Effects of deficit irrigation frequency on plant growth, water use and physiology of *Pelargonium* x *hortorum* and tomato (*Solanum lycopersicum* L. cv. Ailsa Craig)

Richard Kevin Alexander Boyle

B. Sc. (Hons) Zoology, University of Dundee, Scotland

MRes Crops for the future, James Hutton Institute, University of Dundee, Scotland

This thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Lancaster University

Lancaster
University

This project was supported by the Centre for Global Eco-Innovation and is part financed by the European Regional Development Fund.

Centre for Global Eco-Innovation

EUROPEAN UNION
Investing in Your Future
European Regional
Development Fund 2007-13

www.cgeinnovation.org

In dedication to my father, Kevin David Boyle

Declaration

I declare that the contents in this thesis are my own work and have not been submitted in the same form for the award of higher degree at any other institution.

Richard Boyle

Lancaster, UK, August 2015

Acknowledgments

I am sincerely grateful to my supervisors Professor Ian Dodd and Dr Martin McAinsh, who provided me with the opportunity to do this PhD, but have given me so much more through their incredible advice, wisdom and expertise throughout. The PhD was challenging at times (both scientifically and logistically), and I could not have achieved it without their support. I feel I have come out of the other end a much improved and rounded scientist.

I would also like to thank all the members of the Plant Physiology Lab group (and beyond) who have provided me with support and guidance on this project, but also much needed laughs and enjoyment! In particular I would like to thank Dr Shane Rothwell who has been patient and helpful for countless problems and questions, as well as those who have given me valuable practical help and advice, including Dr Jaime Puértolas, Dr Antje Fiebig, Joe Fennell, Holly Butler, Nurul Maz Juhairah Manjul, Dennis Touliatos, Dr Rhydian Beynon-Davies, Dr Ros Jones, Dr Annette Ryan and Dr Geoff Holroyd.

I am particularly thankful for having received funding from the Centre for Global Eco-Innovation (CGE) and ERDF, who not only provided the financial support which allowed this research to be carried out, but also valuable training in a wide array of skills that will be useful for my future career. I would also like to thank the Agriculture and Horticulture Development Board (AHDB), who, along with Arden Lea Irrigation Ltd, kindly provided additional funding (PO 017) and an industrial partner respectively at a critical point nine months into my PhD. Many thanks go to Dr Debbie Wilson and Dr Sarah Fairhurst from AHDB and Arden Lea Nurseries Ltd respectively for their horticultural advice, communications and source of seed throughout! I am also grateful to the Faculty of Science and Technology (Lancaster University), who provided me with a student travel grant to attend and present at an international conference in Spain.

I would like to thank all of my family, including my gran, granddad and Dougie for everything they have done for me, which has involved regular visits to Lancaster, and being there for me in Scotland. In particular, my mum has been a continual source of support, as well as inspiring me to follow a career in science, which started me on a path towards my PhD. Finally, I couldn’t have done this without Laurna, who has been caring, supportive and encouraging throughout, as well as providing me with much needed relief from the project.

Abstract

In horticultural sectors where water is a threatened resource, altering irrigation frequency may present a viable approach to reduce water use, without any negative effect on crop yield and/or quality. However, our understanding of the physiological impact of this approach in containers in a peat based substrate is limited. *Pelargonium x hortorum* Bullseye plants were grown in glasshouse conditions under well-watered (WW; daily replacement of 100% of evapotranspiration (ET)), frequent (FDI), or infrequent (IDI) deficit irrigation regimes (50% of ET supplied daily or cumulatively every 4 days, respectively) for four weeks. Both FDI and IDI resulted in short-term increases in water use efficiency, and longer term increases in plant quality (canopy compactness) compared to WW plants. From a physiological perspective, stomatal conductance (g_s) decreased similarly under both FDI and IDI, but there were treatment differences in leaf water potential (Ψ_{leaf}). FDI resulted in a more positive Ψ_{leaf} compared to WW plants, whilst Ψ_{leaf} under IDI was typically the lowest. Given the lack of a consistent response for Ψ_{leaf}, this suggested another mechanism was regulating stomata in *P.hortorum*. Under a single drying cycle, different components of the xylem sap were measured. Xylem sap pH, Ca^{2+} and NO_3^- did not change, but the plant hormone abscisic acid (ABA) increased in the xylem sap ($[X\text{-}ABA]_{leaf}$) under both irrigation treatments as soil moisture decreased, and showed a strong relationship with g_s both *in vivo* and in a detached leaf transpiration bioassay. However, when plants were irrigated daily at a percentage of daily ET (adapted from FDI), plants showed an attenuated ABA response compared to when irrigation was withheld (adapted from IDI). It was hypothesised that this may have been a root-derived response due to spatial variation in soil moisture distribution, which was investigated in tomato (*Solanum lycopersicum*). Similar results were found where g_s decreased as $[X\text{-}ABA]_{leaf}$ increased, but again the ABA response was attenuated. Furthermore, stomata showed similar sensitivity to ABA under both irrigation treatments. However, similar results were found for root tissue ($[ABA]_{root}$) and xylem ($[X\text{-}ABA]_{root}$) ABA, and modelling revealed that both localised root water uptake and soil moisture content are important for explaining the variation in $[X\text{-}ABA]_{root}$ between irrigation treatments. This research furthers the fundamental understanding of ABA signalling and suggests that irrigation frequency can be altered for a short period of the growing cycle to deliver specific grower objectives.

Publications arising from this work:

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*. (*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*. (*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*. (*Submitted*).

Oral/poster presentations from this work:

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*)

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

Improving resource use efficiency in bedding plants through alternative irrigation and lighting. *Agriculture and Horticulture Development Board*, Studentship Conference. Droitwich Spa, September 2015 (*Oral & Poster*)

Root zone CO_2 enrichment: A suitable approach to vegetable production? *Horticultural Development Company*. Studentship Conference. Pershore, September 2013 (*Poster*)

Stomatal closure of *Pelargonium* × *hortorum* in response to soil water deficit is associated with decreased leaf water potential only under rapid soil drying. *Horticultural Development Company*. Studentship Conference. York, September 2014 (*Poster*)

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*)

Table of Contents:

Page

Chapter 1 – General Introduction

Chapter 2 – Decreased irrigation frequency in *Pelargonium* x *hortorum* can reduce water use, maintain leaf growth and anthocyanin concentrations, and increase canopy compactness

Chapter 3 – Stomatal closure of *Pelargonium* × *hortorum* in response to soil water deficit is associated with decreased leaf water potential only under rapid soil drying

Chapter 4 – Frequent soil drying and re-wetting attenuates root ABA concentrations throughout the soil profile, thereby decreasing long-distance ABA signalling in tomato

List of Figures

Page

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Tables

Page

Chapter 2

Chapter 3

Chapter 4

Abbreviations

A - Photosynthesis

ABA – Abscisic acid

$[\mathrm{ABA}]_{\mathrm{leaf}}$ – Leaf tissue abscisic acid concentration

$[\mathrm{ABA}]_{\mathrm{root}}$ – Root tissue abscisic acid concentration

g_s – Stomatal conductance

E - Transpiration

ET – Evapotranspiration

FDI – Frequent deficit irrigation

IDI – Infrequent deficit irrigation

PRD – Partial root-zone drying

r – Hydraulic resistance

RDI – Regulated deficit irrigation

RWU – Root water uptake

RZ – Root-zone layer

SDI – Sustained deficit irrigation

TR – Transpiration rate

VPD – Vapour pressure deficit

WUE – Water use efficiency

$\mathrm{WUE}_{\mathrm{plant}}$ –Whole plant water use efficiency

$\mathrm{WUE}_{\mathrm{leaf}}$ – Leaf level water use efficiency (A/g_s)

WW – Well-watered

$[\mathrm{X\text{-}ABA}]_{\mathrm{leaf}}$ – Leaf xylem abscisic acid concentration

$[\mathrm{X\text{-}ABA}]_{\mathrm{root}}$ – Root xylem abscisic acid concentration

θ_{pot} – Gravimetric water content

Ψ_{leaf} – Leaf water potential

Ψ_{root} – Root water potential

Ψ_{soil} – Soil water potential

Ψ_w – Water potential

Chapter 1 - Introduction

1.1 Why is water management within the agricultural sector important?

Horticulture, and agriculture as a whole, faces a number of different challenges. There is widespread famine, and yet the world population is expanding rapidly, expected to reach approximately 9 billion by 2050 (Pardey et al., 2014). There is also the issue of producing plants in an ever changing environment, with pressures of climate change and reduced water availability. Many of the current methods within agriculture often involve extremely intensive management regimes, looking to maximise outputs by applying high resource inputs. This includes extensive applications of nutrient fertilisers and herbicides, as well as inefficient methods of irrigation (Conway and Barbier, 2009).

Agricultural water withdrawals for irrigation typically exceed crop water demands. This can be due to the large losses through transport, storage and utilization of water (Frenken and Gillet, 2012), which may be damaging for the surrounding environment. For instance, excessive groundwater withdrawal has resulted in depletion of the water table (in some sites by over 100 metres) in areas such as the North China Plain and the High Plains Aquifer in the USA (Dennehy et al., 2002, Changming et al., 2001). Whilst theoretically these aquifers are “renewable resources”, these water bodies are slow to replenish via rainfall (Gleeson et al., 2012), and current rates of water extraction seem unsustainable. The focus should therefore be on developing sustainable irrigation strategies, with the aim of preventing unnecessary over-irrigation (Gheysari et al., 2009), or minimising water inputs in arid or semi-arid regions. Understanding how these proposed practices impact upon different species of plants at a morphological, physiological and genetic level can have significant impacts upon the way in which we design and implement irrigation (Dodd, 2009).

1.2 The ornamental industry and water use

Water shortage for irrigation is a problem that faces most cropping sectors, and it will be important for these industries to develop strategies to adapt. One area that has received relatively little attention so far is the ornamental nursery sector (Miralles Crespo et al., 2010). In this industry, the vast majority of plants are grown in containers. This means plants have a limited rooting zone, making them susceptible to drought stress if under-watered. Consequently, this leads to growers often over-irrigating their crops, which can limit plant growth (Fiebig and Dodd, 2015). Furthermore, accurate and scheduled irrigation regimes are rare, with a huge number of nurseries using inefficient, overhead sprinkler systems (Briercliffe et al., 2000). With water requirements for the ornamental sector likely to increase (Grant et al., 2011), but greater competition for the resource, this has led to a gradual change in thinking.

Many growers are now focused on implementing irrigation systems that are more efficient (Cameron et al., 2008), such as replacing overhead systems with drip irrigation (Goodwin et al., 2003), both for the environmental and financial incentives. One general complication in progressing with alternative irrigation approaches is an often widespread lack of understanding as to the water relations and requirements of the plants. Thus growers have a limited knowledge as to the impact of altering water availability on plant productivity and quality. Ultimately, this makes it essential to carry out further research to be able to further educate the grower community.

One area that stands out within the ornamental sector is the production of bedding plants. Bedding plants are extremely popular with the general public, and are considered valuable within the UK and worldwide – in 2012-2013 (Figure 1.1), 26% of the entire UK ornamental industry value consisted of bedding plants (Denny and Dimmock, 2014). Some of the most common bedding plants include marigold (*Tagetes* spp.), pansy (*Viola* spp.) and geraniums (*Pelargonium* spp.). Growers of bedding plants face an unpredictable and erratic market, which requires careful planning and strategies to ensure high quality plants are produced within a tight timeframe (HTA, 2012). However, this area of horticulture presents an opportunity to significantly improve water savings (to expand bedding plant production, or to allocate water to other areas of agriculture) along with additional economic benefits.

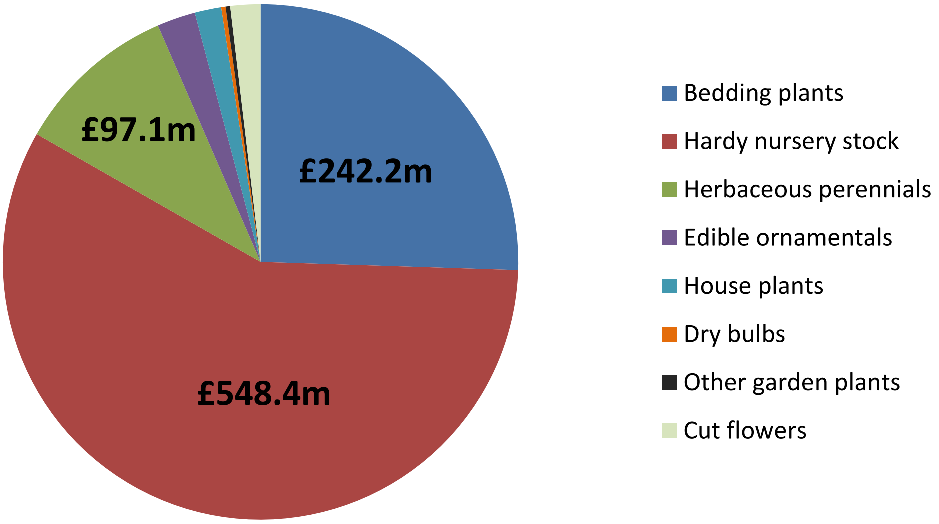


Figure 1.1. Estimated value of UK ornamentals production by crop and sector (July 2012-July 2013). Values of the three most profitable industries are included (£ million). Figure adapted from Denny and Dimmock (2014).

Pelargonium

The *Geraniaceae* is an ornamental, bedding plant family that includes up to eleven genera, and around 750 species, with the *Geranium* and *Pelargonium* genera the most popular as colourful and prolific flowering garden plants (Ávila et al., 2013, Jones et al., 2001). *Pelargonium* are annual, and herbaceous perennial plants that have been bred for a wide variety of leaf colours and shapes, good consumer quality and disease resistance. The most cultivated of the pelargoniums are *Pelargonium zonale* hybrids and *Pelargonium peltatum* (García-Sogo et al., 2012). The zonal geranium *Pelargonium x hortorum* is particularly popular amongst gardeners, due to vibrant flowers and a dark ‘zonal’ band on an otherwise green leaf. *P.hortorum* has also been shown to be drought-tolerant (by withstanding periods of drought stress), making it an ideal candidate for alternative irrigation strategies (Álvarez et al., 2013, Sánchez-Blanco et al., 2009). Despite this, the mechanisms behind this drought tolerance are less well understood.

1.3 Alternative strategies for irrigation management?

To address issues of water availability for agricultural use will require a drastic rethink in approaches to irrigation management and scheduling on a global scale. Conventional irrigation practices of container-based ornamental plants often involves high frequency irrigation events to prevent excessive soil drying given the limited rooting zone (Beeson, 1992), which can be unpredictable if environmental conditions vary. This can be further complicated if growers use outdated approaches to assess water availability (such as feeling how dry the soil is by hand), which is inaccurate and does not account for the water status of the plant. Furthermore, it is still common for ornamental growers to use overhead sprinklers which are inefficient for irrigation placement and thus consume large volumes of water (Cameron et al., 2008). Increasing pressure from consumers, competition from other industries for water availability, and rising water prices has led to a change in the attitude of growers to begin adopting more sustainable approaches to irrigation (Knox et al., 2007).

1.3.1 Deficit irrigation

One strategy that has been considered a potential, sustainable alternative approach to conventional irrigation management is ‘deficit irrigation’. Deficit irrigation is the application of water at a lower rate and/or volume than the plants evapotranspirative (ET) demand (Álvarez et al., 2013). In theory this may limit yield, but will reduce the volume of water used (Geerts and Raes, 2009). In water limited situations this is a particularly useful approach for farmers to increase the productivity of water use (as opposed to maximising yield) of the plant (Fereres and Soriano, 2007).

As an alternative water management technique, deficit irrigation has been applied to a variety of crops. Perhaps the greatest success has been found in tree and vine crops (Kirda et al., 2004), where reductions in overall biomass are less critical than many field grown crops (Jensen et al., 2010). In some species, a period of drought stress can enhance allocation of resources to reproduction, leading to early flowering (Kozlowski and Pallardy, 2002) and increased fruiting (Nora et al., 2012). Thus, deficit irrigation has had positive effects (in terms of water productivity (biomass produced per volume of water)) on a variety of species, including wheat, sunflower and potato (Ali et al., 2007, Karam et al., 2007, Liu et al., 2006a). If implemented correctly, the major benefit of deficit irrigation is improved plant water use without significant loss of yield (Grant et al., 2011), but plant quality may also be enhanced (e.g. fruit with higher sugar, compact ornamental plants) and greater control over growth can be achieved.

'Sustained' deficit irrigation (SDI) involves the gradual imposition of a soil water deficit over the course of a season or growing period (Fereres and Soriano, 2007). Further modifications to this approach include 'regulated' deficit irrigation (RDI). This involves the application of deficit irrigation at specific stages of development during the growing cycle (Kang et al., 2000). This may occur during periods of slow growth, or growth stages in which species are less susceptible to water stress. This approach was first proposed for use in peach orchards, which reduced shoot growth without negatively impacting upon fruit yield or quality (Chalmers et al., 1981). Maintaining irrigation at normal levels during higher risk periods prevented excessive losses of (or even improves) yield or crop quality (Wakrim et al., 2005). For instance, in winegrape RDI can improve taste and berry quality, and increase water productivity (Romero et al., 2013, dos Santos et al., 2007). In pistachio, RDI applied at certain stages of growth resulted in increases in yield along with water savings, which enhanced water productivity (Goldhamer and Beede, 2004).

Applying deficit irrigation to bedding plant species is of interest as losses in overall yield aren't critical, and reduced vegetative growth can improve the ornamental value (Cameron et al., 2008). This potentially allows greater flexibility to implement these strategies, provided there aren't overall losses in quality. Growers will often favour smaller plants which are considered ornamentally more attractive, and are easier to transport without significant damage. Economically this is important, as avoiding excessive growth may increase the value of the plant (Álvarez et al., 2009), whilst reducing the use of chemical growth regulators (Clifford et al., 2004). Deficit irrigation may also enable the plant to tolerate periods of drought at later stages, including distribution in the supply chain (Cameron et al., 2008). For instance, recent work has focused on studying drought-tolerant ornamental plants for sustainable landscape gardening, often in arid environments (Toscano et al., 2014, Franco et al., 2006).

Deficit irrigation can potentially enhance the quality of ornamental crops by decreasing vegetative extension, producing more compact plants (Cameron et al., 2006). This can be dependent upon the severity and timing of the deficit irrigation treatment. Large reductions in soil water availability can be damaging to plant development, affecting aspects such as flowering, leaf pigmentation and plant size (Mieszkalska and Lukaszewska, 2011, Cameron et al., 2008, Sánchez-Blanco et al., 2009, Álvarez et al., 2013), and hence it is important to understand the water requirements of individual species to optimise the irrigation strategy.

For instance, in bed-grown *Petunia* and *Impatiens*, a soil water deficit (allowing pot weight to decrease by 45% before re-irrigating) resulted in non-significant reductions in plant growth and flowering (Andersson, 2011). Significant reductions in growth and flowering were observed in the same species by irrigating the plants at 25 % ET (Blanusa et al., 2009) and in growth of *Cotinus* and *Forsythia* irrigated at 50 % ET (Cameron et al., 2008). When irrigation was applied at either 60% or 30% of soil water content however, no change was found in growth or flowering in *Pelargonium* (Chyliński et al., 2007). Root growth in *Impatiens* and *Pelargonium* significantly increased in both species at the lowest soil moisture availability (through enhanced root proliferation), potentially alleviating the stress conditions (Chyliński et al., 2007). In *Dianthus*, plant growth decreased as the intensity of soil water deficit increased (in plants receiving 70% and 35% of irrigation provided to control plants), but plants only showed a reduction in flower number and floral quality under the most severe soil moisture stress (Álvarez et al., 2009). Perhaps the biggest challenge for growers is how to apply these deficit irrigation techniques successfully on a commercial scale.

In *Rhododendron*, RDI applied after floral initiation promoted greater flowering, but can inhibit development of flowering if applied too early (Sharp et al., 2009). In contrast, both mild and severe (60% and 40% of water applied to control plants respectively) soil water deficits significantly reduced flowering in *Phlomis purpurea*, whilst plant growth and development was also reduced (Álvarez et al., 2012). Water consumption by Rhododendron was more than halved by applying deficit irrigation at 75 % ET, without any decline in growth (Cameron et al., 1999). In rose plants, deficit irrigation decreased the number of floral stems, but did not impact upon plant quality and increased water productivity (Bolla et al., 2010). The above work highlights that by adapting a suitable deficit irrigation strategy, there can be significant water savings whilst also improving the ornamental quality (and thus increased value) of the plants.

1.3.2 Irrigation frequency

Limiting irrigation frequency is an alternative approach which attempts to further control water use, reduce irrigation inputs and maintain plant yield and quality. Irrigation frequency strategies are most commonly utilised in arid and semi-arid regions where water is particularly scarce or there are economic constraints which may limit irrigation water quality (Oktem et al., 2003). However, similar strategies have been adopted in other areas such as containerised production (Beeson, 2006, de Matos Pires et al., 2011). These strategies involve delaying the application of water, typically in an attempt to regulate transpiration, limit excessive loss of water through evaporation or through leaching, and to regulate water inputs in situations where growers over-irrigate plants (Wang et al., 2001).

Limiting irrigation frequency requires a collective understanding of its impacts on both plant growth and water use, particularly at a physiological level, the latter of which has perhaps been undervalued. Environmental variation can impact significantly upon plant ET demands (Askri et al., 2014), which requires flexibility when irrigation frequency is reduced. Specifically, increased atmospheric vapour pressure deficit (VPD) causes a higher rate of transpiration, which may lead to water stress and a reduction in growth due to the inability of the plant to sustain water uptake (Leonardi et al., 2000, Grange and Hand, 1987, Clifton-Brown and Jones, 1999). Furthermore, the substrate water holding capacity can affect the required frequency of irrigation. For instance, in soils that are particularly susceptible to drying, a higher irrigation frequency may be required to prevent excessive moisture depletion (Howell et al., 1997). Altering the frequency of irrigation may also have longer term consequences on soil properties such as causing hysteresis of the soil moisture release curve, impacting upon microbial communities and influencing gas fluxes (Abalos et al., 2014, Holland et al., 2014).

As these strategies will often be applied in water scarce situations, improving water productivity of crops is often a key aim, but inconsistent responses are reported within the literature. Of those studies where comparable volumes of irrigation were applied, plant water productivity (which typically reflected increases or decreases in yield) increased with limited irrigation frequency in maize (El-Hendawy and Schmidhalter, 2010, Mbagwu and Osuigwe, 1985) and cucumber (Wang et al., 2009), and decreased in summer squash (Ertek et al., 2004), potato (Wang et al., 2006) and sorghum (Saeed and El-Nadi, 1998). Inconsistent results were found in bell pepper, which showed either minimal change (Assouline et al., 2006) or larger decreases (Sezen et al., 2006) in water productivity (due to higher irrigation frequency in the former study). Authors of the studies in which water productivity increased as irrigation frequency decreased suggest that this is a consequence of optimal soil moisture conditions being achieved (i.e. plants were not over-watered), whilst a decrease in water productivity may be attributed to plants being subject to too severe a period of stress.

