Pelargonium* x *hortorum* in response to soil water deficit is associated with decreased leaf water potential only under rapid soil drying. *Physiologia Plantarum*. Doi: 10.1111/ppl.12346

Boyle, R.K.A., McAinsh, M., and Dodd, I.C. 2015. Increased irrigation frequency attenuates xylem abscisic acid concentration and sustains growth in *Pelargonium* x *hortorum* compared to periods of soil drying and re-wetting. *Physiologia Plantarum*. (*Publication, Submitted*)

Boyle, R.K.A., McAinsh, M., and Dodd, I.C. 2015. Frequent soil drying and re-wetting attenuates root ABA concentrations throughout the soil profile, thereby decreasing long-distance ABA signalling in tomato. *Plant, Cell & Environment*. (*Publication, Submitted*).

Stomatal closure of *Pelargonium* × *hortorum* in response to soil water deficit is associated with decreased leaf water potential only under rapid soil drying. *Society of Experimental Biology*. Manchester, July 2014 (*Oral presentation*)

Can altered irrigation frequency increase water use efficiency and plant quality in *Pelargonium* x *hortorum*? Lleida, June 2015 (*Oral presentation*)

Can irrigation frequency modify bedding plant water use and quality? *Association of Applied Biologists*. Knowledge exchange: from research to the food supply chain. Lancaster, June 2015 (*Poster presentation*)

References

Abidi, f., girault, t., douillet, o., guillemain, g., sintes, g., laffaire, m., ben ahmed, h., smiti, s., huche-thelier, l. & leduc, n. 2013. Blue light effects on rose photosynthesis and photomorphogenesis. Plant biology, 15, 67-74.

Alvarez, s., banon, s. & sanchez-blanco, m. J. 2013. Regulated deficit irrigation in different phenological stages of potted geranium plants: water consumption, water relations and ornamental quality. Acta physiologiae plantarum, 35, 1257-1267.

Applegren, m. 1991. Effects of light quality on stem elongation of pelargonium in vitro. Scientia horticulturae. 45: 345-351

Buckley, t. N. 2005. The control of stomata by water balance. New phytologist, 168, 275-291.

Casal, j. J. 2013. Photoreceptor signaling networks in plant responses to shade. In: merchant, s. S. (ed.) Annual review of plant biology, vol 64.

Christie, j. M. 2007. Phototropin blue-light receptors. Annual review of plant biology.

Currey, c. J. & lopez, r. G. 2013. Cuttings of impatiens, pelargonium, and petunia propagated under light-emitting diodes and high-pressure sodium lamps have comparable growth, morphology, gas exchange, and post-transplant performance. Hortscience, 48, 428-434.

Dodd, i. C., egea, g., watts, c. W. & whalley, w. R. 2010. Root water potential integrates discrete soil physical properties to influence aba signalling during partial rootzone drying. Journal of experimental botany, 61, 3543-3551.

Dougher, t. A. O. & bugbee, b. 2004. Long-term blue light effects on the histology of lettuce and soybean leaves and stems. Journal of the american society for horticultural science, 129, 467-472.

Fan, x.-x., xu, z.-g., liu, x.-y., tang, c.-m., wang, l.-w. & han, x.-l. 2013. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. Scientia horticulturae, 153, 50-55.

Fernandes-silva, a. A., ferreira, t. C., correia, c. M., malheiro, a. C. & villalobos, f. J. 2010. Influence of different irrigation regimes on crop yield and water use efficiency of olive. Plant and soil, 333, 35-47.

Fukuda, n., fujita, m., ohta, y., sase, s., nishimura, s. & ezura, h. 2008. Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition. Scientia horticulturae, 115, 176-182.

Heo, j. W., lee, c. W., murthy, h. N. & paek, k. Y. 2003. Influence of light quality and photoperiod on flowering of cyclamen persicum mill. Cv. 'Dixie white'. Plant growth regulation, 40, 7-10.

Islam, m. A., kuwar, g., clarke, j. L., blystad, d.-r., gislerod, h. R., olsen, j. E. & torre, s. 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) lamps. Scientia horticulturae, 147, 136-143.

Ismail, m. R., davies, w. J. & awad, m. H. 2002. Leaf growth and stomatal sensitivity to aba in droughted pepper plants. Scientia horticulturae, 96, 313-327.

Jeong, s-w, das, p.k., jeoung, s.c., song, j-y., lee, h, k., kim, y-k., kim, w.j., park, y.i., yoo, s-d., choi, s-b., choi, g., park, y-i. 2010. Ethylene suppression of sugar-induced anthocyanin pigmentation in arabidopsis. Plant physiology. 154: 1514-1531

Jeong, s. W., park, s., jin, j. S., seo, o. N., kim, g.-s., kim, y.-h., bae, h., lee, g., kim, s. T., lee, w. S. & shin, s. C. 2012. Influences of four different light-emitting diode lights on flowering and polyphenol variations in the leaves of chrysanthemum (chrysanthemum morifolium). Journal of agricultural and food chemistry, 60, 9793-9800.