This variation highlights that limiting irrigation frequency can affect species differently, and in some situations may not be suitable. Improved physiological understanding (which appears limited in the irrigation frequency literature) may provide more insight for applying this sort of a strategy on a wider basis.

1.4 Plant responses to soil water deficits

1.4.1 Plant growth

In response to a soil water deficit, plants will show changes in growth. Reduced soil moisture availability will affect many processes, including the uptake and transport of water, nutrients and plant hormones, which will impact on plant growth and physiology (Munns, 2002). The most consistent response on plant growth is a reduction in cell division and expansion (Tardieu et al., 2000), which is often a consequence of reduced turgor pressure and/or cell wall extensibility (Blum, 2011). In turn, this decreases cell size and number, and thus limits leaf expansion. This hydraulically driven process is typically due to a decrease in water status throughout the plant (originating at the roots), and/or a decrease in root hydraulic conductivity (Ehlert et al., 2009, Tardieu et al., 2012). Initial reductions in leaf expansion often also precede any decrease in photosynthetic activity (Blum, 2011, Ashraf and Harris, 2013).

Despite the hydraulic argument above, there is evidence for non-hydraulic regulation of plant growth. In wheat plants where leaves were maintained at full turgor via pneumatic pressure applied on the roots, leaf expansion rate continued to decrease in response to soil drying, suggesting the presence of a root-source signal (Passioura, 1988). Furthermore, in maize plants, leaf expansion was found to decrease without a decrease in leaf water status, suggesting a role for an inhibitor transported in the xylem stream (Saab and Sharp, 1989). It has also been shown that the plant hormone abscisic acid (ABA) may have a role in regulating leaf growth (Bacon et al., 1998), although in contrast there is evidence of growth-promoting role for ABA (Tardieu et al., 2012). Evidence for both hydraulic and non-hydraulic regulation of plant growth suggests there is large species-specific variation.

Ultimately, shoot growth will likely be reduced to prevent unnecessary use of resources and energy (Chaves and Oliveira, 2004), and to limit the total leaf area for water loss (Savé et al., 1994). Resource allocation for root growth (to increase capacity for water uptake) usually increases at the expense of shoot production, impacting upon the root:shoot ratio (Stagnari et al., 2014).

1.4.2 Plant water use

Deficit irrigation can potentially reduce the volume of water required by (and thus applied to) the plant, without any significant declines in yield (Fereres and Soriano, 2007). In areas where water is scarce (e.g. arid or semi-arid regions), or if the target is to improve the sustainability of irrigation practices, this can lead to an increase in water use efficiency (WUE), or '*more crop per drop*' (Cifre et al., 2005). Rather than maximising productivity per cropping area, growers target an increase in productivity with regards to water availability. This approach requires careful management to prevent excessive losses in growth or quality that outweigh the benefit of reducing water inputs.

The concept of WUE can be defined by the specific targets of the grower. For instance, at the whole plant level (WUE_{plant}; biomass of crop produced per unit of water used), this may be focused towards the harvestable biomass of the plant (Blum, 2005), or at the physiological level, which is the ratio of photosynthesis (A) to transpiration (E) (Hatfield et al., 2001). Measuring WUE_{plant} provides a simple means to assess the plants water productivity over an entire growing period. Understanding how plants regulate the balance between water loss and carbon gain through stomatal control, particularly under certain stress conditions, is often a key aim for improving WUE (Easlon et al., 2014). Despite this, a physiological estimate of WUE at the leaf level (WUE_{leaf} - A/E) may fail to account for temporal variation over a long growth period, and in many cases measurements at the leaf level are not representative of whole plant measurements (Tomás et al., 2012). Therefore, physiological improvements in WUE_{leaf} may not necessarily translate to increased whole canopy/yield WUE (Wilkinson et al., 2012).

Deficit irrigation induces different changes in WUE_{plant} depending on the ornamental species: it increases in *Callistemon* (Álvarez and Sánchez-Blanco, 2013) and *Catharanthus* (Jaleel et al., 2008) as a measure of plant biomass to water use (due to more efficient stomatal conductance (g_s)); decreases in *Pelargonium capitum* due to a reduction in essential oil yields (Eiasu et al., 2012); and has no effect in *Pelargonium zonale* as dry matter was proportional to irrigation inputs (Andersson, 2001). These findings are typically a consequence of the balance between stomatal closure limiting water loss and carbon assimilation.

1.4.3 Physiological adaptations

Many of the morphological responses under deficit irrigation are a result of alterations in the physiology of the plant to counter periods of reduced water availability. These include stomatal closure and reduced transpiration that will prevent further water loss, but may also limit photosynthetic activity (Flexas and Medrano, 2002). Stomata are pore like structures found on plant leaves, consisting of two guard cells surrounding the stomatal pore (McAinsh et al., 1990). Stomata are the primary structure regulating water loss from the plant via transpiration, and to allow entry of CO_2 to drive photosynthesis. Transpiration is the process in which water (and subsequently nutrients) is transported along gradients through the plant via the xylem to the leaves. This mechanism cools the leaves, and its rate is affected by a number of structural (stomatal density, leaf anatomy) and environmental (temperature, water availability, light) factors (Collatz et al., 1991). Stomatal closure is one of most widely observed responses to drought, acting to prevent excessive water loss, and to maintain turgor pressure and the water status of the plant at non-lethal levels (Brodribb et al., 2003).

Reductions in stomatal aperture are brought about through two mechanisms which can act in concert. Active control is via a series of cascading cellular-biochemical events, typically induced by antitranspirant phytohormones (Acharya and Assmann, 2009, Wilkinson and Davies, 2010). A second mechanism involves passive regulation of guard cell turgor through changes in water potential within the leaf (Franks, 2013). Longer term exposure to a stress can typically lead to reduced stomatal development (including stomatal number and density) in younger leaves (Hamanishi et al., 2012).

Plants can be described as homeohydric, in that they are able to regulate a water-balance within tissue (Buckley, 2005). The general response to reduced soil water availability however can be typically classed as either avoidance (isohydric; maintained leaf water status) or tolerance (anisohydric; reduced leaf water status), or occasionally a combination of the two (Gallé et al., 2013). Avoidance mechanisms can include larger and deeper roots, enhanced stomatal closure, reductions in transpiration, adaptive morphological characteristics (such as leaf hairs (trichomes)) and alterations in plant architecture (for example, a reduced leaf area). These functions act to maintain an internal water status, even under stress conditions (Sade et al., 2012). On the other hand, mechanisms to allow tolerance to water deficits can involve maintaining turgor potential through osmotic adjustment, altering the shoot-root ratio, and changes in cellular elasticity (Touchette et al., 2007).

Any irrigation strategy that withholds water will reduce soil water availability, altering the soil moisture content and potentially affecting physiological processes, thus requiring careful management (Cameron et al., 2008). In response to soil water deficits, hydraulic signals are a result of increased xylem tension (Comstock, 2002), but also active chemical signals, often before the hydraulic signal as a pre-emptive alert for the plant (Goodger et al., 2005). In doing so, the plants can potentially improve water productivity by maintaining leaf water status, and decreasing transpiration (Eiasu et al., 2012, Garcia-Navarro et al., 2004).

1.5 Plant signalling under soil water deficits

The impact of soil water deficits on plant growth and physiology is affected by direct alterations in crop water relations (hydraulic signalling), but also through chemical signalling within the plant (Dodd, 2005). Plants use a wide range of chemical signals in response to water stress (such as plant hormones, including ABA), and the mechanistic understanding of these signals is currently subject to a considerable amount of research and debate (Wilkinson et al., 2012).

1.5.1 Hydraulic signalling

Hydraulic signals were initially proposed as being central in long distance regulation of stomata in response to soil drying (Comstock, 2002). Changes in water potential (Ψ_w) can be transmitted throughout the plant via the xylem (Fig 1.2), which is influenced by the decrease in soil water potential (Ψ_{soil}) as soil moisture decreases (Ritchie, 1981). Within the plant, decreased Ψ_w initially leads to a loss of turgor (passive cellular dehydration), which may be followed by a longer term increase in osmotic potential (through active increases in solute concentration (albeit there is considerable variation in this, with a limited role in some species)), and a change in water tension, which is detected by the plant (Christmann et al., 2013). Root water potential (Ψ_{root}; Fig 2.1) is highly dependent upon soil hydraulic conductivity and root density (Caldeira et al., 2014). When Ψ_{soil} decreases, or there is an increase in vapour pressure deficit (VPD), this will result in a series of drops in Ψ_w throughout the plant (Buckley, 2005, Pantin et al., 2012, Peak and Mott, 2011). Hydraulic failure occurs when the plant is unable to move water due to xylem vessel cavitation leading to embolism (Barigah et al., 2013, Tyree and Sperry, 1989). This is a consequence of the rate of transpiration exceeding the plants critical level of Ψ_w, or leaf hydraulic conductance reaching zero (McDowell et al., 2008).

Decreased Ψ_{leaf} can be sensed by the stomata (through a loss of turgor by the guard cells), which will act to regulate this decrease by adjusting stomatal aperture (Sperry et al., 2002), but in turn, maintenance of stomatal opening is dependent upon high Ψ_{leaf} (Sack and Holbrook, 2006). This suggests a negative feedback mechanism (Buckley, 2005), where plants will act to prevent xylem embolism (Cochard, 2002), and may further adjust Ψ_{leaf} to limit cell expansion and leaf growth. A series of experiments that applied a pneumatic pressure to the roots of plants grown in drying soil increased Ψ_{leaf} and caused stomatal opening in bean (Mencuccini et al., 2000), *Hymenoclea salsola* (Comstock and Mencuccini, 1998), Douglas fir and alder (Fuchs and Livingston, 1996).

Furthermore, although some species can show an initial hydraulic response (Rodrigues et al., 2008), there is often still the requirement of ABA to regulate stomatal closure (Wilkinson and Davies, 2002), whilst Ψ_{leaf} can enhance stomatal sensitivity to ABA (Tardieu and Davies, 1992), although this is not a universal response (Tardieu and Simonneau, 1998). In *Arabidopsis* exposed to osmotic stress (osmotic stress medium at Ψ_w = -1.0 MPa) around the roots, rapid stomatal closure occurred in response to hydraulic signalling, with ABA being synthesised in the leaves after an initial drop in cell turgor pressure (Christmann et al., 2007). These results are in contrast to findings in wheat and sunflower where plants that were maintained at full turgor (xylem sap on the verge of bleeding from a cut surface) under drying soil (by applying pneumatic pressure to the roots) showed stomatal closure, indicating other root sourced signals (Gollan et al., 1986).

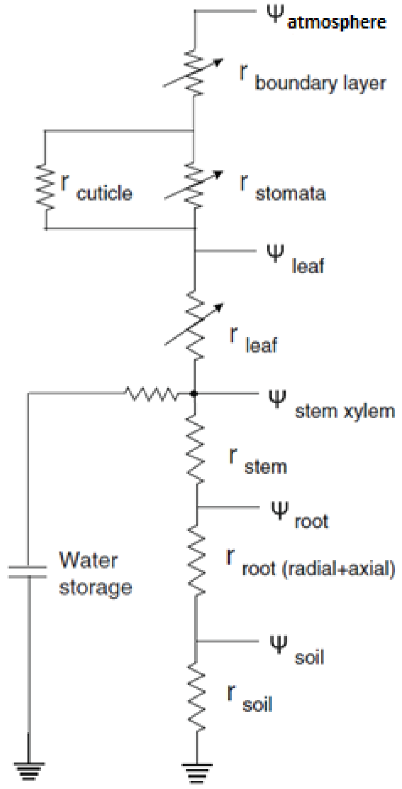


Figure 1.2 Resistance diagram of soil-plant-atmosphere continuum showing water potentials (Ψ) and hydraulic resistance (r). Figure modified from Blum (2011).

Stomatal closure without a decrease in leaf turgor was also found in plants exposed to partial root-zone drying (PRD; laterally irrigating half of the root, whilst irrigation is withheld from the other half) in apple (Gowing et al., 1990), sycamore (Khalil and Grace, 1993) and grape (Stoll et al., 2000). These findings have contributed to the debate by highlighting that ABA can be synthesised and transported from the roots without any reduction in Ψ_{leaf} (due to half of the roots receiving full irrigation). In addition, in certain studies stomata were found to close in response to a soil water deficit without a change in leaf water status (Bates and Hall, 1981), or prior to decreased Ψ_{leaf}, indicating a primary role for chemical signalling in some species. In grafted tomato plants, stomata closed in response to soil drying which was regulated by shoot-sourced ABA, without a change in leaf water status (because of root pressurisation; Holbrook et al., 2002). Recently it has been highlighted that leaf vascular tissue hydraulic conductance can be reduced by ABA, which also plays a role regulating stomatal closure (Pantin et al., 2013).

1.5.2 Chemical signalling

The work above focused on chemical signalling, along with early research which highlighted the significance of the plant hormone ABA as a root-to-shoot signal (where root ABA accumulated prior to a decrease in Ψ_{leaf}; Zhang et al., 1987, Zhang and Davies, 1989b), challenged the traditional view of hydraulic signalling regulating stomatal closure as the soil dried. A root-to-shoot signal (Fig 1.3) travels apoplastically or symplastically from the roots (the first site to sense a decline in soil moisture status) to the shoots, and induces a physiological response in a region remote from the site of synthesis (Dodd, 2005).

As such, a wide array of chemical signals has since been shown to have a role in the signalling response of plants to soil drying. This includes a prominent role for ABA as discussed, but other hormones may include the ethylene precursor ACC (1-Aminocyclopropane-1-carboxylic acid), gibberellins, jasmonates and cytokinins, for example (not discussed below; however, ABA will be used as an example of a root-to-shoot signal, to demonstrate supporting evidence of its significance). The transport and effect of these signals can act as direct stress regulators, or may interact with other hormones and compounds to modulate sensitivity of stomata, for example (Jia and Zhang, 2008). Further components of the xylem sap shown to have important roles in signalling, and may also interact with plant hormones, include nutrients and xylem sap pH. Drought stress typically decreases the uptake of nutrients by the roots and the subsequent loading of nutrients into the xylem, which may serve as a stress indicator at the shoot (Pérez-Alfocea et al, 2011).

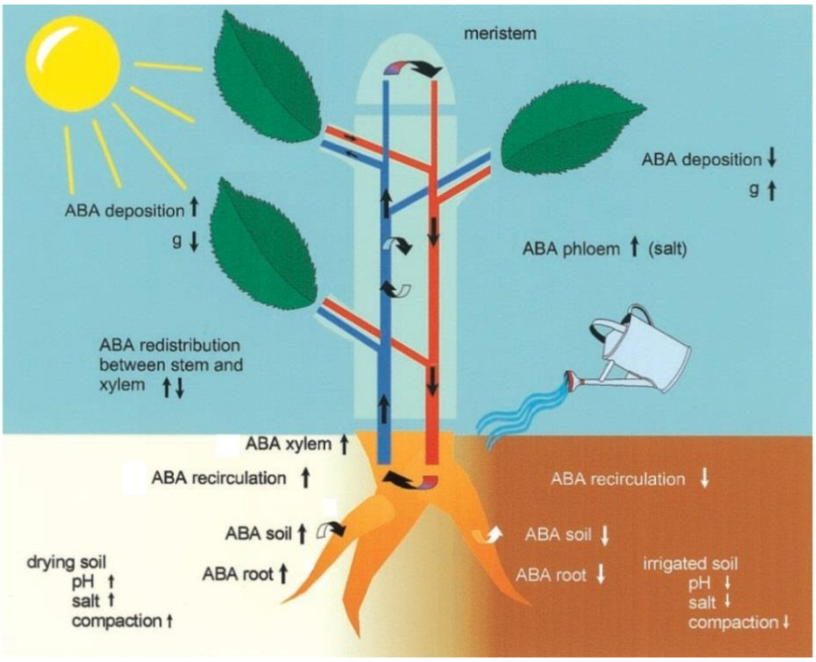


Figure 1.3 ‘Root-to-shoot’ signalling diagram showing the synthesis of ABA in the roots in response to drying soil, subsequent transport to the shoot via the xylem, effect on stomatal conductance (g), and recirculation within the phloem. Figure modified from Sauter et al. (2001).

1.5.3 Abscisic Acid

ABA is a sesquiterpenoid that is a product of the synthesis of carotenoid precursors (Nambara and Marion-Poll, 2005). An important plant hormone, it is widely accepted as one of the key, long-distance signals involved with various stress responses (for example drought and salt stress), but also has roles in developmental processes. These include cell division, seed maturation, and seed dormancy, germination and leaf growth rate, as well as regulating stomatal conductance, root development and canopy expansion (Wilkinson and Davies, 2010, Acharya and Assmann, 2009).

ABA has a central role in plant responses to water stress (Zhang and Davies, 1991, Cutler and Krochko, 1999, Nambara and Marion-Poll, 2005). Catabolism of ABA involves hydroxylation and conjugation as metabolic pathways, which occurs rapidly to ensure high turnover of the hormone, particularly when stress situations have been alleviated (Nambara and Marion-Poll, 2005). The function of ABA as a stress hormone is dependent upon the plant’s ability to regulate biosynthesis and degradation in parallel (Zhang et al., 2006).

ABA can also be stored in vacuoles and apoplastically in an inactive form, ABA glucose ester (ABA-GE). The glucose ester is cleaved by β-glucosidase to release ABA under drought stress (Nambara and Marion-Poll, 2005, Lee et al., 2006, Hirayama and Shinozaki, 2010). ABA-GE may act in long distance ABA transport (as it is more effectively transported than the un-conjugated form, due to its resistance to ABA 8’-hydroxylase), with evidence of increased ABA-GE in the xylem sap of drought stressed plants (Schachtman and Goodger, 2008, Sauter et al., 2002).

Regulating stomata is an important function under stress conditions, and ABA has two distinctive roles – i) preventing stomatal opening; and ii) maintaining stomatal closure (Kim et al., 2010), with the ultimate goal of preventing excessive water loss. It is known that at elevated concentrations under stress conditions, ABA acts upon stomata by binding to receptors (many of which are thought to be cytosolic (Guo et al., 2011)). This can induce a signal transduction cascade in the stomata which leads to an influx, or redistribution, of Ca^{2+} in the cytoplasm of the cell (McAinsh et al., 1990), preventing stomatal opening by decreasing osmotic potential of the guard cells (Assmann and Shimazaki, 1999, Wilkinson et al., 2001). Alternatively, stomatal closure may be initiated through ABA mediated K^+ efflux via the plasma membrane, which reduces the turgor of the guard cells (Chen et al., 2012).

The original hypothesis for ABA action in the leaves was that a change in Ψ_{leaf} released ABA from the leaf mesophyll (Loveys, 1977), and that leaf ABA biosynthesis may increase in response to a loss of leaf turgor (Pierce and Raschke, 1980). However, subsequent work led to ABA being re-defined as a root-derived, and subsequent 'root-to-shoot' signal. Drought-stressed roots can act as the primary site for transport to the aerial parts of the plant (Zhang and Davies, 1987), and in drought stressed *Phaseolus*, ABA increased in the roots prior to an increase in the xylem sap or leaves (Trejo and Davies, 1991). When exposed to a soil water deficit, sunflower showed elevated synthesis of ABA within the roots, which was suggested as being essential in the initial increase in ABA within the xylem sap (Neales and McLeod, 1991). In *Commelina* grown under PRD, ABA only increased in roots under drying soil, whilst those that were irrigated showed minimal change in ABA (Zhang et al., 1987).

Establishing whether or not root sourced ABA was transported to the shoot (and subsequently acted upon stomata) was a critical step. In *Commelina*, it was shown that dehydrated roots loaded with externally supplied ABA increased leaf ABA concentrations and induced stomatal closure (Zhang and Davies, 1987). These findings were supported by work in sunflower which showed that root ABA export increased under soil drying and significantly increased xylem ABA concentrations, which corresponded with decreasing g_s (Shashidhar et al., 1996). In *Ricinus communis* L., ABA delivery rate (the product of xylem ABA concentration and sap flow rate) was increased 5-fold in response to drought, demonstrating an active response (root ABA biosynthesis and xylem loading) that was not simply a function of reduced sap flow (Jokhan et al., 1996).

However, work has often challenged the idea of ABA primarily being a root sourced signal. Firstly, key studies showed that ABA can be synthesised in the leaves in response to soil drying (Zhang and Davies, 1989b, Zhang et al., 2006). Research with grafted tomato plants strengthened this argument. When water was withheld, stomata closed in the absence of either a root derived ABA source or a reduction in Ψ_{leaf} (maintained under artificial pressure) in wild-type (WT) tomato scions grafted to either *flacca* or *sitiens* (ABA deficient) mutant rootstocks (with ABA either very low or often undetectable in well-watered *sitiens* plants) (Holbrook et al., 2002). The authors therefore attribute increased leaf ABA concentrations to another root derived signal. Additional support for this was that root genotype did not affect soil-drying induced stomatal closure when both sunflower and tomato WT shoots were grafted onto either WT or ABA-deficient roots (Fambrini et al., 1995, Jones et al., 1987).

In *Arabidopsis* it was found that during water stress, ABA was mainly synthesised in the leaves, and some of the ABA from the leaves was transported to the roots via the phloem and recycled in the xylem (Munns and Cramer, 1996, Ikegami et al., 2009). Furthermore, ABA in both the roots and xylem sap has been found to increase in response to soil drying (Wang et al., 1999), whilst a strong linear correlation can be found between the two, suggesting a regulatory role for the roots in ABA transport to the shoot (Liang et al., 1997). ABA within the xylem sap was strongly correlated with g_s (Tardieu and Davies, 1992, Borel et al., 2001, Sobeih et al., 2004), highlighting the significance of transported ABA in regulating the stomata. In maize, a similar relationship was observed between g_s and xylem ABA when ABA concentration *in vivo* increased endogenously or artificially (stem feeding to intact plants in the field), and in detached leaves fed with ABA (Tardieu et al., 1993). Conversely, in wheat and barley plants, *in vivo* ABA concentrations were insufficient to inhibit transpiration in detached leaf bioassays (Munns and King, 1988; Munns, 1992). However, experiments in which ABA was removed from xylem sap using an immunoaffinity column showed transpiration rates consistent with well-watered plants (Zhang and Davies, 1991), highlighting species-specific variation in stomatal regulation.

Given the above inconsistencies on the effects of ABA on stomata, it is perhaps unsurprising that there are other chemical components within the xylem sap that may act as localised or root-to-shoot messengers, either acting independently of, or directly influencing ABA signalling (Wilkinson and Hartung, 2009). This may be due to differences between detached leaves and those *in vivo* perceiving an endogenous ABA signal, with greater stomatal sensitivity to ABA *in vivo* (Dodd, 2003, Correia and Pereira, 1995). Additional antitranspirant signals can include malate, sulphate, pH, calcium and nitrate (Goodger and Schachtman, 2010). Through previous molecular work using ABA-insensitive mutants, it has been revealed that ABA also interacts with the signalling pathways of other phytohormones, such as ethylene and cytokinins, which in turn impacts upon ABA-induced stomatal closure (Anderson et al., 2004, Wilkinson and Davies, 2010, Wilkinson et al., 2012). Applying hormones in the xylem that are known to interact with the ABA pathway (e.g. cytokinins) has also been shown to prevent or reverse the effects of ABA in the leaves (Hansen & Dörffling, 2003), further indicating the significant crosstalk between signalling pathways.

Drought-induced ABA accumulation can be the result of an increase in xylem sap pH, which may become more alkaline with a drought-induced decrease in xylem nitrate concentration (Schachtman and Goodger, 2008). However, soil drying can also increase xylem nitrate concentration, which may lead to stomatal closure through alkalisation of the xylem sap (Goodger et al., 2005, Wilkinson et al., 2007). ABA is a weak acid and as such accumulates under alkaline conditions (Daie and Wyse, 1983). Alkalisation of the xylem sap can result in increased xylem and apoplastic ABA concentrations, whilst also increasing the transport to the guard cells, and the sensitivity of stomata to ABA (Patonnier et al., 1999, Wilkinson and Davies, 2002, Wang et al., 2012). ABA becomes deprotonated as the xylem sap becomes more alkaline, and cannot be transported passively to the mesophyll tissue, where the subsequent accumulation of ABA in the apoplast causes stomatal closure (Schachtman and Goodger, 2008). It has been reported that there are at least three ABA receptors, one of which is an extracellular plasma-membrane bound receptor (GCRP2 – G-PROTEIN COUPLED RECEPTOR 2), suggesting plants can detect extracellular ABA (Liu et al., 2007, Shen et al., 2006, Schachtman and Goodger, 2008), whilst signalling within the guard cells is primarily through the PYR/PYL/RCAR proteins (Cutler et al., 2010). Conversely, in well-watered plants, a more acidic pH is typically found which prevents the apoplastic accumulation of ABA, and often promotes stomatal opening (Wilkinson et al., 2007).

Another potential messenger in the response of plants to water stress is calcium, with a well-defined role for the Ca^{2+} ion as a secondary signalling messenger (Chasan, 1995). It is widely reported that stomata will shut in response to an increase in concentrations of cytosolic Ca^{2+} in the guard cells (MacRobbie, 1992), whilst ABA has been shown to induce an increase in stomatal Ca^{2+} concentrations

(McAinsh et al., 1990). Cytosolic Ca^{2+} concentrations are affected by the uptake of Ca^{2+} by the roots, and by concentrations in the xylem (Nagata et al., 2004). Furthermore, in *Commelina*, a 3-fold increase in xylem sap Ca^{2+} was observed by increasing Ca^{2+} concentration in the provided nutrient solution from 1 mol m^{-3} to 8 mol m^{-3}, resulting in a decrease in g_s (Atkinson et al., 1990). Additional work in *Commelina* revealed a reduction in g_s when the Ca^{2+} concentration in nutrient solution was increased from 4 mM to 15 mM (Ruiz et al., 1993). Drought stress has been shown to result in both decrease (Munns and King, 1988) and increased (Goodger et al., 2005) xylem Ca^{2+} concentrations, which may result in stomatal closure. However, it was found that a range of anions and cations measured in the xylem sap (including Ca^{2+}) exhibited no correlation with decreased g_s in *Helianthus* in response to soil drying (Gollan et al., 1992). Rather it was proposed that changes in xylem Ca^{2+} may increase plant sensitivity to ABA, and thus Ca^{2+} transported in the xylem may act as a secondary signal to regulate stomata (Schurr et al., 1992, Gollan et al., 1992).

1.6 Aims of the study

The aim of this research was to investigate the possible role of varying irrigation frequency on the production and water use of an ornamental bedding plant species, *Pelargonium* x *hortorum*. Initial experiments (Chapter 2) studied the effect of limiting water application on the leaf gas exchange, whole plant WUE and growth of *P.hortorum* by applying two irrigation frequencies: 1) frequent deficit irrigation (irrigation daily at 50 % ET of WW plants); 2) infrequent deficit irrigation (irrigation cumulatively every 4 days at 50 % of WW plants ET demand). This was followed by a series of experiments focused on the signalling mechanisms that controlled gas exchange and leaf water status in *P.hortorum* under the contrasting soil water deficit treatments that occurred as a result of the different irrigation frequencies (Chapter 3). Finally, tomato (*Solanum lycopersicum*) was used as a model species to investigate if differences in physiological responses to different irrigation frequencies (observed in *P.hortorum*) was determined by the spatial distribution of soil moisture under the different irrigation treatments, thereby altering root-to-shoot ABA signalling (Chapter 4). Tomato was selected for this series of experiments due to the difficult of obtaining root tissue samples and xylem sap from *P.hortorum*.

The major objectives of this study were –

1. To assess the impact of different irrigation frequencies on leaf gas exchange, plant development and water use efficiency in *Pelargonium* x *hortorum*
2. To investigate whether there was a difference in the signalling mechanisms (both chemical and hydraulic) regulating leaf gas exchange in *P.hortorum* in response to different rates of soil drying (through different irrigation frequencies)

3. Using tomato as a model species, to understand whether the response observed in *P.hortorum* in response to different soil water deficit regimes was mediated by altered root-to-shoot ABA signalling

Chapter 2 – Decreased irrigation frequency in *Pelargonium* x *hortorum* can reduce water use, maintain leaf growth and anthocyanin concentrations, and increase canopy compactness

2.1 Introduction

Adapting irrigation scheduling is an essential agronomic approach that can be utilised to regulate crop growth and water use. Ornamental growers have often received criticism for inefficient approaches to irrigation, but many are now adopting more sustainable strategies (HTA, 2007), such as replacing overhead sprinklers with drip systems, which ensures more accurate placement of irrigation (Owen and Stoven, 2010). Altering irrigation frequency is one aspect of scheduling that may present a viable option to growers, particularly in nurseries with irrigation systems that can accurately monitor soil water availability and/or plant water status. Manipulating irrigation frequency has been used in arid or semi-arid regions where water is limited (Oktem et al., 2003), or in golf course management (Fu and Dernoeden, 2009), but has received little attention in the production of ornamental plants.

Ornamental growers aim to produce high-quality, high-value plants. In general, quality can be broadly defined in terms of plant compactness, enhanced foliar and floral characteristics (e.g. pigment composition), rooting characteristics and/or enhanced shelf life, although this will vary between species (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 increased costs and awareness of environmental and health effects (along with pressure from consumers) may reduce the long-term viability of this approach (Lütken et al., 2012). The environmental impact of plant production is now a major consideration for consumers (Khachatryan and Choi, 2014). Consequently, growers have looked increasingly to manipulate other aspects of plant production, such as regulating water availability, which often has positive results controlling growth and quality.

Implementing a controlled soil water deficit can tightly regulate growth by preventing vegetative expansion (Cameron et al., 2008). Evidence of success using this approach has been found in different species. In the flowering shrub *Bougainvillea*, applying a soil water deficit at 50 % of the plants daily evapotranspirative (ET) demand increased plant quality (increased floral index) and water productivity (Cirillo et al., 2014). In *Petunia* and *Impatiens*, whilst a 25 % ET water deficit decreased growth and flowering, water productivity (number of flowers per unit volume of water used) increased (Blanusa et al., 2009). Manipulating irrigation frequency may enable the imposition of mild soil water deficits, without imposing too severe a stress on the plants.

Pot-grown species are of particular interest as they typically require frequent irrigation events to prevent excessive soil drying (Warsaw et al., 2009). However, growers often overcompensate for this, which may lead to over-watering and can be damaging for plant production (Hamdy et al., 2003). By understanding the physiological effect of altering irrigation frequency, it may be possible to tailor the regime to the specific requirements of the plant. This may result in significant water savings; it has been estimated that increased efficiency of irrigation practices could reduce the water applied to containerised nursery crops by 25% (Regan, 1999). In addition, this may lead to improvements in plant WUE, although this has shown mixed responses in ornamental species. For example, WUE increased in poinsettia (Morvant et al., 1998), but decreased in rose (Katsoulas et al., 2006) and *Pelargonium capitatum* (Eiasu et al., 2012). As increased WUE may be achieved by reducing irrigation inputs (whilst maximising characteristics associated with water use), this presents a challenge for growers (particularly if plant quality can be included in WUE calculations).

Currently, limited understanding of the impact of alternative irrigation strategies at the physiological level is a major barrier to their adoption (Morison et al., 2008). Many growers are still reliant on gained experience or basic measurements of soil moisture availability, although these techniques may fail to provide a sufficient indication of the water status of the plant. Simple measurements of plant physiology such as Ψ_{leaf}, g_s and ET provide more valuable indicators of irrigation requirements (Jones, 2004). Monitoring physiological status under reduced irrigation frequency can allow growers to accurately schedule irrigation to ensure plants don't receive severe stress, and to allow regular recovery periods from the imposed stress (Mielke et al., 2003, Souza et al., 2004).

To date, the impact of irrigation frequency on physiological responses has been shown to vary between species. In pot grown mango, decreasing irrigation frequency from daily to every 2 days resulted in reduced g_s, A and E, which negatively impacted upon plant growth (Ouma, 2007), whilst in coleus, similar reductions in irrigation frequency had no impact upon g_s, A or E (Scheiber et al., 2008). Furthermore, as irrigation frequency decreased, Ψ_{leaf} increased in pistachio (likely through a reduction in g_s) (Pour et al., 2005), decreased in cotton (Radin et al., 1989) and St. Augustinegrass (Peacock and Dudeck, 1984), and did not change in maize (despite a decrease in g_s) (Zhang et al., 1998). In addition, in *Pelargonium capitatum*, decreasing irrigation frequency pre-conditioned plants to be less susceptible to later periods of drought by maintaining Ψ_{leaf}, g_s, E and leaf relative water content (Eiasu et al., 2012). These results suggest that irrigation frequency can induce a specific response in different species, further strengthening the argument that it should be adapted to the requirements of the plants.

The aim of study was therefore to assess the impact of limiting irrigation frequency on the production of *Pelargonium* x *hortorum*, and to determine whether physiological assessments of plant requirements can be used to better regulate irrigation inputs. Two irrigation frequencies were examined, including a frequent (irrigating plants daily at 50 % ET; FDI) and infrequent (delayed deficit irrigation; IDI) deficit irrigation programme, both of which provided the same volume of water to plants over the treatment period. 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 whole plant water use efficiency was investigated.

2.2 Materials and Methods

2.2.1 Plant culture

Pelargonium x *hortorum* BullsEye (zonal geranium) seeds were sown and germinated (three seeds initially, with two removed after germination) in individual 13 cm x 11.3 cm (1.05 L) 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 an average temperature of 30 ^{o}C. Experiments were carried out in a naturally lit glasshouse compartment (5 m x

3 m), 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 37 °C with a night temperature of 17 °C, and the average daily relative humidity was 35.6±0.9 %. Daily VPD during sampling (1100 h-1300 h) is reported in Fig. 2.1. Environmental conditions in the centre of the glasshouse were recorded using a Hortimax growing solutions Ektron II (Pijnacker, The Netherlands). For each irrigation treatment (which were applied 6 weeks after germination) four plants were sampled every two days (48 plants total), and the experiment was repeated twice.

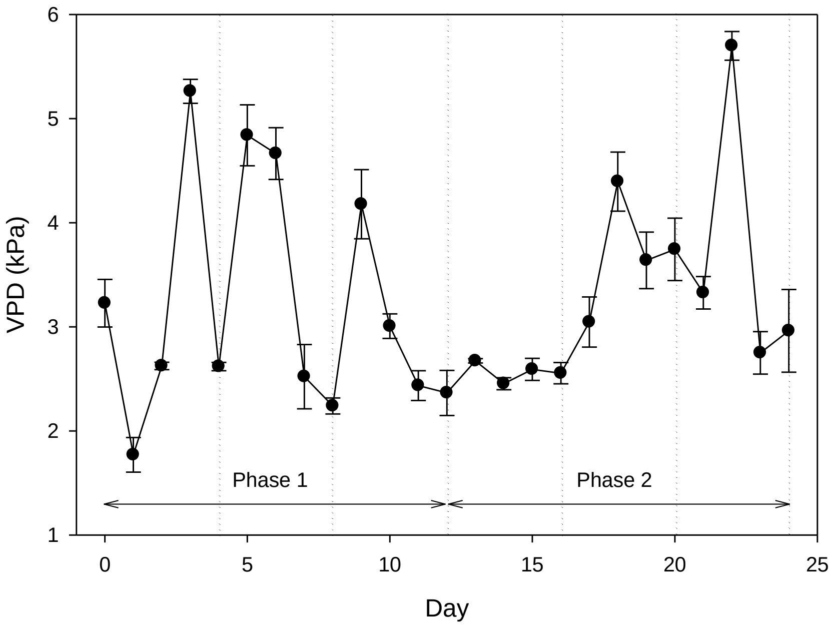


Figure. 2.1. Vapour pressure deficit (VPD) in the greenhouse during sampling (1100 h-1300 h) over the entire experimental period. Bars represent means ± SEM (n=3). Vertical lines indicate each re-watering event for the infrequent deficit irrigation (IDI) treatment, and each Phase is indicated by horizontal lines.

Figure. 2.2. Images of Pelargonium x hortorum plants under well-watered (WW), infrequent (IDI) or frequent (FDI) deficit irrigation over the experimental period on days 8, 14, 20 and 24.

2.2.2 Irrigation Treatments

Prior to starting treatments, individual plants and pots were weighed using a balance with a 0.1 g resolution (Scout Pro Portable balance, Ohaus, Switzerland) and watered daily to container capacity. Pot weight was measured at 0800 h each day to calculate daily ET of each plant, by accounting for any irrigation supplied in the previous 24 h. To achieve well-watered (WW) conditions, all plants were watered until drainage was visible from the bottom of the pot. Plants were then left to freely drain overnight, before being weighed to determine container capacity. Two groups of plants were subject to different irrigation regimes (Fig. 2.3a); infrequent (IDI; withholding water with regular re-watering events), and frequent deficit irrigation (FDI; daily irrigation at 50% of WW plants ET). After 4 days of with-holding water, plants subject to IDI received the accumulated irrigation volume supplied to plants under FDI over the same cycle. To prevent any water loss, closed base trays (which tightly fitted the pot base) were placed under each pot at the point of re-watering to prevent evaporation and to ensure re-absorption of any leachate. Thus plants under both irrigation treatments received the same volume of water during the treatment period. Irrigation regimes were applied at week 6 after germination for both treatments (on plants selected with 14-15 leaves), over 24 days. Plants were exposed to six drying and re-watering cycles (IDI) or sustained irrigation at 50 % ET (FDI), and experiments were repeated twice (Fig. 2.2).

2.2.3 Stomatal conductance (g_s) measurements

g_s was measured using a porometer (Model AP4, Delta-T Devices, Cambridge, UK). Two readings of g_s were taken either side of the mid-rib on the youngest, fully expanded abaxial side of one leaf per plant. Measurements of g_s were carried out every 2 days from the beginning of the treatment period. Additional measurements of g_s were made on all plants over the duration of a drying-re-watering cycle (1 day prior to re-watering, and on 3 subsequent days). Diurnal measurements of g_s were made after re-watering at the end of each drying period every 2 h from 0800 h to 1600 h.

2.2.4 *Leaf water (Ψ_{leaf}) potential*

Ψ_{leaf} was measured immediately after measurements of g_s 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). Detached leaves were transported in a sealed bag to the laboratory, and placed in the pressure chamber within 15 s of excision. Once in the chamber, the cut petiole was cleaned with deionised H_2O and filter paper to remove cellular debris. Pressure was raised in the chamber at a rate of 0.02 MPa s^{-1}, and Ψ_{leaf} was recorded when xylem sap collected on the surface of the cut petiole.

2.2.5 Leaf pigment analysis

Leaf tissue samples for pigment analysis were collected from the anthocyanic zonal band (see Fig. 2.2) of the leaf at the end of each drying cycle. Leaf anthocyanin concentrations were determined spectrophotometrically. Frozen leaf tissue (15-20 mg with an area of 0.6 cm^2) was ground with 600 µl of Methanol 1% HCl (v/v), 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 Methanol 1% HCl (v/v):Milli-Q water (60:40 v/v), and the absorbance was then measured using a spectrophotometer. Anthocyanin absorbance (A_{530}-0.25*A_{657}) was used to calculate concentration of anthocyanin per gram fresh weight (Rabino and Mancinelli, 1986), which was used as an indicator of foliage quality.

2.2.6 Growth, biomass & water use efficiency

Canopy volume, which was measured as the total height, width and breadth of the plant, was determined at the end of each 4 day drying period in order to assess the overall compactness of the plant. Plant material was harvested every 2 days after physiological measurements. Shoot fresh weight, which was separated into leaf and stem (including petioles), and root fresh weight (after separation from soil and careful cleaning) was measured. Leaf number was recorded, and total leaf area was 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 weight. WUE was determined as the ratio of plant dry mass to the total volume of water applied (applied WUE), or to plant evapotranspiration (evapotranspirative WUE).

2.2.7 Statistical analysis

Differences between irrigation treatments on each day of sampling were determined 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 were 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. The six drying and re-wetting cycles were separated into two experimental phases, each comprising three drying and re-watering cycles to determine statistically whether experimental duration altered treatment effects of physiological responses. The effect of irrigation treatment and irrigation phase on the relationship between plant and soil variables was tested using a three-way analysis of covariance (ANCOVA). Altered sensitivity of the y-variable to the x-variable is indicated by a significant interaction term. All graphs were created using Sigmaplot 8 (Systat Software Inc.).

2.3 Results

WW plants showed a steady increase in plant evapotranspiration over the experimental period (Fig. 2.3c). FDI resulted in a more stable ET, which was typically lower than WW plants, whilst IDI showed a series of declines and peaks in ET, which corresponded with each re-watering event. Recovery time of ET was similar for plants under IDI under each cycle, generally increasing over 48 h after re-watering, before declining over the subsequent 24-48 h. IDI and FDI plants received the same volume of water over the experimental period (Fig. 2.3b), which corresponded to approximately 51% of that supplied to WW plants.

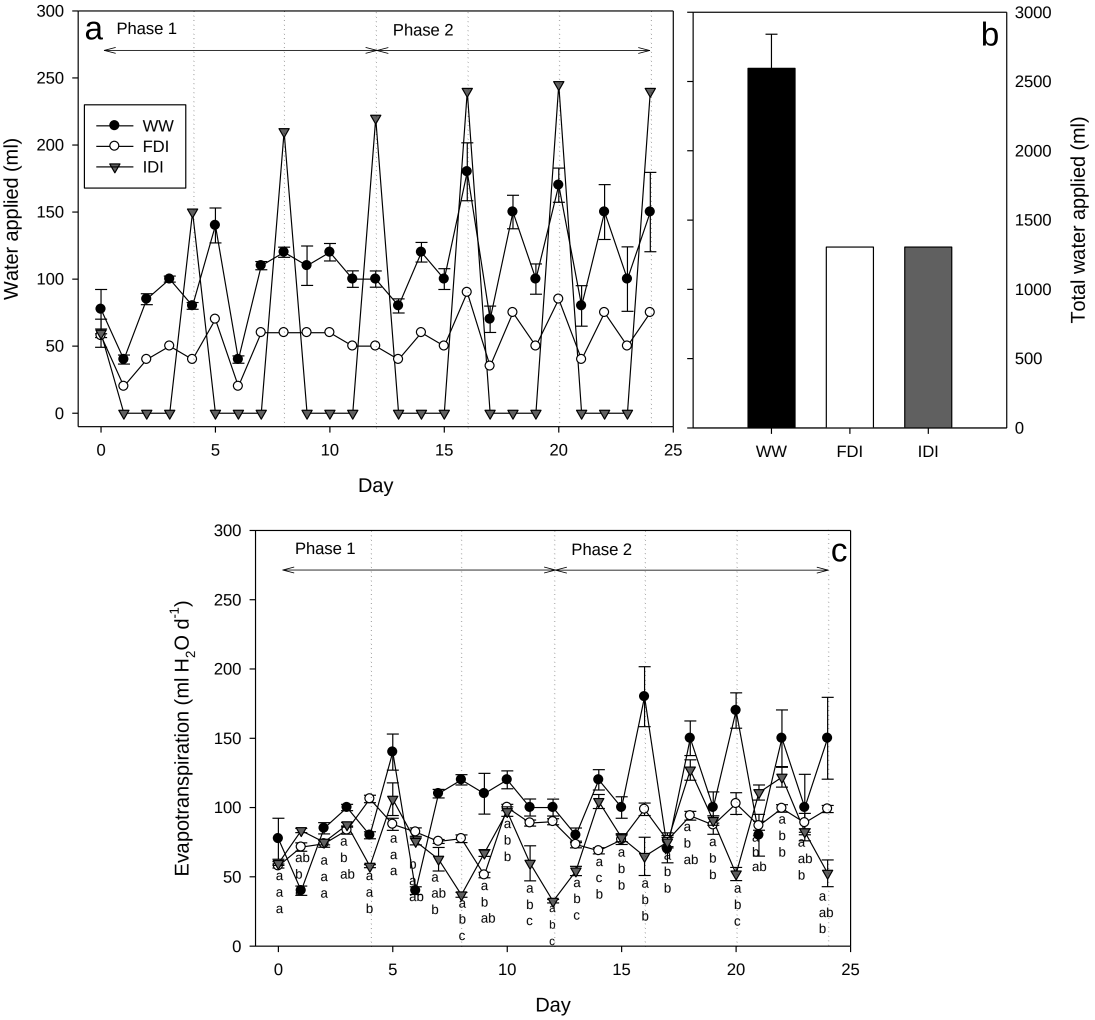


Figure. 2.3. a) Volume of water applied per treatment per day; b) total volume of water applied to each treatment over the entire experimental period; c) daily evapotranspiration for *P.hortorum* plants subject to well-watered (WW) conditions, frequent (FDI) or infrequent (IDI) deficit irrigation. Data are means ± SEM (n=13). 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 Phase is indicated by horizontal lines.

Stomatal conductance was measured every 2 days over the experimental period (Fig. 2.4a), and was on average 691±102 mmol m^{-2} s^{-1} in WW plants (although there was a large decrease in g_s in WW plants during the last week of the experiment – possibly as a consequence of environmental variation, or a limited rooting zone due to container size). FDI resulted in a gradual decrease in g_s, which only showed significant reductions compared to WW plants from Day 8 (after which it was ~17% of WW plants over the sampling period). In contrast, IDI plants showed a rapid decrease in g_s by Day 4, after which it remained at ~15% of WW plants for the remainder of the sampling period. Diurnal measurements of g_s on the day after re-watering of IDI plants revealed that although plants showed recovery within the first cycle (Table. 2.1), in subsequent cycles g_s didn’t increase within 24 h of re-watering, but rather within 48 hours, similar to the relationship observed for ET (Fig. 2.4b). WW plants had the highest mean diurnal g_s over the sampling period (Fig. 2.4c). At 0800 h and 1600 h, there was no significant differences between FDI and IDI plants, but at 1200 h FDI plants showed a peak of g_s which wasn’t observed under IDI. Stomatal conductance decreased consistently with decreasing soil moisture under both deficit irrigation treatments (Fig. 2.5a), with no differences between phases (no-significant treatment x phase x θ_{pot} interaction; Table. 2.2).