Kim, t. H., bohmer, m., hu, h. H., nishimura, n. & schroeder, j. I. 2010. Guard cell signal transduction network: advances in understanding abscisic acid, co2, and ca2+ signaling. Annual review of plant biology, vol 61, 61, 561-591.

Liu, x., guo, s., xu, z., jiao, x. & tezuka, t. 2011. Regulation of chloroplast ultrastructure, cross-section anatomy of leaves, and morphology of stomata of cherry tomato by different light irradiations of light-emitting diodes. Hortscience, 46, 217-221.

Loveys, b. R., robinson, s. P. & downton, w. J. S. 1987. Seasonal and diurnal changes in abscisic-acid and water relations of apricot leaves (prunus-armeniaca l). New phytologist, 107, 15-27.

Mccarthy, m. G. 1997. The effect of transient water deficit on berry development of cv. Shiraz (vitis vinifera l.). Australian journal of grape and wine research, 3, 2-8.

Merilo, e., joesaar, i., brosche, m. & kollist, h. 2014. To open or to close: species-specific stomatal responses to simultaneously applied opposing environmental factors. New phytologist, 202, 499-508.

Pantin, f., monnet, f., jannaud, d., costa, j. M., renaud, j., muller, b., simonneau, t. & genty, b. 2013. The dual effect of abscisic acid on stomata. New phytologist, 197, 65-72.

O'carrigan, a., hinde, e., lu, n., xu, x. Q., duan, h. L., huang, g. M., mak, m., bellotti, b. & chen, z. H. 2014. Effects of light irradiance on stomatal regulation and growth of tomato. Environmental and experimental botany, 98, 65-73.

Park, y. G., oh, h. J. & jeong, b. R. 2013. Growth and anthocyanin concentration of perilla frutescens var. Acuta kudo as affected by light source and dif under controlled environment. Horticulture environment and biotechnology, 54, 103-108.

Perez-alfocea, f., ghanem, m. E., gomez-cadenas, a. & dodd, i. C. 2011. Omics of root-to-shoot signaling under salt stress and water deficit. Omics-a journal of integrative biology, 15, 893-901.

Quarrie, s. A., whitford, p. N., appleford, n. E. J., wang, t. L., cook, s. K., henson, i. E. & loveys, b. R. 1988. A monoclonal-antibody to (s)-abscisic acid - its characterization and use in a radioimmunoassay for measuring abscisic-acid in crude extracts of cereal and lupin leaves. Planta, 173, 330-339.

Sampson, j. 1961. A method of replicating dry or moist surfaces for examination by light microscopy. Nature. 191, 932-933.

Sanchez-blanco, m. J., alvarez, s., navarro, a. & banon, s. 2009. Changes in leaf water relations, gas exchange, growth and flowering quality in potted geranium plants irrigated with different water regimes. Journal of plant physiology, 166, 467-476.

Savvides, a., fanourakis, d., & van ieperen, w. 2012. Co-ordination of hydraulic and stomatal conductances across light qualities in cucumber leaves. Journal of experimental botany. 63(3): 1135-1143

Scholander, p. F., hammel, h. T., bradstre.ed & hemmings.ea 1965. Sap pressure in vascular plants - negative hydrostatic pressure can be measured in plants. Science, 148, 339-&.

Shimazaki, k., doi, m., assmann, s. M. & kinoshita, t. 2007. Light regulation of stomatal movement. Annual review of plant biology.

Stutte, g. W., edney, s. & skerritt, t. 2009. Photoregulation of bioprotectant content of red leaf lettuce with light-emitting diodes. Hortscience, 44, 79-82.

Sumanta, n., haque, c.i., nishika, j., & suprakash, r. 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Research journal of chemical sciences. 4(9): 63-69.

Tardieu, f. & davies, w. J. 1992. Stomatal response to abscisic-acid is a function of current plant water status. Plant physiology, 98, 540-545.

Terfa, m. T., solhaug, k. A., gislerod, h. R., olsen, j. E. & torre, s. 2013. A high proportion of blue light increases the photosynthesis capacity and leaf formation rate of rosa x hybrida but does not affect time to flower opening. Physiologia plantarum, 148, 146-159

Wilkinson, s. & davies, w. J. 1997. Xylem sap ph increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. Plant physiology, 113, 559-573.

Wilkinson, s. & davies, w. J. 2002. Aba-based chemical signalling: the co-ordination of responses to stress in plants. Plant cell and environment, 25, 195-210.

Wilkinson, s. & davies, w. J. 2010. Drought, ozone, aba and ethylene: new insights from cell to plant to community. Plant cell and environment, 33, 510-525.