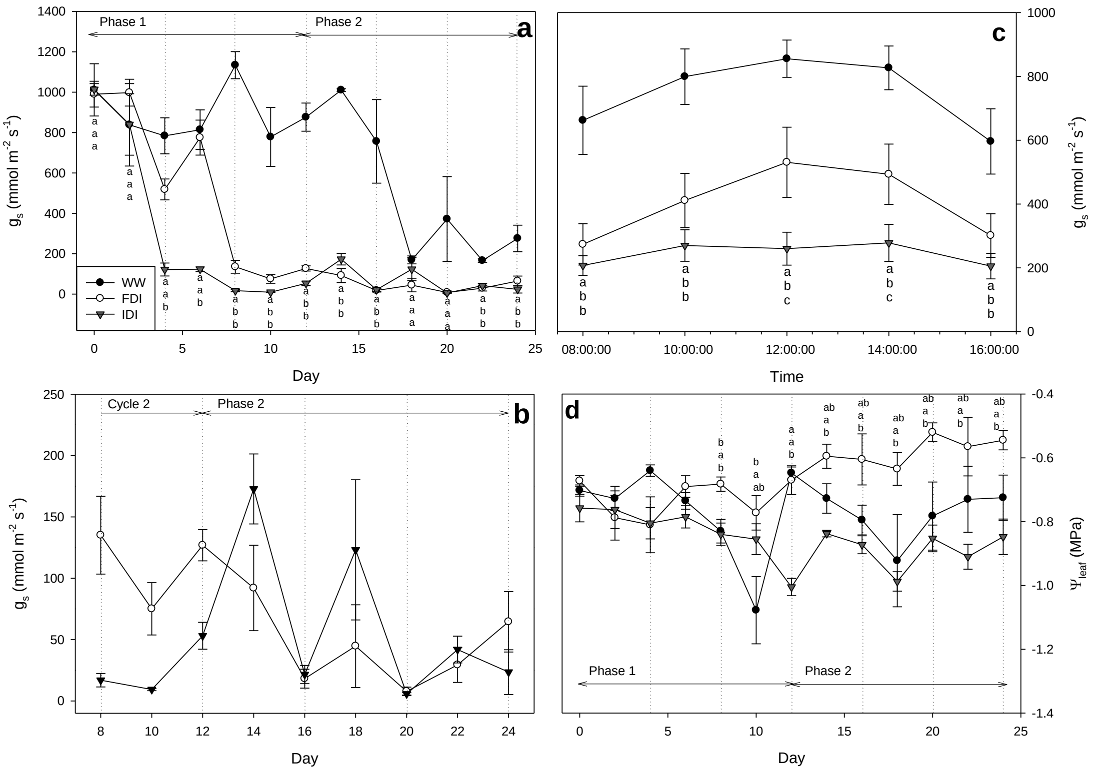


Figure. 2.4. Stomatal conductance (g_s) every two days over a) the entire experimental period (n=4); b) frequent (FDI) or infrequent (IDI) deficit irrigation from Day 8 (n=4); c) mean diurnal stomatal conductance (g_s) 24 h after re-watering (n=25); d) leaf water potential (Ψ_{leaf}) every two days (n=4) of *P.hortorum* plants subject to well-watered (WW) conditions, FDI or IDI. 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. Diurnal g_s data in panel c) is the mean of five diurnal measurement cycles over the experimental period. Different letters indicate significant differences between irrigation treatments on each day according to a one-way ANOVA ($p<0.05$).

		Time				
Day	Treatment	0800	1000	1200	1400	1600
5	WW	714±58	614±61	771±55	839±89	677±92
	FDI	503±56	581±37	679±75	656±68	497±81
	IDI	443±97	436±63	598±97	676±119	588±102
9	WW	748±111	958±70	1002±3	1047±34	899±88
	FDI	266±120	492±106	768±114	538±124	156±114
	IDI	193±24	455±96	473±88	545±139	196±54
13	WW	672±141	752±126	544±129	521±116	501±94
	FDI	182±15	301±50	269±88	189±19	454±97
	IDI	135±12	200±39	90±36	107±16	137±30
17	WW	581±120	767±108	921±66	728±104	348±115
	FDI	275±119	334±129	403±141	425±129	194±24
	IDI	119±8	144±28	74±22	24±7	68±5
21	WW	597±105	906±70	1040±40	1000±10	556±122
	FDI	142±14	348±103	535±132	656±133	204±27
	IDI	148±12	115±21	66±16	38±10	38±10

Table. 2.1. Diurnal measurements of stomatal conductance (g_s) of *P.hortorum* plants subject to either well-watered (WW) conditions, frequent (FDI) or infrequent deficit irrigation (IDI). Data are means SEM of all measurements (n=5) carried out during the sampling period for all irrigation treatments.

The average Ψ_{leaf} in WW plants over the course of the sampling period was -0.8±0.1 MPa (Fig. 2.4d), but it decreased on Days 10 and 18, which coincided with increased VPD (Fig. 2.1). Ψ_{leaf} of FDI plants gradually increased over the sampling period which, from Day 8 was generally more positive than WW plants (except on Day 12). This is in contrast to IDI plants, which showed a decrease in Ψ_{leaf} and after Day 12 was lower than WW plants (significantly lower on Days 4 and12). There were no phase effects for Ψ_{leaf} (Table. 2.2), but consequently, Ψ_{leaf} did not decrease as soil moisture decreased under IDI (Fig. 2.5b) and thus did not correlate with g_s (Fig. 2.5c). In contrast, FDI showed an increase in Ψ_{leaf} over the experimental period (significant phase x θ_{pot} interaction), and was correlated with g_s.

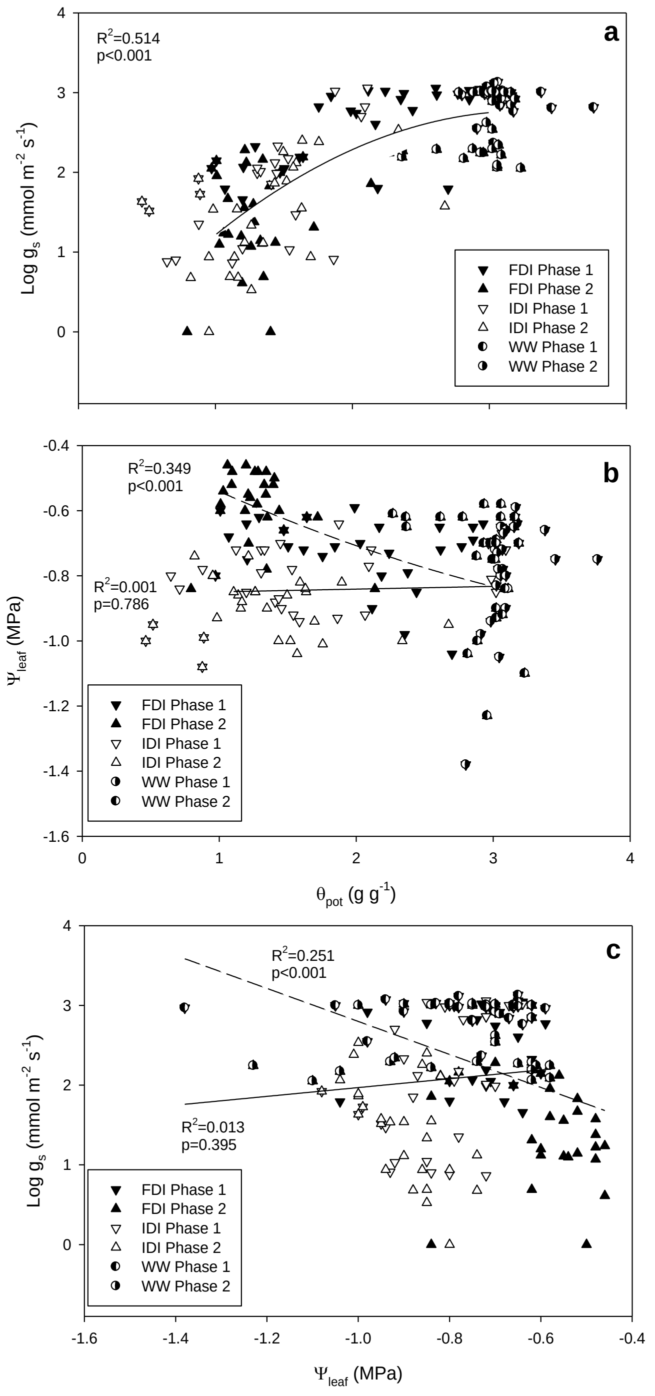


Figure. 2.5. a) Log stomatal conductance (g_s); and b) leaf water potential (Ψ_{leaf}) in response to soil drying; c) relationship between Ψ_{leaf} and g_s of *P.hortorum* under different irrigation treatments over two experimental phases. Open symbols show data from plants subject to frequent deficit irrigation (FDI), whilst closed symbols show data from plants subject to infrequent deficit irrigation (IDI) (n=56). Half & half symbols show data from well-watered (WW) plants (n=28). Data points are paired individual samples, regression line is fitted for data where θ_{pot} ranged from 1-3 g g^{-1}, and P values are reported.

	θ_{pot} *vs* g_s		θ_{pot} *vs* Ψ_{leaf}			Ψ_{leaf} *vs* g_s	
	F value	*P value*	*F value*	*P value*		*F value*	*P value*
Treatment	3.897	**0.051**	21.840	**<0.001**	Treatment	3.834	**0.053**
θ_{pot}	119.002	**<0.001**	11.002	**0.001**	Ψ_{leaf}	1.503	**0.223**
Phase	4.969	**0.028**	0.271	**0.604**	Phase	1.531	**0.218**
Treatment*θ_{pot}	2.419	**0.123**	9.854	**0.002**	Treatment* Ψ_{leaf}	6.106	**0.015**
Phase*Treatment	1.229	**0.270**	4.971	**0.028**	Phase*Treatment	3.910	**0.050**
Phase*θ_{pot}	0.309	**0.579**	1.064	**0.304**	Phase* Ψ_{leaf}	0.484	**0.488**
Phase*Treatment *θ_{pot}	0.558	**0.457**	3.366	**0.069**	Phase*Treatment* Ψ_{leaf}	3.380	**0.069**

Table. 2.2. *F*- and *P*-values from a three-way ANCOVA testing the interactive effects of applying either frequent (FDI) or infrequent (IDI) deficit irrigation to *P.hortorum* plants on stomatal conductance (g_s) and leaf water potential (Ψ_{leaf}), and the relationship between Ψ_{leaf} and g_s (Fig. 2.5). Interactive effects were tested on data over two experimental phases, and from data where θ_{pot} ranged between 1–3 g g^{-1}.

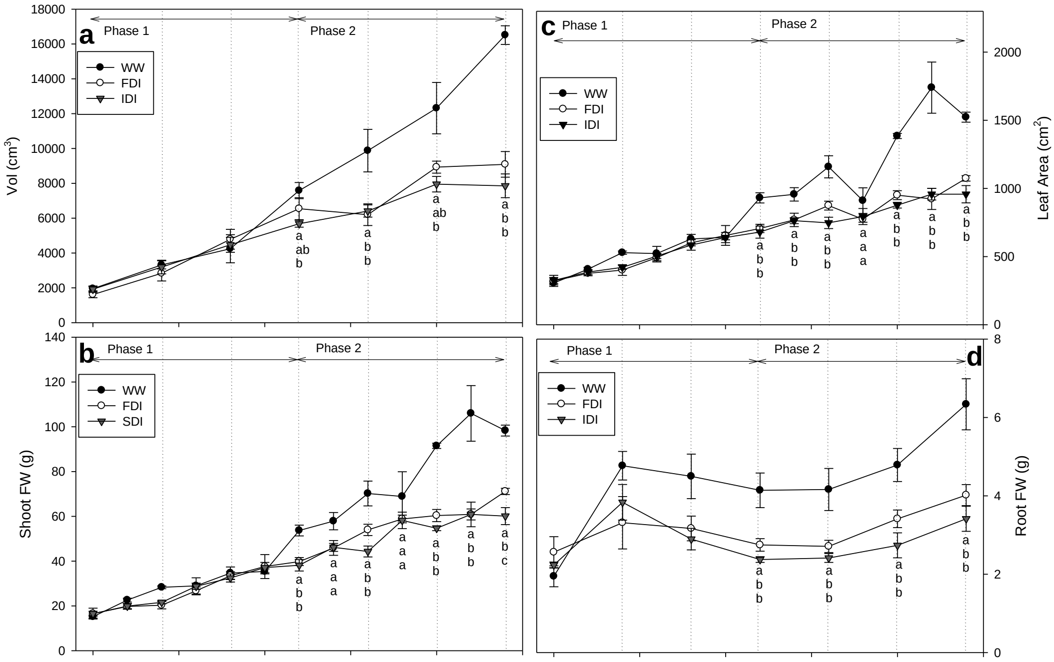


Figure. 2.6. a) Canopy volume (Vol); b) shoot fresh weight (Shoot FW); c) leaf area; and d) root fresh weight (Root FW) every two-four days of *P.hortorum* plants subject to well-watered (WW) conditions, frequent (FDI) or infrequent (IDI) deficit irrigation. Data are means ± SEM (n=4-5). Different letters 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 Phase is indicated by horizontal lines.

Plant growth, assessed by canopy volume, was similar for all treatments until Day 10 (Fig. 2.6a). However, by the end of the second week, the volume of the WW plants was approximately 30% larger than under either IDI or FDI. Similar reductions for both deficit irrigation treatments were observed after Day 10 for shoot fresh weight (Fig. 2.6b) and leaf area (Fig. 2.6c). By the end of the experimental period, FDI plants had significantly greater shoot fresh weight than IDI plants (71±1 g and 60±4 g respectively). By Day 8, root fresh weight of both IDI and FDI had decreased compared to WW plants (Fig. 2.6d). These findings for biomass corresponded with WUE being initially higher under both IDI and FDI compared to WW plants up to Day 10 (Fig. 2.7a & b). On Days 6 and 10 IDI plants had a significantly higher applied WUE than WW plants (Fig. 2.7a), and a higher ET WUE on Days 4 and 12 (Fig. 2.7b). However, from Day 18 WW plants had the highest applied WUE, which corresponded with the relationship of shoot DW (Fig. 2.7c) and the total volume of water applied to each plant up to the point of harvest (Fig. 2.7d).

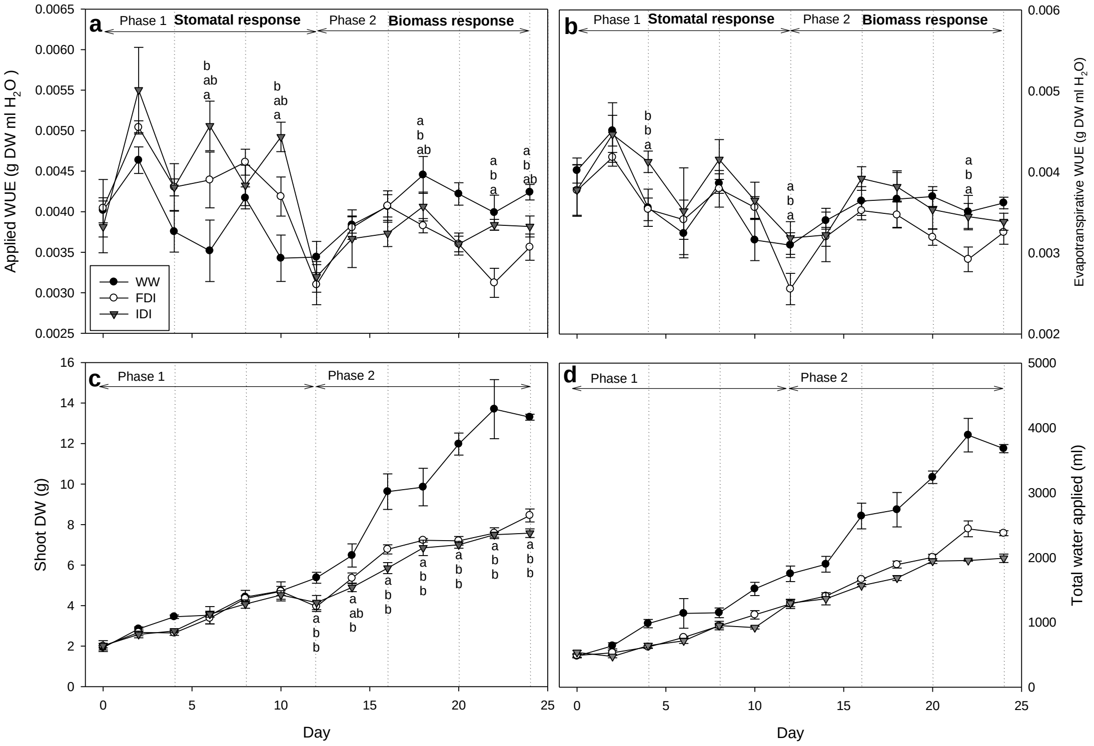


Figure. 2.7. a) Applied water use efficiency (WUE); b) evapotranspirative WUE; c) shoot dry weight (Shoot DW); and d) total water applied over the entire experiment (cumulatively until harvest) every two days of *P.hortorum* plants subject to well-watered (WW) conditions, frequent (FDI) or infrequent (IDI) deficit irrigation over the entire experimental period. Data are 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 Phase is indicated by horizontal lines.

The mean anthocyanin concentration over the experimental period for WW plants was 26.6±1.8 mg cm^2. No significant differences were found between IDI and WW plants for anthocyanin concentration over the entire experimental period (Fig. 2.8). However, FDI had a significantly higher anthocyanin concentration than both WW and IDI plants on Day 8, and was lower than WW plants on Days 16 and 20 (albeit no significant differences were observed on the remaining sampling days).

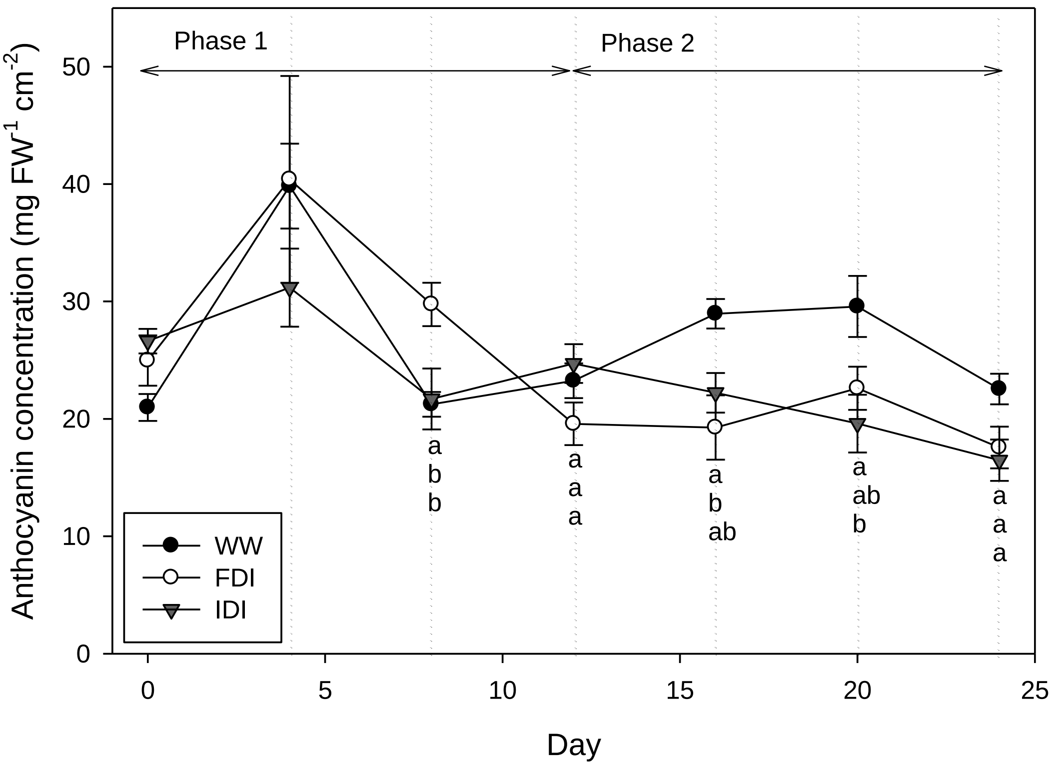


Figure. 2.8. Leaf anthocyanin concentration every four days of *P.hortorum* plants subject to well-watered (WW) conditions, frequent (FDI) or infrequent (IDI) deficit irrigation. Data are means ± SEM (n=4). Different letters indicate significant differences between irrigation treatments on each day according to a one-way ANOVA ($p<0.05$). Vertical lines indicate each re-watering event for the IDI treatment, and each Phase is indicated by horizontal lines.

2.4 Discussion

Modifying irrigation frequency is a strategy that could potentially be implemented in nurseries as a water saving technique (Beeson, 2006). Both deficit irrigation treatment groups received 50% of WW plants ET demand (cumulatively at the same volume), but at different irrigation frequencies (Fig. 2.3b). Plants were grown in an organic peat-based compost, typical of the substrate used in approximately 40% of horticultural growing products (DEFRA, 2010). This substrate is favoured because of its high water holding capacity, but it can also quickly become hydrophobic, making it difficult for re-watering (Michel et al., 2001). Reducing irrigation frequency, particularly in a container based system, must therefore be considered carefully to provide adequate re-watering, and also to prevent excessive loss of moisture.

Reducing the irrigation frequency created a situation where plants under IDI showed a series of increases in ET after re-watering (typically within 24 h), followed by a decrease over the subsequent 24 h (Fig. 2.3c). 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 a rapid (1-2 days), partial recovery of leaf gas exchange upon re-watering (Fig. 2.4c). As the soil in each pot was almost entirely covered by the canopy at the time the different irrigation treatments were implemented, soil evaporative losses will be minimal across irrigation treatments.

Stomatal closure is a well characterised response to soil drying in *P.hortorum*, which is tightly regulated to limit water loss (Álvarez et al., 2013, Sánchez-Blanco et al., 2009). In the current study, g_s decreased as soil moisture decreased under both IDI and FDI (Fig. 2.5a), but stomatal closure occurred earlier in IDI plants (Fig. 2.4 a). This is possibly a consequence of the initially quicker depletion of soil moisture under IDI, but also the length of time in which water was with-held, likely enhancing the severity of the stress. Decreased g_s, followed by partial recovery upon re-watering (typically occurring 24-48 h after re-watering (Fig. 2.4b & c)), indicating that stomatal closure can partially explain the fluctuations observed in ET (Čerekovic et al., 2013). This supports the understanding that the stomata of *P.hortorum* are particularly sensitive to soil moisture availability (Sánchez-Blanco et al., 2009, Arora et al., 1998), providing an important mechanism to prevent excessive water loss under soil water deficits.

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} even as soil moisture decreased (Fig. 2.5b), IDI showed reductions in Ψ_{leaf} compared to WW plants (Fig. 2.4d), albeit non-significant. This suggests that over the course of the experimental period, IDI does not impose too severe a water deficit (out of the range of control plants) and may actually provide pre-conditioning to other stressful conditions such as VPD (Fig. 2.1), which may explain the decrease in Ψ_{leaf} of WW plants on Day 10 for *P.hortorum* (particularly as Ψ_{leaf} has been shown to be sensitive to changes in VPD (Kramer, 1988)). In contrast under FDI (Table. 2.2), 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. 2.4d & 2.5b, c). *P.hortorum* has previously been shown to have a low lethal Ψ_{leaf} threshold but particularly sensitive stomata, which provide a regulatory mechanism for water loss (Augé et al., 2003). This may have a useful application in conditioning plants for future, unexpected periods of drought stress, although the lower Ψ_{leaf} observed under IDI may have more deleterious effects on plant growth and development. Given g_s decreased similarly between the irrigation treatments without a consistent change in Ψ_{leaf}, the unifying regulatory mechanism behind this response is unclear, and may point towards a chemical signal (Dodd, 2003).

Significant differences in growth and biomass were detected between WW plants and those under both IDI and FDI by Day 12 (Fig. 2.6), which is consistent with previous work examining a drought stress and recovery response in *P.hortorum*

(Sánchez-Blanco et al., 2009). By the end of the experimental period shoot FW was significantly reduced in IDI plants compared to FDI plants, implying that prolonged exposure to cycles of withholding irrigation and re-watering eventually decreased plant growth. This may be due to limited leaf expansion at a lower Ψ_{leaf} (Munns et al., 2000), although leaf expansion can decrease without a decrease in Ψ_{leaf} (Martin-Vertedor and Dodd, 2011). However, plants that are smaller and more compact are favoured by growers for their aesthetic value, and because they are more suitable for transport and handling by retailers (Cameron et al., 2008). In addition, regulating plant growth via deficit irrigation frequency may reduce the use of chemical growth retardants. Taken together, this shows that the ornamental (and perhaps economic) value of plants can be increased by altering deficit irrigation frequency.

The above changes in biomass correlate with two distinct periods of plant WUE during the experimental period. As no differences were observed for shoot DW until Day 12 (Fig. 2.7d), both IDI and FDI plants had higher applied and ET WUE (Fig. 2.7a & b) than WW plants (Phase 1 – driven by stomatal responses), but this effect was lost by the end of the experimental period (Phase 2 – biomass driven response). There is limited information regarding the effects of irrigation frequency on WUE. Of those, greenhouse-based studies with the same irrigation volumes showed varied responses. When irrigation frequency was reduced, WUE increased in cucumber (Wang et al., 2009), had no effect on lettuce (Xu et al., 2004), and decreased in rose plants (Katsoulas et al., 2006), highlighting large variation between species. However, WUE was not monitored continuously throughout these studies, and thus there may be optimal periods for WUE. For instance, in pot grown tedera plants subject to a single period of drying and re-watering, there was a peak of intrinsic WUE (iWUE; leaf photosynthesis/g_s) during the drying phase (Foster et al., 2015).

Therefore, it is clear that this requires further research across more species, particularly if irrigation frequency can deliver short-term benefits in WUE (such as in *P.hortorum*).

Another important ornamental characteristic of *P.hortorum* is the anthocyanic ‘zonal’ banding on the leaves (Liakopoulos and Spanorigas, 2012). There is little evidence that foliar anthocyanin accumulation can be directly increased though soil water deficits (Steyn et al., 2002). Nevertheless, it has been suggested that the presence of high anthocyanin may correlate with drought tolerance (or rather the presence of stress conditions) (Chalker-Scott, 1999), although this has been questioned (Manetas, 2006). In this study, no significant differences in anthocyanin concentration were found between IDI or FDI plants (Fig. 2.8), indicating that decreasing irrigation frequency does not negate leaf quality. This is consistent with previous work in *P.hortorum* where no loss in leaf colour was found under regulated deficit irrigation treatments (Álvarez et al., 2013), suggesting that water savings can be achieved whilst maintaining foliar quality (which may in fact have more value for ornamental growers).

2.5 Conclusions

Taken together, these results show that growers can adapt their irrigation scheduling dependent upon whether their aims are to reduce water consumption, improve WUE, or increase ornamental quality. Less frequent deficit irrigation resulted in a series of peaks and declines in ET, earlier reduction in g_s and a lower Ψ_{leaf} compared to plants subject to FDI. Neither deficit irrigation treatment diminished foliage quality. IDI and FDI both result in decreased plant growth over time compared to WW plants, with IDI plants the smallest by the end of the experimental period. This was reflected in WUE, which was higher under both IDI and FDI over the first 10 days, but was lower after Day 10. However, in an ornamental species such as *P.hortorum*, the increased ornamental quality (decreased canopy volume) along with reduced water inputs may be considered more favourable than any change in WUE. Therefore, it is clear that irrigation volume can be decreased whilst maintaining foliar quality and increasing canopy compactness, thus increasing the ornamental value of the plants, reducing irrigation costs, and ultimately increasing the sustainability of irrigation practices. If this irrigation strategy can be adopted on a commercial scale, there may be significant environmental and economic benefits for the growers.

Chapter 3 - Stomatal closure of *Pelargonium* x *hortorum* in response to soil water deficit is associated with decreased leaf water potential only under rapid soil drying

3.1 Introduction

The increasing scarcity of freshwater water resources world-wide makes the development of sustainable irrigation practices a key challenge for agriculture. An improved understanding of the physiological mechanisms by which plants respond to reduced water availability and how these can be manipulated is therefore essential to improve the efficiency of plant water use (Álvarez et al., 2013). Stomatal closure is a primary response to water deficit that decreases transpirational water loss, thereby contributing to plant survival during periods of drought (Bahrun et al., 2002), but can limit photosynthesis and biomass accumulation. Partial stomatal closure without limiting photosynthesis represents an important target for increasing water use efficiency (WUE).

Hydraulic and chemical signals have both been implicated in regulating stomatal responses to water deficit (Wilkinson and Davies, 2010). There is evidence that stomata close in response to decreased Ψ_{leaf}, acting as a regulatory feedback mechanism, possibly as a consequence of reduced Ψ_{leaf} increasing stomatal sensitivity to ABA (Tardieu and Davies, 1992, Buckley, 2005). In addition, root pressurisation experiments in bean (*Phaseolus vulgaris*) and *Hymenoclea salsola* grown in drying soil show stomatal opening coincident with recovery of Ψ_{leaf} (Comstock and Mencuccini, 1998, Mencuccini et al., 2000) suggesting that stomatal closure occurs in response to leaf water deficits. However , there is also evidence that stomatal closure can occur in the absence of any change in Ψ_{leaf} (Bates and Hall, 1981, Sobeih et al., 2004), and that stomatal closure can maintain Ψ_{leaf} under water deficits thereby maintaining leaf turgor (Sperry et al., 2002). In Chapter 2, stomata of *Pelargonium* x *hortorum* closed in response to soil drying under two different deficit irrigation frequencies, without a consistent response of Ψ_{leaf} (Fig. 2.5). Furthermore, root pressurisation (that returned leaves to full turgor) in wheat and sunflower failed to prevent stomatal closure in response to soil drying (Gollan et al., 1986). These conflicting findings suggest the existence of root-supplied chemical signals that regulate stomatal behaviour.

The plant hormone ABA is known to regulate stomatal conductance in response to reduced water availability. ABA is synthesised either in the roots and transported to the shoots, or locally in the leaves, where it activates a complex signalling network leading to stomatal closure (Kim et al., 2010, Merilo et al., 2014). In many species stomatal closure is correlated with increased leaf xylem ABA concentration ($[X\text{-}ABA]_{leaf}$) (Wilkinson and Davies, 2002). However, it can be difficult to distinguish whether increased $[X\text{-}ABA]_{leaf}$ is a cause or a consequence of stomatal closure, since a slowing of transpiration rate (and thus sap flow) is expected to increase the concentration of all xylem sap constituents (Dodd et al., 2008). Nevertheless, in maize (Zhang and Davies, 1991) and lupin (Correia and Pereira, 1995) there is a consistent relationship between leaf transpiration and $[X\text{-}ABA]_{leaf}$ in both detached leaves and intact plants. Furthermore, removing ABA from maize xylem sap removed its antitranspirant activity (Zhang and Davies, 1991). In contrast, although $[X\text{-}ABA]_{leaf}$ increases under water stress in wheat, feeding synthetic ABA at these concentrations to detached wheat leaves does not elicit stomatal closure (Munns and

King, 1988). Additionally, wheat xylem sap retains its antitranspirant effect following removal of ABA, suggesting the presence of other antitranspirant compounds in xylem sap (Munns and King, 1988). Taken together, 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, Buckley, 2005).

Other chemical signals have also been implicated in regulating stomatal closure. Alkalisation of the xylem sap affects the partitioning of ABA between apoplast and symplast, causing an increase in apoplastic ABA accumulation, thereby increasing both the delivery of ABA to the guard cells and also stomatal sensitivity to ABA (Patonnier et al., 1999, Schachtman and Goodger, 2008, Wilkinson and Davies, 2002, Wilkinson et al., 2007). Nitrate concentrations in the xylem sap can decrease in response to soil drying (Bahrun et al., 2002), and decreased nitrate concentrations are often associated with alkalisation of the xylem sap (Dodd et al., 2003). However, xylem nitrate can also increase under soil drying (Goodger et al., 2005), and this can also lead to alkalisation of the xylem sap and stomatal closure (Wilkinson et al., 2007). Soil drying can either increase (Ernst et al., 2010, Goodger et al., 2005) or decrease (Munns and King, 1988) xylem Ca^{2+} concentrations, whilst increases in Ca^{2+} within the xylem (Kim et al., 2010, Merilo et al., 2014, Ruiz et al., 1993) or the guard cell cytosol (Gilroy et al., 1991, McAinsh et al., 1990) can cause stomatal closure. In addition, other plant hormones such as ethylene may have a role in regulating stomatal closure, either directly or through interactions with ABA (Wilkinson and Davies, 2010, Chen et al., 2013).

Despite evidence for both hydraulic and chemical signals decreasing stomatal conductance in response to reduced water availability, different species may adopt different signalling mechanisms to initiate stomatal closure (Wilkinson and Davies, 2010). One of the clearest examples is that apricots (*Prunus armeniaca*) show minimal change in $[\text{X-ABA}]_{\text{leaf}}$ in response to soil drying, indicating that ABA does not have a key role in stomatal regulation, whereas in grapevine (*Vitis vinifera*), $[\text{X-ABA}]_{\text{leaf}}$ increases in response to soil drying and is tightly linked to stomatal closure (Loveys, 1984, Loveys et al., 1987). These findings highlight the need to understand how physiological responses to water availability differ between species (Sharp and Davies, 2009), to assist in designing appropriate irrigation management systems for different species.

Irrigation scheduling is an important tool for modifying crop quality and yield, and several approaches can be adopted. Imposing water deficits (typically called 'deficit irrigation') is one such strategy, although there can be considerable variation in this approach. Water deficits may be 'transient' (McCarthy, 1997), where water is completely withheld for a defined period of time, or 'sustained', where plants are supplied with a percentage of daily ET requirements (Fernandes-Silva et al., 2010). This raises the question as to whether these contrasting approaches have similar effects on crop physiological responses.

To date, applying deficit irrigation strategies to bedding plant species has received relatively little attention (Álvarez et al., 2013). *Pelargonium* x *hortorum* is an annual bedding plant species, popular for its attractive ornamental characteristics (both flowers and foliage). Whilst stomata of this species close in response to soil drying allowing plants to survive under prolonged water stress (Álvarez et al., 2013), little is known about the mechanisms regulating this closure. Previous results (Chapter 2; Figs. 2.4 & 2.5) highlighted that stomata of *P.hortorum* would close in response to soil drying, but Ψ_{leaf} was not consistently related to stomatal closure (Fig. 2.5c). Therefore the role of long-distance chemical signals including ABA and other xylem-borne antitranspirants (as well as investigating potential secondary roles for Ψ_{leaf}) in stomatal closure of *P.hortorum* subject to different types of soil drying was investigated by comparing physiological responses to withholding irrigation *versus* supplying a fraction of crop evapotranspirational needs.

3.2 Materials and Methods

3.2.1 Plant culture

Pelargonium x *hortorum* BullsEye (zonal geranium) seeds were germinated under similar conditions as reported in Chapter 2 (Section 2.2.1). Plants were grown at an average temperature of 24 ^{o}C, with a daily maximum temperature in the greenhouse of 27 °C and a night temperature of 17 °C. Environmental conditions in the centre of the glasshouse were recorded using a Hortimax growing solutions Ektron II (Pijnacker, The Netherlands), and were used to estimate atmospheric vapour pressure deficit (VPD) during the sampling period (1100h to 1300 h; Fig. 3.1a). Twenty plants were grown per irrigation treatment, with five plants per irrigation treatment measured per day for each of the variables described below. Consistency of plant material was based on the leaf number of individual plants. The experiment was repeated on three occasions.

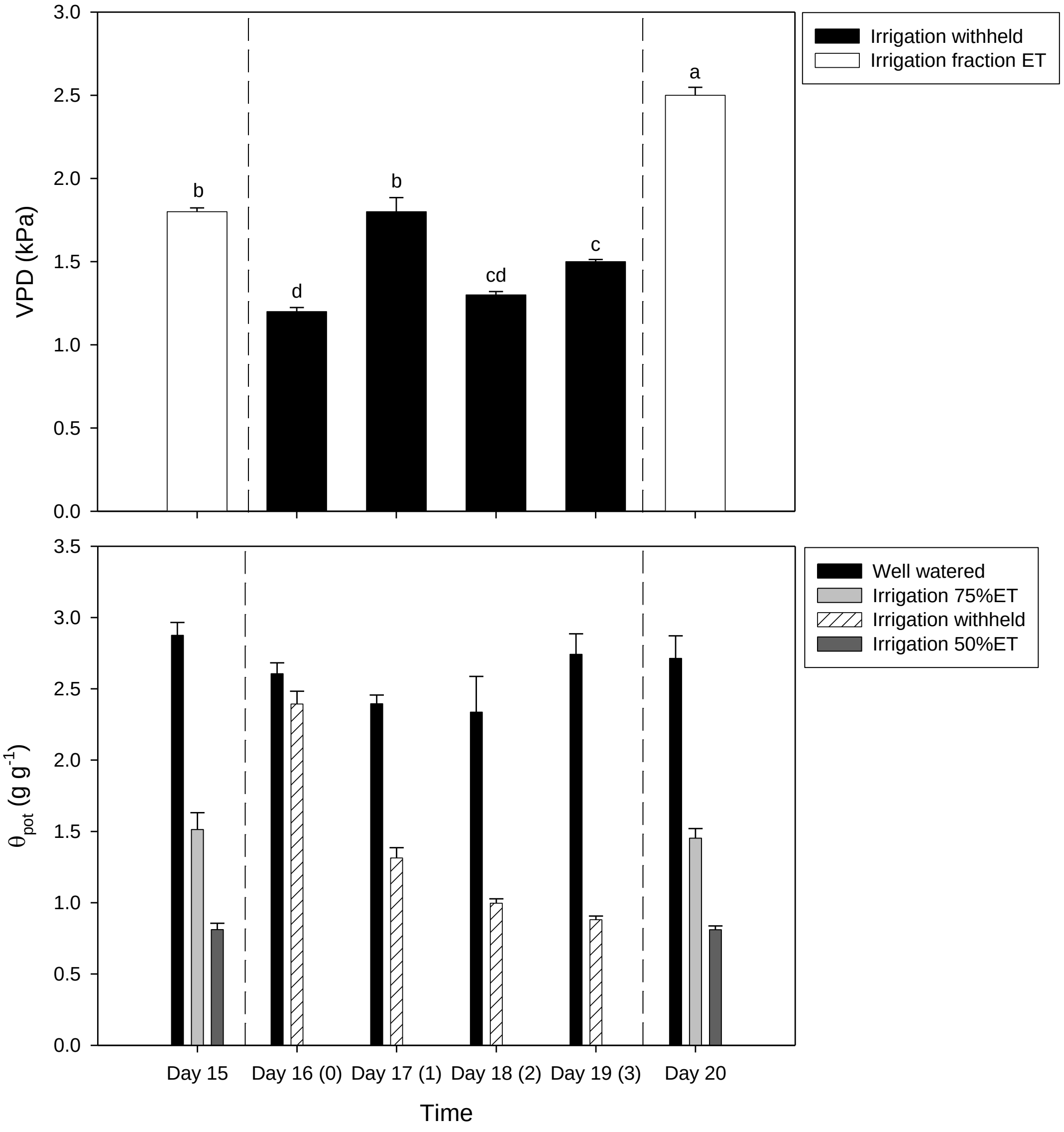


Figure. 3.1. a) Vapour pressure deficit (VPD) in the greenhouse when plants were measured (1100 h to 1300h) where irrigation was withheld (Days 16-19; days of withholding water in parenthesis) or supplied at a fraction of daily evapotranspiration (ET; both 75% and 50% ET)) (Days 15 & 20). Bars represent means ± SEM (n=3). Different letters within panel indicate significant differences according to a one-way ANOVA ($p<0.05$). **b)** Whole-pot gravimetric water content (θ_{pot}) of *P.hortorum* where irrigation was withheld (Days 16-19; days of withholding water in parenthesis) or where irrigation was supplied at a fraction of daily ET (Days 15 & 20). Data are means ± SEM (n=5).

3.2.2 Irrigation Treatments

Irrigation treatments applied were adapted from Chapter 2 (Section 2.2.2). During plant establishment, all pots were weighed using a balance with a 0.1 g resolution (Scout Pro Portable balance, Ohaus, Switzerland) and watered daily to container capacity. Pot weight was measured at 0800 h each day and was used to calculate daily ET, by accounting for any irrigation supplied in the previous 24 h. Immediately prior to starting treatments, all plants were watered at 1600 h until drainage was visible from the bottom of the pot. Plants were then left to freely drain overnight, before being weighed to determine (WW) container capacity. During the treatment period, all plants were watered twice daily (0800 h and 1600 h) to maintain water availability at either WW or treatment level, and to prevent excessive soil drying overnight. Plants were subject to two different deficit irrigation treatments (Fig. 3.2) by completely withholding irrigation for 4 days, or long term application of irrigation at a fraction of plant ET (irrigated at either 75 % or 50 % of WW ET) for 20 d. Irrigation treatments were applied 7 and 9 weeks after germination respectively to ensure that sampling was carried out on plants of the same chronological age (on plants with approximately 50 leaves). A third group of plants were maintained under WW (100 % ET supplied daily) conditions and used as a reference. Sampling occurred in week 9, two weeks after one group of plants had received daily irrigation at a fraction of ET, and one day prior to irrigation being withheld in the remaining group of plants, and experiments were repeated on three occasions. All physiological measurements were taken between 1100 h and 1300 h.

3.2.3 Stomatal (g_s) conductance

g_s was measured as reported in Chapter 2 (Section 2.2.3) using a porometer (Model AP4, Delta-T Devices, Cambridge, UK). Measurements of g_s were carried out over a 4 day period for plants where irrigation was withheld (Days 16-19 of the treatment period), and at Days 15 and 20 for plants receiving irrigation at a fraction of daily ET. WW plants were measured every day.

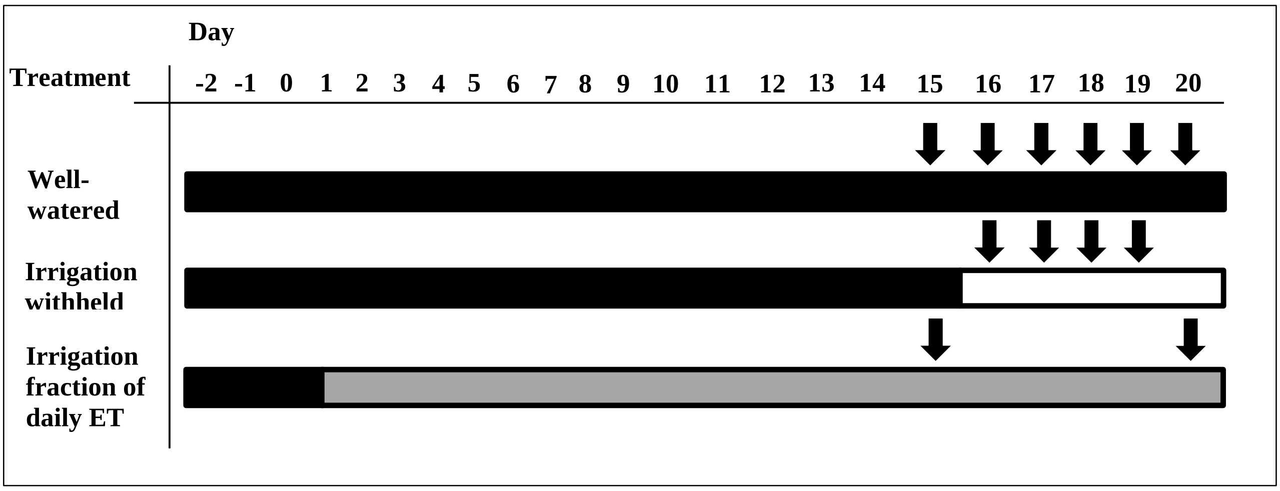


Figure. 3.2. Timetable of irrigation treatments and sampling. Irrigation was applied to plants at a fraction of ET on Day 0, and irrigation was withheld from a separate group of plants on Day 16. Black bar indicates plants were well-watered (WW); grey bar indicates irrigation was supplied at a fraction of daily evapotranspiration (ET); white bar indicates irrigation was withheld. Black arrows indicate the day in which sampling was carried out for plants under each irrigation treatment.

3.2.4. Leaf water (Ψ_{leaf}) potential

Ψ_{leaf} was measured immediately after measuring g_s on the same leaf, using a pressure chamber (Model 3000F01 Plant Water Status Console; Soil Moisture Equipment Corp. Santa Barbara, CA, USA) as reported in Chapter 2 (Section 2.2.4). After xylem sap was collected on the surface of the cut petiole, an overpressure of

0.4 MPa was then applied to each leaf to collect xylem sap. The initial droplets of sap were discarded and each cut petiole was cleaned using filter paper, before sap was sampled for 3 min using a pipette. Xylem sap samples were stored in a 1.5 ml microfuge tube, immediately analysed for pH and different ionic components, and then frozen in liquid nitrogen. To investigate whether there were differences between localised and whole leaf Ψ_{leaf}, an additional group of plants were either subject to irrigation supplied at a fraction of daily ET, or irrigation withheld. Whole leaf Ψ_{leaf} measurements were carried out as described previously. Localised Ψ_{leaf} was measured by thermocouple psychrometry according to Martin-Vertedor and Dodd, 2011 at two leaf positions by excising 8mm diameter leaf discs from each side of the leaf where g_s was measured.

3.2.5 *Xylem sap analysis*

After measuring Ψ_{leaf}, sap samples from each leaf were immediately assayed using a twin compact pH meter (Model B-212), and Ca^{2+} (Model B-751) and NO_3^- (Model B-34X) ion-selective electrodes (Horiba Instruments Ltd, Northampton, UK). Sap samples were removed from the probe after each measurement, and returned to the microfuge tube prior to freezing and storage for further analysis. $[X\text{-}ABA]_{leaf}$ were determined by radioimmunoassay with the MAC252 monoclonal antibody (Quarrie et al., 1988).

3.2.6 Soil water status

After measuring g_s and Ψ_{leaf}, the growth substrate (including plant roots) was removed from the pot and was weighed, dried in the oven for 7 days, and then reweighed to calculate gravimetric soil water content (θ_{pot}; Fig. 3.1b). To assess the variation in moisture status within the soil profile, soil moisture content (θ) was measured using an ML2x ThetaProbe interfaced with a HH2 moisture meter (Delta-T Devices; Cambridge, England) at 5 cm and 10 cm below the soil surface, over a 4 day period when plants were either irrigated with a fraction of plant daily ET, or when irrigation was withheld. Measurements of θ at each depth were then used to calculate θ_{pot} via a calibration curve.

3.2.7 *Transpiration bioassay of detached leaves*

The youngest, fully expanded leaves from individual WW plants that had been kept in the dark overnight were removed using a razor blade, and the petiole was immediately recut under distilled water to avoid embolism. Leaves were then immediately transferred to individual 10 ml conical flasks containing artificial xylem sap solution, and allowed to stabilise in the dark for 2 h in a growth chamber. The top of each conical flask was sealed with parafilm to reduce evaporative losses (but with a small cut to allow access for the petiole), and contained 10 ml of artificial xylem sap solution : 1 mM KH_2PO_4, 1 mM K_2HPO_4, 1 mM $CaCl_2$, 0.1 mM $MgSO_4$, 3 mM KNO_3, and 0.1 mM $MnSO_4$ (Dodd et al., 2003). After 2h, leaves were then transferred to labelled conical flasks containing artificial sap augmented with 0, 10 or 50 nM ABA, and were returned to the controlled growth chamber under artificial metal halide light (HR5005H, Siemens, Munich, Germany) delivering 250 µmol m^{-2} s^{-1} at 24 °C, with a VPD of 0.62±0.05 kPa. Each flask (and leaf) was weighed initially with a four point balance, and then re-weighed every 50 min over a 200 min period. After the assay, g_s of all leaves was measured. Individual leaf area was then measured using a leaf area meter (LI-3100C Area Meter, LI-COR Inc., Lincoln, NE, USA) to calculate transpiration rate.

3.2.8 Statistics

Data from a single representative experiment are reported. Differences between irrigation treatments, and between days 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 were Log transformed and re-tested. If values were again found not to be normally distributed, a non-parametric Kruskal-Wallis test determined whether significant differences occurred between treatments and days. Analysis of Covariance (ANCOVA) determined whether irrigation treatment 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.).

3.3 Results

At the time of measurement, whole pot θ_{pot} (Fig. 3.1b) of WW plants was stable throughout the sampling period. Similarly, θ_{pot} was maintained when irrigation was applied at both 75 % and 50 % of plant ET but significantly lower (53% and 29% respectively) than the value in WW soil. Withholding irrigation significantly reduced θ_{pot} within 24 h, which continued to decline (albeit at a slower rate) throughout the experiment. θ_{pot} was also measured in the upper and lower levels of the soil profile (Table. 3.1). When plants received irrigation at a fraction of daily ET, soil moisture was higher within the upper level of the soil than the lower level, but still lower than in WW plants. When irrigation was withheld, soil moisture content decreased rapidly in both the upper and lower levels, with greater effects in the upper levels.

Level	Upper				Lower			
Treatment	Day 16 (0)	Day 17 (1)	Day 18 (2)	Day 19 (3)	Day 16 (0)	Day 17 (1)	Day 18 (2)	Day 19 (3)
WW	3.2±0.1 a	3.0±0.2 a	2.9±0.1 a	2.9±0.1 a	3.0±0.1 a	2.7±0.1 a	2.7±0.1 a	2.6±0.1 a
Irrigation withheld	3.1±0.2 ab	1.0±0.1 b	0.5±0.0 b	0.5±0.0 b	2.9±0.1 a	1.1±0.1 b	0.7±0.0 b	0.7±0.0 b
Irrigation at 75 % ET	2.7±0.2 ab	2.2±0.3 a	2.7±0.3 a	2.6±0.3 a	1.9±0.0 b	1.5±0.3 b	1.1±0.1 b	1.1±0.1 b
Irrigation at 50 % ET	2.1±0.3 b	1.8±0.3 b	2.2±0.3 a	2.2±0.3 a	0.8±0.5 c	0.9±0.4 b	0.2±0.0 c	0.2±0.0 b

Table. 3.1. Gravimetric water content (θ_{pot}; g g^{-1}) of *P.hortorum* plants subject to well-watered conditions (n=5), or from which irrigation was withheld (n=5) or supplied at a fraction of plant daily evapotranspiration (ET) (n=10) over a 4 day period. Sampling was at Days 16-19 after supplying irrigation at a fraction of plant daily ET was first applied, with days of withholding irrigation in parenthesis. Measurements were carried out in the upper (5 cm below the soil surface) and lower (10 cm below the soil surface) levels of the soil profile. Different letters within a column indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ±SEM of all measurements.

In WW plants, average stomatal conductance was 99±16 mmol m^{-2} s^{-1} during the study. Withholding irrigation significantly reduced g_s (by 56 % compared to WW plants) within 24 hours, with further decreases over time (Fig. 3.3a). In plants where irrigation was supplied at a fraction of daily ET, g_s decreased (compared to WW plants) over the two days of sampling by 69 % and 85 % at 75 % ET and 50 % ET, respectively (Fig. 3.3b). Stomatal closure of *P.hortorum* under both deficit irrigation treatments correlated with decreased θ_{pot} (Fig. 3.4). However, the relationship between g_s and θ_{pot} was similar in both deficit irrigation treatments (there was no significant treatment x θ_{pot} interaction) especially at $\theta_{pot} > 1$g g^{-1}.

Xylem sap pH, and xylem NO_3^- and Ca^{2+} concentrations did not change significantly with soil drying irrespective of how irrigation treatments were imposed (Table. 3.2). When irrigation was withheld, $[X\text{-}ABA]_{leaf}$ significantly increased (6-fold) within 24 h of ceasing irrigation, and $[X\text{-}ABA]_{leaf}$ continued to increase over subsequent days (Fig. 3.3c). On both sampling dates, $[X\text{-}ABA]_{leaf}$ significantly increased in plants provided with 50 % ET (Fig. 3.3d). $[X\text{-}ABA]_{leaf}$ increased significantly with decreasing θ_{pot} under both deficit irrigation treatments (Fig. 3.5). However, the contrasting irrigation treatments resulted in a different response (significant treatment x θ_{pot} interaction) suggesting that plants receiving irrigation at a fraction of daily ET show an attenuated $[X\text{-}ABA]_{leaf}$ response compared to those where irrigation was withheld.

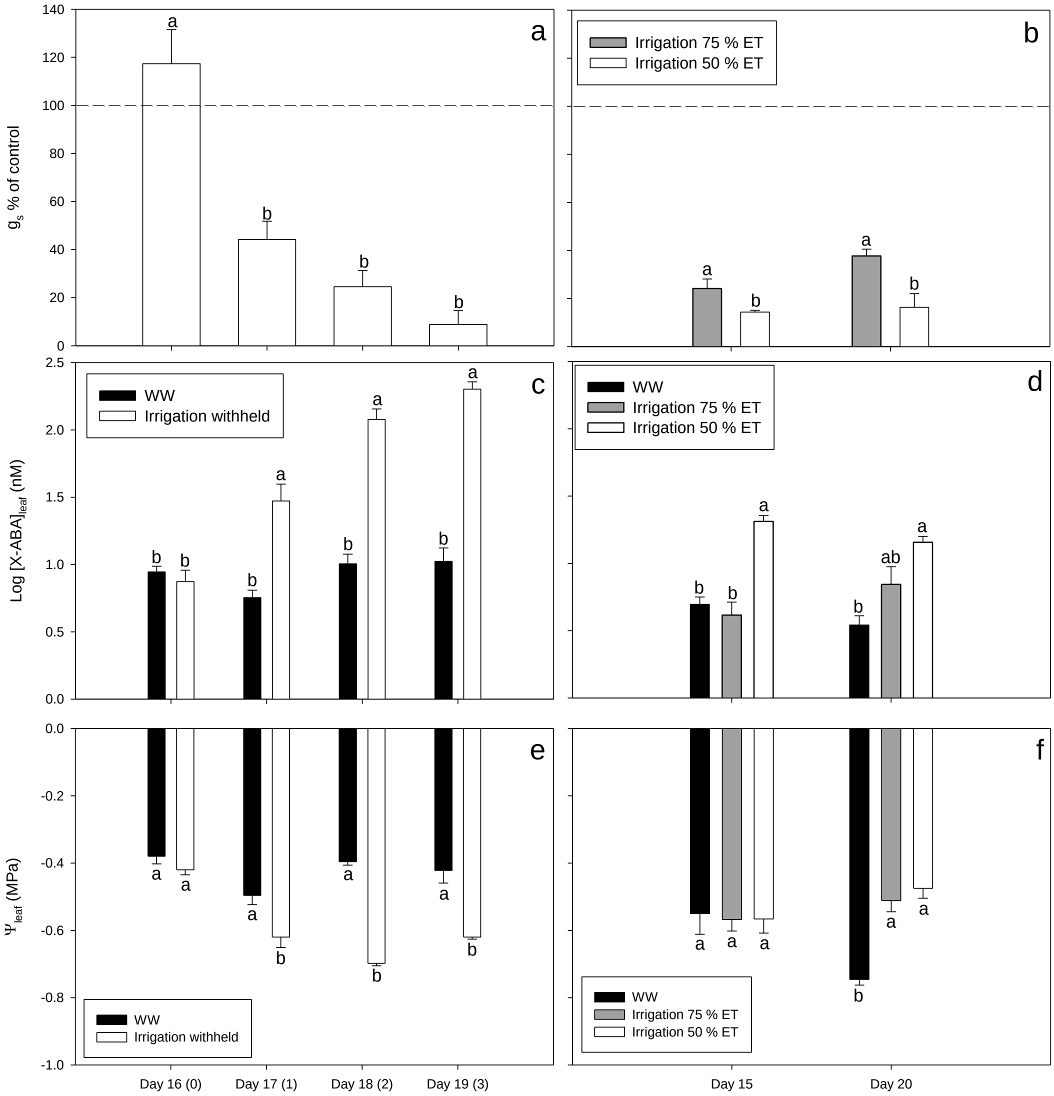


Figure. 3.3. Mean stomatal conductance (g_s) as a percentage of well-watered (WW) controls (a, b), log xylem sap ABA concentrations ($[\text{X-ABA}]_{leaf}$) (c, d) and whole leaf leaf water potential (Ψ_{leaf}) (e, f) in *P.hortorum* plants recorded on each day of sampling where irrigation was withheld (a, c, e) and where irrigation was supplied at a fraction of daily evapotranspiration (ET) (b, d, f). Different letters within a panel indicate significant differences on each day according to a one-way ANOVA ($p<0.05$). Data are means ± SEM (n=5).

Treatment		pH	Ca^{2+} (mM)	NO_3- (mM)
Irrigation withheld		5.9±0.1 a	0.7±0.1 a	0.4±0.0 a
Irrigation at a fraction of daily ET	75 %	6.0±0.5 a	0.5±0.2 a	0.4±0.1 a
	50 %	6.1±0.2 a	0.5±0.3 a	0.4±0.1 a
WW		5.9±0.4 a	0.4±0.1 a	0.4±0.0 a

Table. 3.2. Mean xylem sap pH, calcium (Ca^{2+}; mM) and nitrate (NO_3^-; mM) of *P.hortorum* plants subject to well-watered (WW) conditions (n=20), or from which irrigation was withheld (n=20) or supplied at a fraction of plant daily evapotranspiration (ET) (n=10). Different letters within a column indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ±SEM of all measurements carried out during the sampling period for both deficit irrigation treatments (over a 4 day period when irrigation was withheld, or on 2 days when irrigation was supplied at a fraction of daily ET).

When irrigation was withheld, Ψ_{leaf} significantly decreased within 24 h of treatments being applied, which persisted over the sampling period (Fig. 3.3e). In contrast, plants receiving irrigation at a fraction of daily ET showed no variation in Ψ_{leaf} on the first day of sampling, and on the second day of sampling (Day 20) WW plants actually had a significantly lower Ψ_{leaf} than those where irrigation was applied at a fraction of daily ET (Fig. 3.3f). The decreased Ψ_{leaf} of WW plants (Fig. 3.3f) is likely explained by a higher rate of transpiration, where ET was 3.0±0.2 µl H_2O cm^{-2} h^{-1} and 5.0±0.4 µl H_2O cm^{-2} h^{-1} for WW plants on Days 15 and 20 respectively, possibly due to differences in VPD on these two days. Consequently, the two irrigation treatments differed in the response of Ψ_{leaf} to θ_{pot}, such that Ψ_{leaf} decreased as water availability decreased when irrigation was withheld, whilst no significant relationship was found in plants when irrigation was supplied at a fraction of daily ET (significant treatment x θ_{pot} interaction; Fig. 3.6).

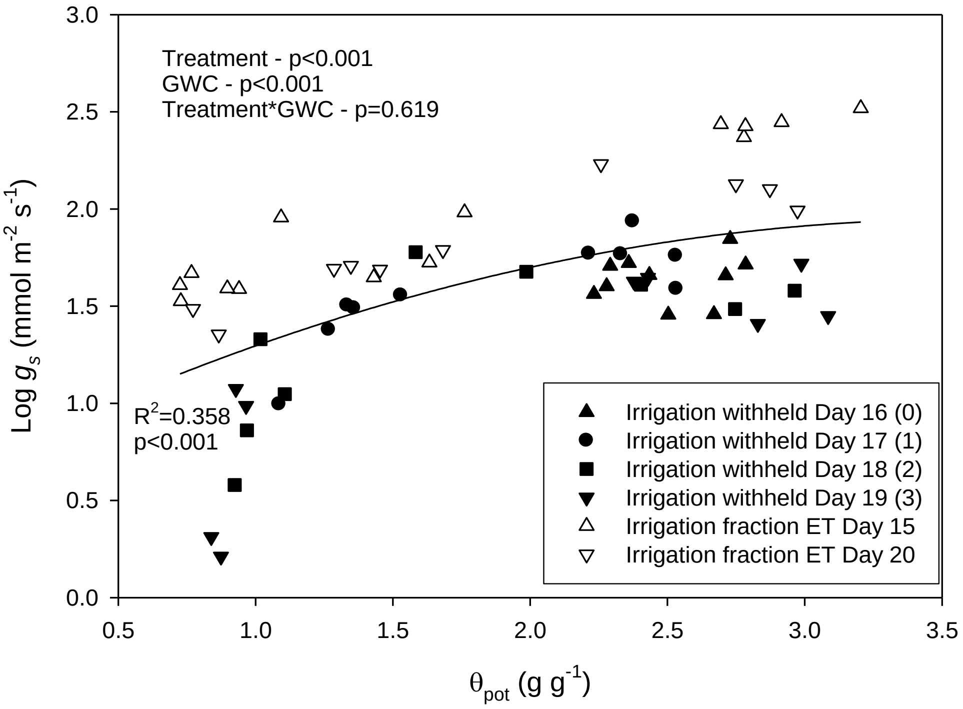


Figure. 3.4. Log stomatal conductance (g_s) of *P.hortorum* in drying soil (whole-pot gravimetric water content - θ_{pot}) under different irrigation treatments. Closed circles show data from plants where irrigation was withheld (n= 38) on Days 16-19 (days of withholding irrigation in parenthesis); open circles show data from plants where irrigation was supplied at a fraction of daily evapotranspiration (ET) (n= 28) on Days 15 & 20. Data points are paired individual samples, regressions line is fitted and P values reported. P values from ANCOVA reported.

Similar relationships were observed when whole leaf and localised measurements of Ψ_{leaf} were compared under each irrigation treatment (Table. 3.3), the only exception being that no decrease in Ψ_{leaf} was observed at the localised level when irrigation was withheld. When irrigation was withheld, decreased whole leaf Ψ_{leaf} correlated with decreased g_s, whilst in plants where irrigation was supplied at a fraction of daily ET, Ψ_{leaf} significantly increased as g_s decreased although the correlation was weak ($R^2 = 0.19$, $p = 0.02$; Fig. 3.7). Decreased Ψ_{leaf} was correlated with increased [X-ABA]$_{leaf}$ in plants where irrigation was withheld, but no relationship was found between Ψ_{leaf} and $[X\text{-}ABA]_{leaf}$ when irrigation was supplied at a fraction of daily ET (Fig. 3.8). Under both deficit irrigation treatments, relationships between Ψ_{leaf}, and both g_s and $[X\text{-}ABA]_{leaf}$ differed depending whether irrigation was supplied at a fraction of daily ET, or withheld (significant treatment x Ψ_{leaf} interactions).

Treatment	Moisture range	Whole leaf Ψ_{leaf} (MPa)	Localised Ψ_{leaf} (MPa)
Irrigation withheld	3.0 – 3.5 g g^{-1}	-0.62±0.02a	-1.08±0.05a
	1.0 – 1.5 g g^{-1}	-0.78±0.04b	-0.97±0.08a
Irrigation at a fraction of daily ET	3.0 – 3.5 g g^{-1}	-0.62±0.02b	-1.08±0.05b
	1.0 – 1.5 g g^{-1}	-0.46±0.03a	-0.83±0.04a

Table. 3.3. Whole leaf (n= 8) and localised (leaf disc; n=16) leaf water potential (Ψ_{leaf}) measured over two soil moisture ranges in plants where irrigation was either withheld, or supplied at a fraction of daily evapotranspiration (ET). Different letters within each treatment and Ψ_{leaf} measurement indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ±SEM of all measurements carried out during the sampling period for both deficit irrigation treatments.

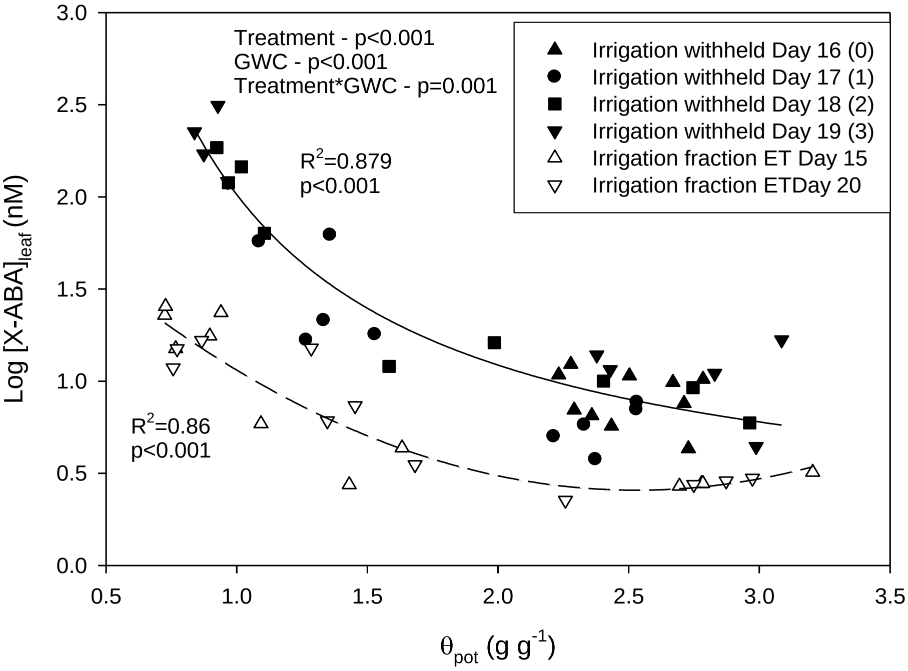


Figure. 3.5. Log leaf xylem abscisic acid concentration ($[\text{X-ABA}]_{\text{leaf}}$) of *P.hortorum* in drying soil (whole-pot gravimetric water content - θ_{pot}) under different irrigation treatments. Closed symbols show data from plants where irrigation was withheld (n= 38) on Days 16-19 (days of withholding irrigation in parenthesis); open symbols show data from plants where irrigation was supplied at a fraction of daily evapotranspiration (ET) (n= 23) on Days 15 & 20. Different symbols indicate each day of sampling. Data points are paired individual samples, regressions lines fitted and P values reported. P values from ANCOVA reported.

Stomatal conductance declined similarly with increasing $[\text{X-ABA}]_{\text{leaf}}$ (Fig. 3.9) under both deficit irrigation treatments (no significant treatment x ABA interaction), explaining 76% of the variation in g_s. To determine whether this relationship was causal, detached leaves were fed synthetic ABA in a growth chamber, which decreased the transpiration rate (TR) as ABA concentrations increased (Fig. 3.10a). Higher ABA concentrations decreased TR more rapidly, with significant decreases in leaf TR (compared to control leaves) detected after 50 and 100 min for 50 nM and 10 nM ABA respectively. At these concentrations, TR decreased by 22% and 39 %

(mean data from 150-200 min) in leaves fed with 10 nM and 50 nM ABA respectively (Fig. 3.10b).

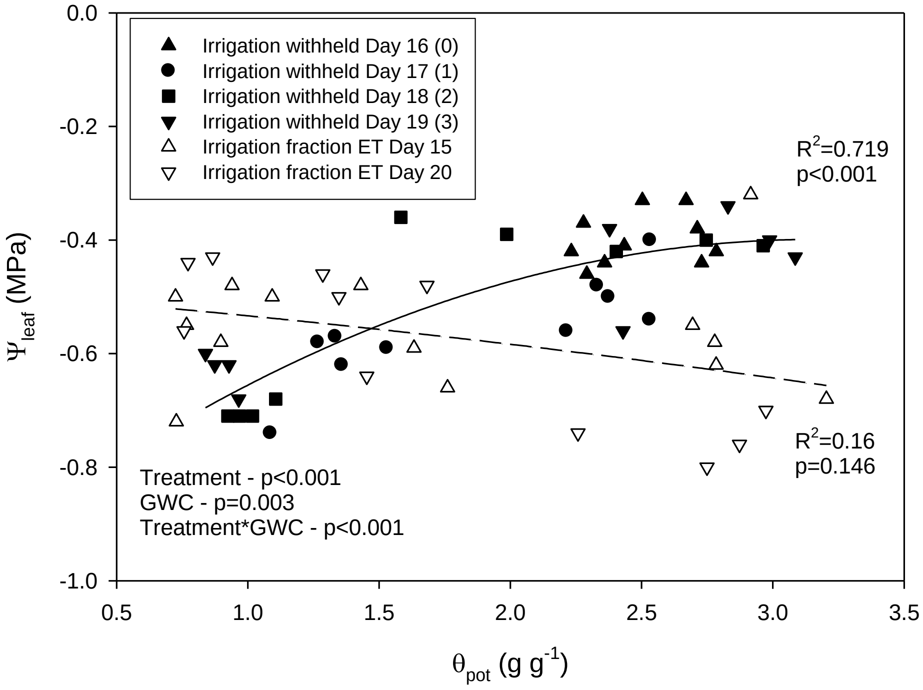


Figure. 3.6. Whole leaf leaf water potential (Ψ_{leaf}) of *P.hortorum* in drying soil (whole-pot gravimetric water content - θ_{pot}) under different irrigation treatments. Closed symbols show data from plants where irrigation was withheld (n= 38) on Days 16-19 (days of withholding irrigation in parenthesis); open symbols show data from plants where irrigation was supplied at a fraction of daily evapotranspiration (ET) (n= 28) on Days 15 & 20. Different symbols indicate each day of sampling. Data points are paired individual samples, regressions lines fitted and P values reported. P values from ANCOVA reported.

At the end of the TR bioassay, g_s of leaves provided with 10 nM and 50 nM ABA was significantly decreased by 30 % and 62 % respectively compared to control leaves (Fig. 3.10c). When g_s of detached leaves was compared to whole plant g_s within a similar range of $[X\text{-}ABA]_{leaf}$ (±5 nM of each ABA concentration used in the bio-assay), g_s was significantly lower in intact plants at lower ABA concentrations (124±18 mmol m^{-2} s^{-1} and 207±29 mmol m^{-2} s^{-1}, for whole and detached leaves respectively). However, this is a likely consequence of the higher VPD in the glasshouse (1.7±0.2 kPa) than the growth chamber (0.6±0.1 kPa). In contrast, there were no significant differences in g_s between detached leaves and attached leaves at $[X\text{-}ABA]_{leaf}$ concentrations of 50 nM (Fig. 3.10c).

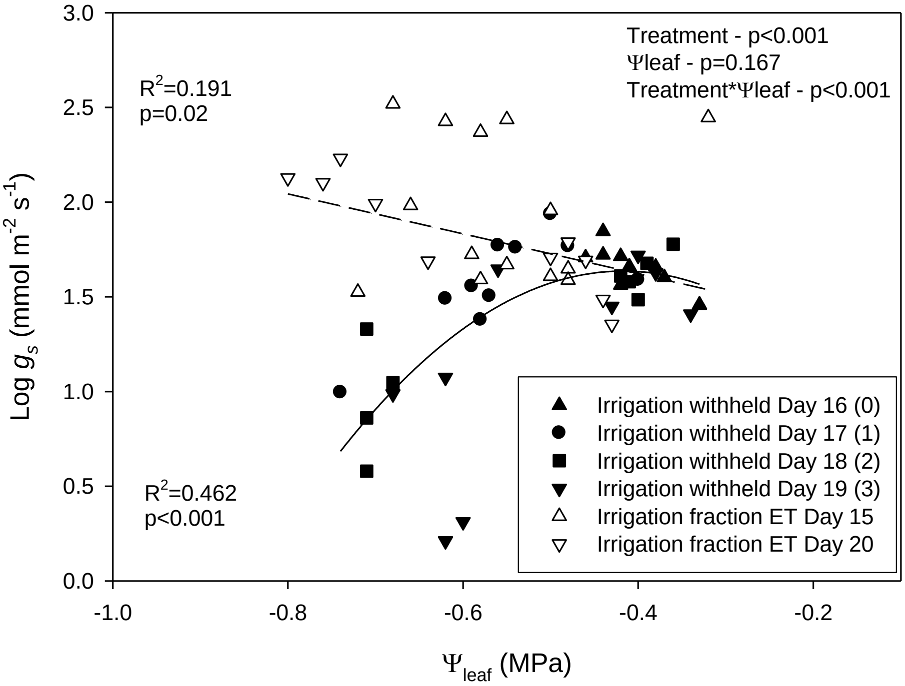


Figure. 3.7. Log stomatal conductance (g_s) of *P.hortorum* in response to whole leaf leaf water (Ψ_{leaf}) potential under different irrigation treatments. Closed symbols show data from plants where irrigation was withheld (n= 38) on Days 16-19 (days of withholding irrigation in parenthesis); open symbols show data from plants where irrigation was supplied at a fraction of daily evapotranspiration (ET) (n= 28) on Days 15 & 20. Different symbols indicate each day of sampling. Data points are paired individual samples, regressions lines fitted and P values reported. P values from ANCOVA reported.

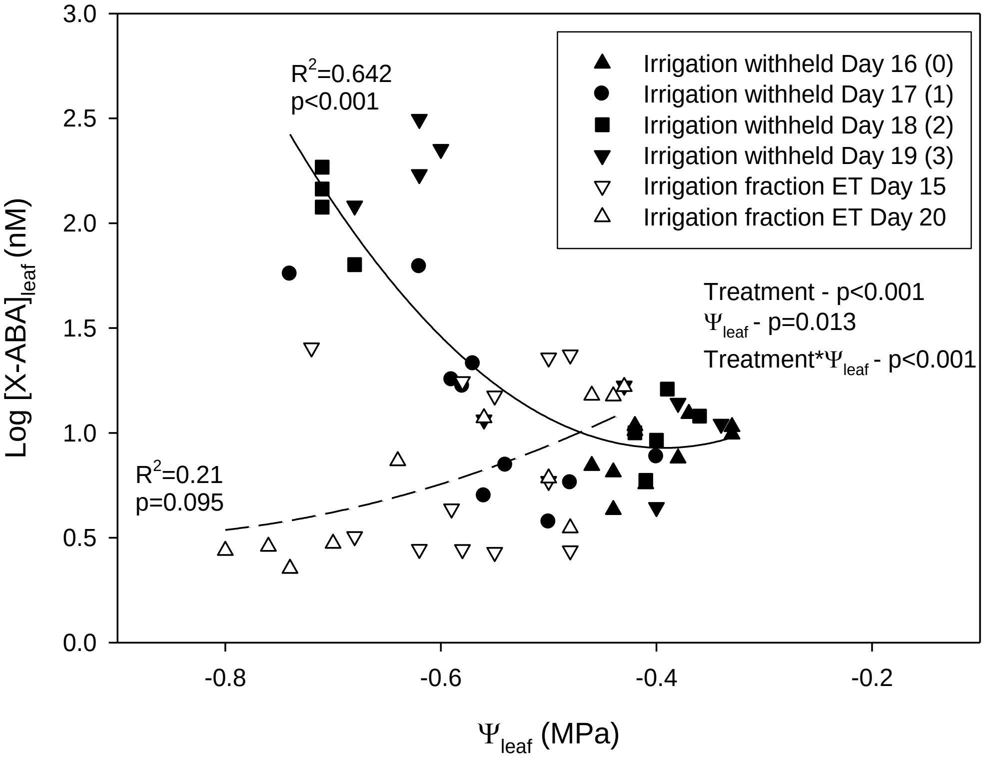


Figure. 3.8. Log xylem sap abscisic acid concentration ($[X\text{-}ABA]_{leaf}$) of *P.hortorum* in response to whole leaf leaf water potential (Ψ_{leaf}) under different irrigation treatments. Closed symbols show data from plants where irrigation was withheld (n= 38) on Days 16-19 (days of withholding irrigation in parenthesis); open symbols show data from plants where irrigation was supplied at a fraction of daily evapotranspiration (ET) (n= 23) on Days 15 & 20. Different symbols indicate each day of sampling. Data points are paired individual samples, regressions lines fitted and P values reported. P values from ANCOVA reported.

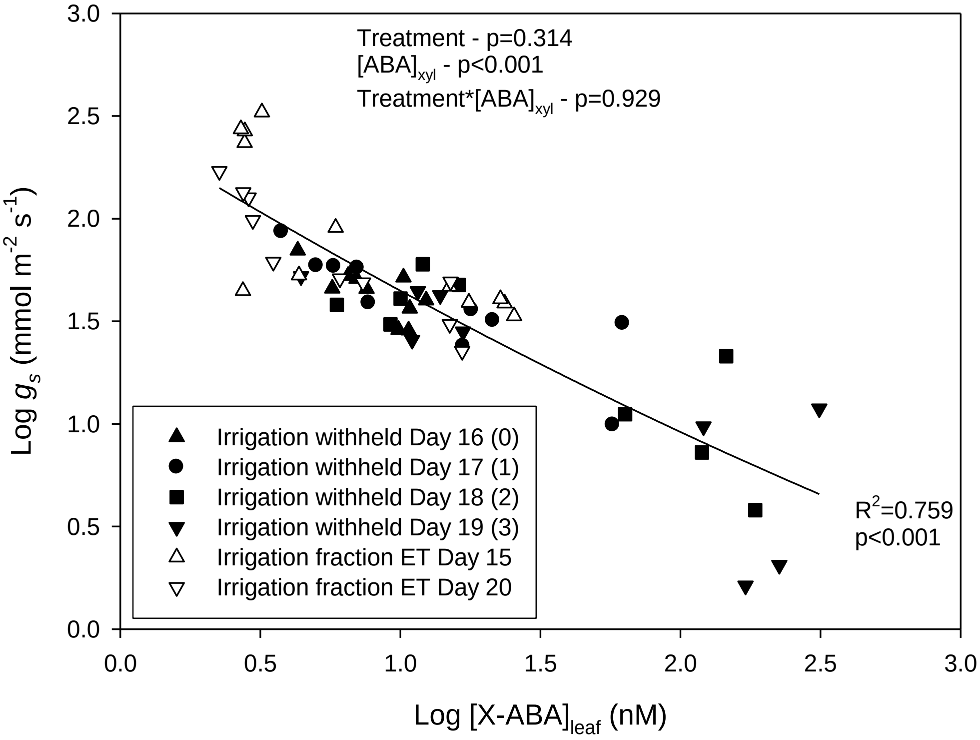


Figure. 3.9. Log stomatal conductance (g_s) of *P.hortorum* in response to log xylem sap abscisic acid concentration ([X-ABA]$_{leaf}$) under different irrigation treatments. Closed symbols show data from plants where irrigation was withheld (n= 38) on Days 16-19 (days of withholding irrigation in parenthesis); open symbols show data from plants where irrigation was supplied at a fraction of daily evapotranspiration (ET) (n= 23) on Days 15 & 20. Different symbols indicate each day of sampling. Data points are paired individual samples, regressions line is fitted and P values reported. P values from ANCOVA reported.

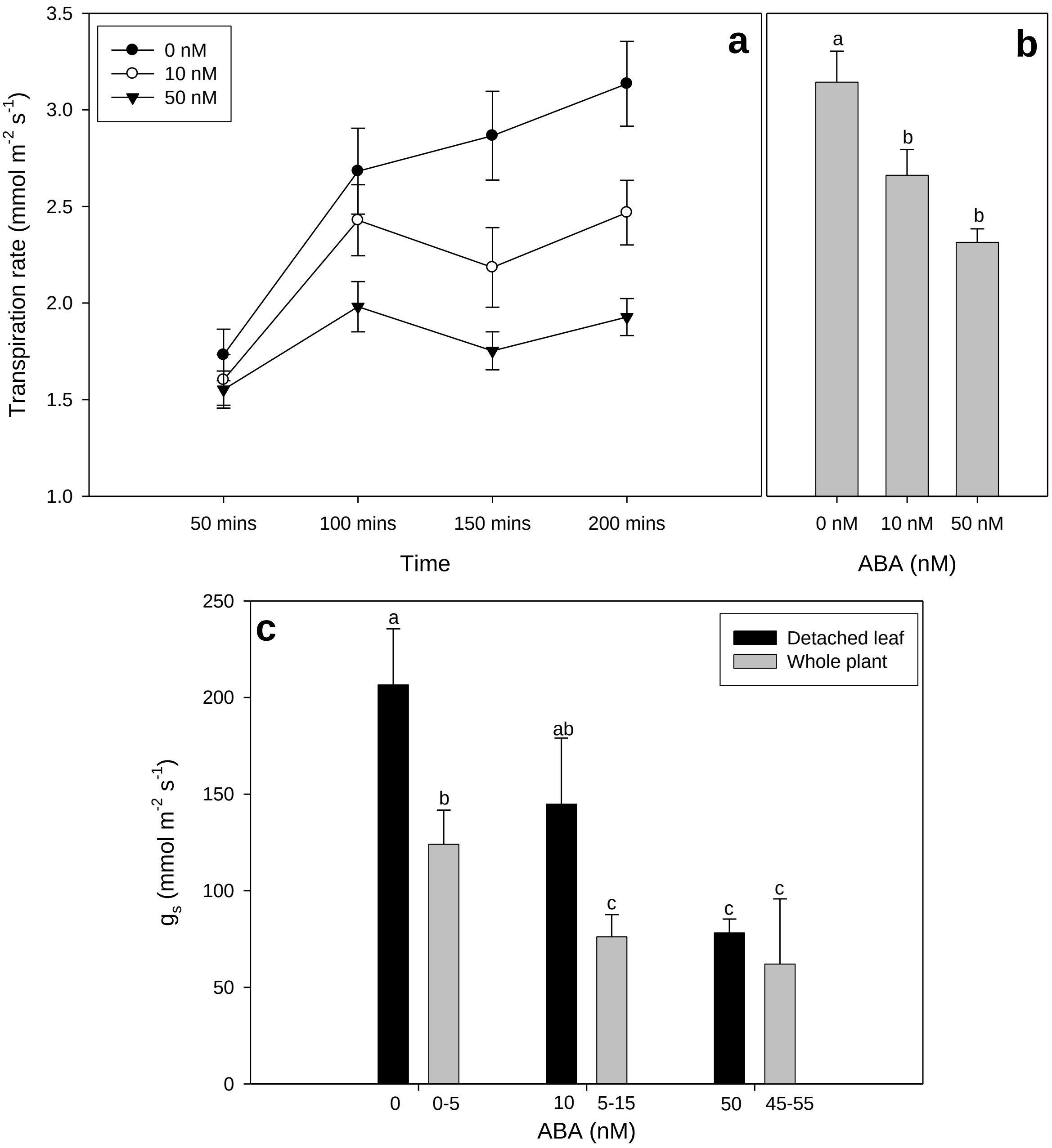


Figure. 3.10. a) Transpiration rate of detached *P.hortorum* leaves fed artificial xylem sap containing abscisic acid (ABA) concentrations at 0 nM (filled circles), 10 nM (open circles) or 50 nM (closed triangles) (n=10). **b)** Mean transpiration rate from 150-200 minutes (n=20). **c)** Mean stomatal conductance (g_s) from both detached leaves (n=5) after the transpiration bio-assay, and from whole plants (n=10) with comparable concentrations of leaf xylem abscisic acid concentrations ($[X\text{-}ABA]_{leaf}$) to those used in the bio-assay (±5 nM). Different letters in a panel indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ± SEM.

3.4 Discussion

Although stomatal closure in response to soil drying is conserved across different irrigation treatments (Fig. 3.4), there may be a divergence in the signalling mechanism involved (cf. Figs. 3.5-8). While stomatal closure can increase Ψ_{leaf} in some species (Dodd et al., 2009, Kudoyarova et al., 2007), there is also evidence that decreased Ψ_{leaf} can directly cause stomatal closure in others (Saliendra et al., 1995). Stomatal closure accompanied by decreased Ψ_{leaf} when irrigation was withheld (Fig. 3.7) implies a regulatory role for Ψ_{leaf}. However, irrigation supplied at a fraction of daily ET allowed plants to acclimate to decreased soil water availability by maintaining Ψ_{leaf} irrespective of whether Ψ_{leaf} was measured in whole leaves or just at the point that g_s was measured (Fig. 3.3f; Table. 3.3). This suggests that Ψ_{leaf} is not a key regulator of g_s in *P.hortorum*. Rather, it is likely that another mechanism initiates stomata closure, thereby maintaining leaf turgor and preventing xylem embolism associated with leaf water deficit (Zhang et al., 2006).

Xylem nutrient concentrations have been implicated in regulating stomatal conductance under water deficits (Dodd, 2005, Ernst et al., 2010). However, xylem sap pH, and the concentrations of NO_3^- and Ca^{2+} in the xylem sap did not change in response to either irrigation treatment (Table. 3.2). As pH, NO_3^- and Ca^{2+} were assayed in sap samples from leaves that had been measured for g_s (paired sampling), this suggests that other chemical signal(s) regulate stomatal responses to soil drying. ABA is widely accepted as a key long-distance chemical signal of soil drying (Dodd, 2005, Schachtman and Goodger, 2008), and was therefore a likely candidate for initiating stomatal closure. Under both deficit irrigation treatments, $[X\text{-}ABA]_{leaf}$ increased as θ_{pot} decreased (Figs. 3.3b & c, 3.5), as in other studies (Correia and

Pereira, 1995, Jarvis and Davies, 1997). Moreover, the similar decrease in g_s as [X-ABA]$_{leaf}$ increased in both intact plants exposed to soil drying, and detached leaves fed synthetic ABA via the transpiration stream (Fig. 3.10) supports the hypothesis that stomatal closure in *P.hortorum* during periods of reduced soil water availability is ABA-mediated.

Similar stomatal closure in response to 50nM ABA, both *in vivo* and in detached leaves, suggests a limited role for other antitranspirants. Indeed, supplying ABA to detached leaves explained 76 % of the variation in g_s (and a 50 % reduction in g_s *in vivo* at the same [X-ABA]$_{leaf}$ when plants were exposed to soil drying; Fig. 3.10c). Although well-watered plants had a lower g_s than detached leaves that were supplied with an ABA-free artificial xylem sap for 5.5 h (Fig. 3.10c), this is likely due to differences in VPD between the glasshouse environment (1.7±0.2 kPa) and the growth chamber (0.6±0.1 kPa). Indeed, in well-watered plants, increased VPD is associated with both reduced stomatal conductance and increased leaf ABA (Bunce, 2006). Taken together, these observations strongly suggest that ABA is a central regulator of stomatal closure in *P.hortorum* in response to reduced soil moisture availability.

When plants were irrigated at a fraction of daily ET, [X-ABA]$_{leaf}$ was lower than when irrigation was withheld (Fig. 3.5) at the same soil moisture levels. This indicates differences in the way *P.hortorum* regulates ABA signalling under contrasting irrigation treatments. However, the mechanism(s) underlying these differences may vary depending upon whether ABA is acting as a localised signal or as a root-to-shoot signal.

ABA may increase locally, with synthesis occurring primarily in the leaves in response to a leaf water deficit (Hartung et al., 2002). Support for a localised signal comes from tomato grafting studies where wild-type scions show increased leaf (Holbrook et al., 2002) and xylem (Dodd et al., 2009) ABA concentration, even when grafted to ABA-deficient rootstocks. Also, ABA catabolism can increase in response to rehydration (Zhang et al., 2006) and a more positive Ψ_{leaf} (Dodd, 2005), which may explain the attenuated $[X\text{-}ABA]_{leaf}$ when irrigation was supplied at a fraction of ET. Furthermore, in Arabidopsis, alleviating leaf water deficit by the localised application of water directly onto leaf surfaces decreased leaf ABA concentration as indicated by down-regulation of ABA-reporter genes (Christmann et al., 2007). Indeed, the attenuated $[X\text{-}ABA]_{leaf}$ response observed when irrigation was supplied at a fraction of daily ET in *P.hortorum* may therefore be due to a more positive Ψ_{leaf} (Fig. 3.8) and lower leaf ABA concentrations, since leaf xylem sap collected by pressurising detached leaves may contain substantial volumes of symplastically-derived sap (Borel and Simonneau, 2002). Further measurements are needed to resolve this mechanism, by measuring leaf ABA concentrations and expression of ABA biosynthesis and catabolism genes, as well as alternative methods to collect leaf xylem sap that minimise symplastic contributions (Holbrook et al., 2002, Netting et al., 2012).

Alternatively, the higher $[X\text{-}ABA]_{leaf}$ observed when irrigation was withheld might be due to similar xylem loading of ABA by root cells but into a smaller sap flux, increasing xylem ABA concentrations (Hartung et al., 2002, Jokhan et al., 1996). Certainly, when irrigation was withheld, plants had lower whole plant evapotranspiration than the 50 % ET irrigation treatment at similar soil moisture (mean values of 1.9±0.2 ml H_2O h^{-1} and 2.2±0.2 ml H_2O h^{-1} respectively).

Furthermore, measurements of root [X-ABA]$_{leaf}$ (at sap flow rates equivalent to whole plant transpiration - Jokhan et al. (1996)) are required to sustain the argument that root ABA export differs according to whether irrigation is withheld or a supplied at fraction of daily ET, but these will be difficult in *P.hortorum* as multiple stems arise from the shoot base.

A further potential explanation is that differences in soil water distribution under the different irrigation treatments (Table. 3.1) might affect root ABA accumulation (Puértolas et al., 2013). Despite whole pot θ_{pot} being similar under both deficit irrigation treatments, soil moisture decreased uniformly throughout the soil profile when water was withheld, whilst daily irrigation at a fraction of ET resulted in higher moisture availability within the upper levels (Table. 3.1). Roots exposed to different levels of soil drying in split pot experiments (Khalil and Grace, 1993) or in vertical gradients of moisture depletion (Zhang and Davies, 1990) have locally increased root ABA concentration. Root density is typically higher in the upper soil layers, which generally results in a higher rate of soil drying (Sharp and Davies, 1985). However, as soil moisture was maintained in the upper layers when irrigation was supplied at a fraction of daily ET, it is possible that the roots in this region have lower ABA concentrations, thereby attenuating the ABA signal from the lower roots by contributing a greater proportion to the total sap flux (Dodd et al., 2008, Puértolas et al., 2013). Similarly, Zhang and Tardieu (1996) suggested that whilst root tips are the primary site of ABA synthesis, the overall mass of older roots may contribute more to root-sourced ABA flux. Furthermore, in Barley plants exposed to PRD, when a greater proportion of roots were in drying soil there was a higher leaf ABA concentration independent of any change in Ψ_{leaf} (Martin-Vertedor and Dodd, 2011). Although measurements of root ABA accumulation are required to substantiate this hypothesis, this was not possible in the current study due to the difficulty of separating fine *P.hortorum* roots from a substrate high in organic matter.

3.5 Conclusions

This study provides evidence that different irrigation treatments significantly affect the relationships between plant gas exchange, ABA status and leaf water status. Ψ_{leaf} decreased in response to reduced water availability only when irrigation was withheld, suggesting it is unlikely to act as a universal regulator of g_s. Instead, when a fraction of crop ET was supplied daily, Ψ_{leaf} increased with stomatal closure suggesting that g_s regulates leaf water status. Further, $[X\text{-}ABA]_{leaf}$ appears to be the central 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 evapotranspiration. Since increased $[X\text{-}ABA]_{leaf}$ can be responsible for stomatal limitation of photosynthesis (Wilkinson and Davies, 2002), attenuating ABA signalling by daily irrigation may maximise carbon gain thereby improving crop water use efficiency as seen in Chapter 2 (Fig. 2.7a & b).

Chapter 4 – Frequent soil drying and re-wetting attenuates root ABA concentrations throughout the soil profile, thereby decreasing long-distance ABA signalling in tomato

4.1 Introduction

Soil drying, whether naturally occurring or artificially imposed, commonly results in heterogeneity in soil moisture content. Higher root densities in (and thus water uptake from) the upper sections of soil, along with evaporative losses from the soil surface, results in surface drying, with higher moisture levels typically found further down the soil profile (Zhang and Davies, 1989b, Sharp and Davies, 1985). The use of irrigation techniques such as PRD can also result in distinct wet and dry zones in the soil (Stoll et al., 2000).

Soil moisture heterogeneity can affect the spatial distribution of the concentration of ABA in the roots ($[ABA]_{root}$), such that $[ABA]_{root}$ can be correlated with localised moisture availability in plants grown under PRD (Khalil and Grace, 1993, Stoll et al., 2000). Withholding irrigation from maize caused a progressive increase in $[ABA]_{root}$ as soil moisture availability in a soil column decreased, with considerable vertical differences in local $[ABA]_{root}$ (Zhang and Davies, 1989a). Alternately, although bulk $[ABA]_{root}$ increased when bean (*Phaseolus vulgaris*) was grown in soil columns, the increase was homogenous and was not accentuated in specific regions of drier soil (Puértolas et al., 2013, Trejo and Davies, 1991). However, in potato, drying the upper part of the soil profile increased local $[ABA]_{root}$ compared to deeper in the soil where soil moisture was higher (Puértolas et al., 2014). Thus there is considerable variation in the spatial distribution of root ABA accumulation in different species.

Development of alternative, water-saving irrigation techniques (such as in Chapters 2, 3) may modify vertical soil moisture profiles, particularly in pot grown plants (Chapter 3, Table. 3.1), which may alter the mechanisms of root-to-shoot signalling in planta. For instance, a reversal of the natural vertical soil moisture profile described above (i.e. upper soil wet, lower soil dry) would result in a higher density of mature roots in wet soil near the surface of the pot, whilst younger roots at the base of the pot would experience gradual soil drying over time. This can be achieved by varying the frequency and volume with which irrigation is applied (Chapters 2 and 3), and may maximise plant water use efficiency (Chapter 2, Fig. 2.7). Furthermore, it has been suggested that older roots contribute relatively more to the overall flux of root-sourced ABA (Zhang and Tardieu, 1996), which may significantly modify ABA signalling under soil drying.

ABA synthesis increases in roots in drying soil (Sauter et al., 2001), and this ABA can be transported to the shoot to induce processes such as stomatal closure (Hartung et al., 2002), whilst roots in wet soil continue to take up water, thereby maintaining plant water status. Withholding irrigation created vertical soil moisture gradients that increased $[\text{X-ABA}]_{\text{leaf}}$ in pot grown maize, thereby inducing stomatal closure (Zhang and Davies, 1990). Moreover, artificially imposing soil moisture heterogeneity by hydraulically segmenting the root system and withholding irrigation from certain segments increased leaf ABA ($[\text{ABA}]_{\text{leaf}}$) in lupin, whilst also reducing growth (Gallardo et al., 1994). Furthermore, increased stem xylem ABA concentrations (Liang et al., 1997), and root xylem ABA ($[\text{X-ABA}]_{\text{root}}$) has been related to changes in shoot physiology, such as decreased gas exchange and leaf expansion (Dodd, 2005). In contrast however, sunflower plants grown under PRD had increased $[\text{X-ABA}]_{\text{root}}$ without an increase in $[\text{X-ABA}]_{\text{leaf}}$ and limited stomatal response (Dodd et al., 2008), suggesting there may be species-specific responses to heterogeneous soil drying.

Long-distance ABA transport and signalling under soil drying likely also depends on plant water status and soil hydraulic properties. A tight balance is maintained between ABA biosynthesis and degradation (Nambara and Marion-Poll, 2005), and as such, frequent re-irrigation events may stimulate ABA catabolism in the roots and shoot (Zhang and Davies, 1989b, Zhang et al., 2006). The distribution of moisture within the soil profile, and hysteresis of the substrate moisture retention curve as a consequence of frequent drying and re-wetting, can influence root development and distribution (Whitmore and Whalley, 2009), and alter root water status (Rhizopoulou and Davies, 1991). In turn, $[ABA]_{root}$ and ABA accumulation rate increased linearly as Ψ_{root} decreases (Simonneau et al., 1998).

To date, the impact of higher moisture in the upper layers of the soil profile (as occurs during frequent deficit irrigation – Chapter 3, Table. 3.1) on localised $[ABA]_{root}$, and in turn its effects on root-to-shoot signalling, and shoot physiology, remains untested. When split-root plants were simultaneously exposed to both dry and wet soil (in separate pots), measurements of sap fluxes and $[ABA]_{root}$ from the different sections of the root zone better explained $[X\text{-}ABA]_{leaf}$ than simply averaging the $[X\text{-}ABA]_{root}$ from both parts of the system (Dodd et al., 2008). Furthermore, exposing more roots within the dry side of a horizontal split pot system increased $[ABA]_{leaf}$, independently of any change in leaf water potential (Ψ_{leaf}) (Martin-Vertedor and Dodd, 2011). However, in bean grown with vertical soil moisture gradients, both g_s and $[X\text{-}ABA]_{root}$ were well correlated with $[ABA]_{root}$, suggesting that the effect of soil drying on the shoot may act independently of irrigation placement (and is instead a function of homogenous changes in Ψ_{root})

(Puértolas et al., 2013). This suggests that vertical *versus* lateral soil moisture heterogeneity can alter root-to-shoot ABA signalling.

The current study aims to establish the mechanism(s) by which irrigation supplied at a fraction of daily ET (compared to when irrigation was withheld) attenuates root-to-shoot ABA signalling (Chapter 2, Fig. 3.5; Boyle et al., 2015). Initial studies using *P.hortorum* found it was impossible to collect root xylem sap in this species due to the canopy being just above the soil profile, whilst root samples could also not be accurately collected due to fine root distribution and the highly organic, peat based substrate. Therefore, tomato (*Solanum lycopersicum*) was selected as a more suitable species for root sampling. Different frequencies of soil drying, withholding irrigation *versus* daily application of irrigation as a fraction of crop ET needs, caused large changes in the distribution of moisture within the soil profile in *P.hortorum*. This heterogeneity in moisture distribution was investigated in tomato, to establish whether irrigation frequency altered soil moisture, Ψ_{root} and ABA accumulation, and whether this affected shoot physiology. Finally, $[X\text{-}ABA]_{leaf}$ and $[X\text{-}ABA]_{root}$ were modelled in aiming to predict the impact of variability in soil moisture and contributions of water uptake from different parts of the soil profile on long-distance ABA signalling in different irrigation treatments.

4.2 Materials and Methods

4.2.1 Plant culture

Tomato (*Solanum lycopersicum* L. cv. Ailsa Craig) seeds were germinated in 84 unit plug trays (3.5 x 3.5 x 5 cm units) in a peat based substrate (Levington M3, Everris, UK) at 27°C, and in complete darkness to induce etiolation of the hypocotyl to ensure plants could easily fit in a pressure chamber. A moisture release curve for this substrate has been published previously (Dodd et al., 2010). Once seedlings were etiolated (1 week, ~6 cm from soil surface to cotyledon), they were transferred to individual 6.9 cm x 24 cm (0.9 l) cylindrical pots containing the same substrate (Fig. 4.1). Pots were plastic tubes with a mesh base designed to fit in a pressure chamber, and were separated in two lengthwise to allow access to the soil and roots (Puértolas et al., 2013). Experiments were carried out in a naturally lit glasshouse compartment (5 m x 3 m). High pressure sodium lamps (Osram Plantastar 600W; Munich, Germany) provided supplementary lighting for a photoperiod of 0600 h-2000 h when ambient PAR was less than 500 µmol m^{-2} s^{-1}. The daily maximum temperature in the greenhouse was 30 °C with a minimum night temperature of 17 °C. Environmental conditions in the centre of the glasshouse were recorded using a Hortimax growing solutions Ektron II (Pijnacker, The Netherlands).

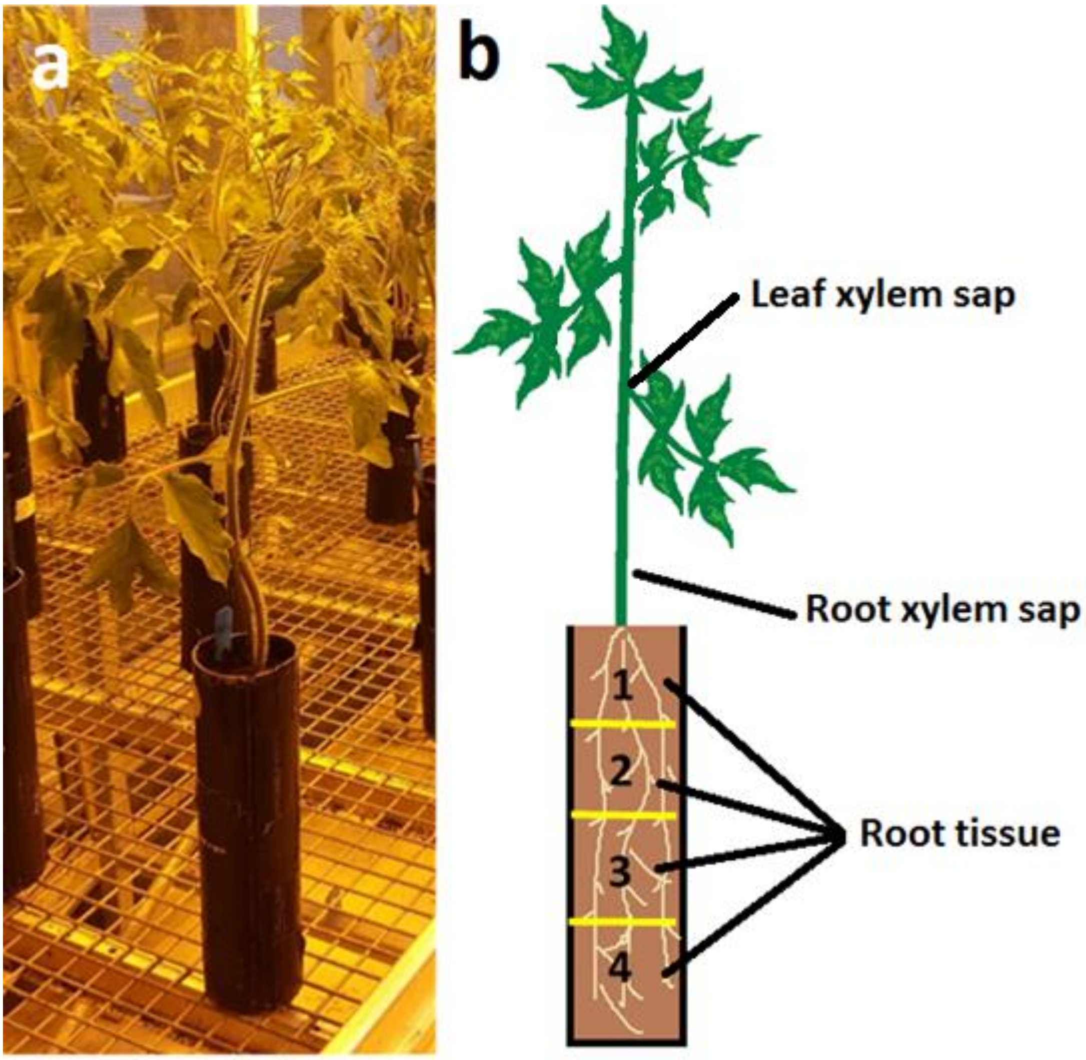


Figure. 4.1. a) Image of tomato plant with etiolated hypocotyl grown in cylindrical pot; and b) diagram indicating the sampling points for tissue and sap. Numbers indicate each of the layers sampled for root tissue.

4.2.2 Irrigation Treatments

Plants were irrigated daily according to Chapter 3 (Section 3.2.2; Boyle et al., 2015) to maintain well-watered conditions until the start of the experiment. Subsequently, plants were grouped into three different irrigation treatments: irrigation applied at 50% of plant daily ET for 10 days and withholding irrigation for 3 days (Figs. 4.2, 4.3 a & b), while a third group of plants were maintained under WW conditions (100

% ET) and used as a reference. Differences in deficit irrigation treatment duration were to allow adequate soil drying when irrigating at 50 % ET to allow sampling at comparable whole pot soil moisture contents. To ensure sampling was carried out on plants of the same chronological age, the first two irrigation treatments were applied 4 and 5 weeks after germination respectively. Volumetric soil moisture content was measured in four layers using four soil moisture sensors (SM300) connected to a data logger (GP1) (Delta-T Devices, Cambridge, UK) for the 10 day experimental period in plants from both irrigation treatments, and the mean value of the pot is reported (Fig. 4.3b). All physiological measurements were taken between 1000 h and 1400 h, with irrigation supplied at 0800 h and 1600 h. Four plants were sampled per irrigation treatment per day for all paired measurements, with the experiment repeated three times.

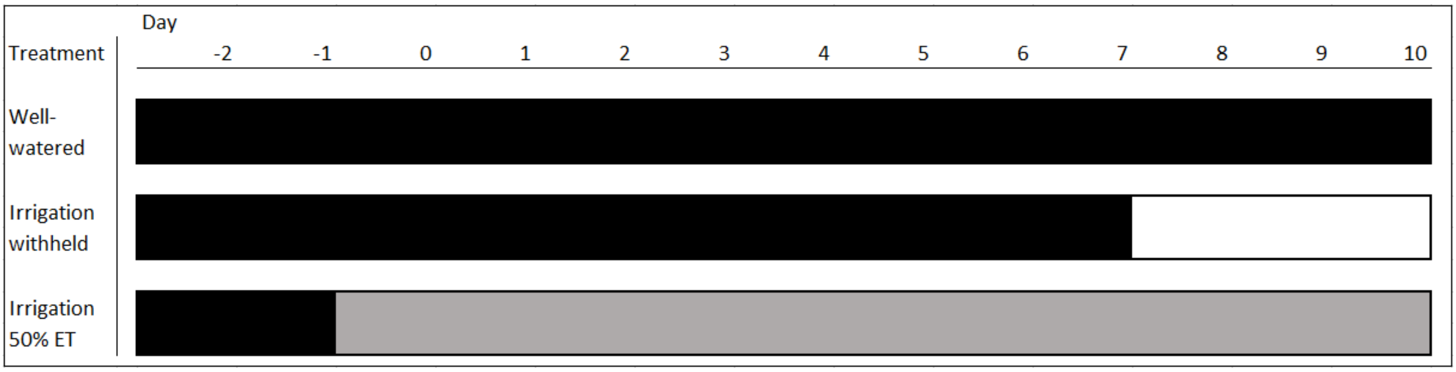


Figure. 4.2. Timetable of irrigation treatments. Irrigation was applied to plants at a 50 % evapotranspiration (ET) on Day 0, and irrigation was withheld from a separate group of plants on Day 8. Black bar indicates plants were well-watered (WW); grey bar indicates irrigation was supplied at 50 % of daily ET; white bar indicates irrigation was withheld. Sampling was carried out for plants under each irrigation treatment on days 8-10.

4.2.3 Gas exchange measurements

Prior to measurements (at 0900 h), the tops of the pots were sealed with duct tape to prevent evaporative losses and weighed, left for 1-2 h, and then re-weighed to determine whole plant transpiration rate. This provided an average sap flow rate by dividing weight loss by time, which was then used to match the flow rate for root xylem sap collection (Rothwell and Dodd, 2014). g_s was measured using a porometer (Model AP4, Delta-T Devices, Cambridge, UK). Two readings of g_s were taken on the 3rd leaf (youngest, fully expanded) from the top of each plant. Measurements of g_s were carried out over a 3 day period for all plants.

4.2.4 Leaf water potential (Ψ_{leaf}) measurements

Ψ_{leaf} was measured immediately after measuring g_s on the same leaf using a pressure chamber (Model 3000F01 Plant Water Status Console; Soil Moisture Equipment Corp. Santa Barbara, CA, USA) as described in Section 2.2.4, and leaf xylem sap samples were collected as described in Section 3.2.4.

4.2.5 Root water potential (Ψ_{root}) measurements

Ψ_{root} was measured immediately after Ψ_{leaf}. The plant was de-topped below the cotyledon, the hypocotyl was wrapped in parafilm to prevent loss of sap, and the whole pot was inserted and sealed in the pressure chamber. Since flow rate can influence xylem ABA concentrations (Jokhan et al., 1996), sap was collected at an over-pressure that generated flow rates equivalent to *in vivo* transpiration as described previously (Rothwell and Dodd, 2014). For plants in the pressure chamber, sap was sampled for 20 s and weighed, which was repeated with increasing pressure at intervals of 0.05 – 0.1 MPa until the correct flow rate was achieved. Sap was then collected for 3 min using a pipette, stored in a 1.5 ml microfuge tube and then frozen in liquid nitrogen.

4.2.6 Root water uptake, soil water status and root density

Root water uptake (RWU) was determined within each layer by measuring dielectric constant readings every 1 min for 1 h between 1000 h and 1400 h. RWU was calculated as the difference between the final and initial readings within the total volume of each layer. After sampling each plant, the pot was opened, and the growth substrate (including plant roots) was separated into 4 individual vertical layers using a circular cutting tool – an upper layer of 5 cm, with the remaining layers 6 cm in height (Fig. 4.1b). Roots were collected from each layer by hand, washed briefly (<10 s) in deionized water and then frozen in liquid nitrogen for analysis of ABA. Each soil layer was then removed and fresh weight determined, then dried in the oven for 7 days, and then reweighed (including roots) to calculate whole-pot θ_{pot} at the time of gas exchange measurements. A separate group of plants, exposed to the treatments described above were sampled for root density. Roots were carefully washed to remove soil, fresh weight determined and then oven dried for 7 days to achieve dry weights.

4.2.7 Xylem sap and tissue ABA analysis

After measuring Ψ_{leaf} and Ψ_{root}, sap samples were stored for analysis of ABA. Root tissue samples were freeze-dried and then finely ground, before being diluted at a ratio of 1:50 weight using deionized water. $[X\text{-}ABA]_{leaf}$, $[X\text{-}ABA]_{root}$ and $[ABA]_{root}$ concentrations were determined by radioimmunoassay (Quarrie et al., 1988). It has been verified previously using GC-MS analysis that tomato has no immunoreactive contamination (Mulholland et al., 1999). To ensure there was no effect of root pressurisation on $[ABA]_{root}$, a separate group of plants were subject to different periods of time under pressurisation in a pressure chamber, which revealed there were no differences across the sampling period in this chapter ($[ABA]_{root}$ of 153±36 ng g DW^{-1}, 154±31 ng g DW^{-1} and 186±41 ng g DW^{-1} was found for 0, 5 and 10 mins pressurisation respectively).

4.2.8 Xylem sap ABA modelling

Measured $[X\text{-}ABA]_{root}$ and $[X\text{-}ABA]_{leaf}$ was compared with that predicted from different models to try to understand the variation in ABA between the different irrigation treatments. As Ψ_{leaf} did not show any relationship with either $[X\text{-}ABA]_{leaf}$ or g_s (Fig. 4.6), it was not included in any of the models. All models were based solely on data from plants where irrigation was withheld as those plants showed homogeneous soil moisture distribution. Predicted data were modelled on an independent group of plants where irrigation was either withheld, or applied at 50 % ET. The models tested were:

i) $[X\text{-}ABA]_{leaf}$ dependent upon whole pot soil moisture content, with the relationship:

$$[X - ABA]_{leaf} = 105.04\theta pot^2 - 738.97\theta pot + 1445.2$$

where θ_{pot} was determined by averaging the soil moisture content from the four layers in each pot.

ii) $[X\text{-}ABA]_{leaf}$ dependent upon the soil moisture content of each layer measured within the soil profile, and accounting for root water uptake within each layer (adapted from a similar model in Dodd et al. (2008)):

$[X-ABA]_{leaf} = (F_{RZ1} * Est[X-ABA]_{leafRZ1}) + (F_{RZ2} * Est[X-ABA]_{leafRZ2}) + (F_{RZ3} * Est[X-ABA]_{leafRZ3}) + (F_{RZ4} * Est[X-ABA]_{leafRZ4})$

where F_{RZx} represents root water uptake from each layer as a fraction of the total root water uptake from the entire soil profile, and $Est[X-ABA]_{leafRZx} = 105.04\theta potRZx^2 - 738.97\theta potRZx + 1445.2$, where $\theta_{pot}RZx$ represents the soil moisture content from each layer within the soil profile.

iii) $[X\text{-}ABA]_{root}$ dependent upon whole pot soil moisture content, with the relationship:

$$[X-ABA]_{root} = 813.24\theta pot^{-3.553}$$

where θ_{pot} was determined by averaging the soil moisture content from the four layers in each pot.

iv) $[X\text{-}ABA]_{root}$ dependent upon θ_{pot} of each layer measured within the soil profile, and accounting for root water uptake within each layer (adapted from Dodd et al. (2008)):

$[X-ABA]_{root} = (F_{RZ1} * Est[X-ABA]_{rootRZ1}) + (F_{RZ2} * Est[X-ABA]_{rootRZ2}) + (F_{RZ3} * Est[X-ABA]_{rootRZ3}) + (F_{RZ4} * Est[X-ABA]_{rootRZ4})$

where F_{RZx} represents root water uptake from each layer as a fraction of the total root water uptake from the entire soil profile, and $Est[X-ABA]_{rootRZx} = 813.24\theta potRZx^{-3.553}$, where $\theta_{pot}\,RZx$ represents the soil moisture content from each layer within the soil profile.

4.2.9 Statistics

Significant differences between the three independent experiments were determined by analysis of covariance (ANCOVA). As no significant interactions between experiments occurred, data were pooled. One-way analysis of variance (ANOVA) at $p<0.05$ established differences between irrigation treatments on each day using SPSS Statistics 20 (IBM). *Tukey's* multiple comparison test was used to discriminate between means where significant differences were found in ANOVA. Shapiro-Wilk test was used to establish normality of data, and when non-normal distribution was found, data were log transformed and re-tested. Kruskal-Wallis test was used to determine significant differences if non-normality was again found in data. Repeated measures ANOVA was used to distinguish significance between irrigation treatment and root zone for root water uptake, with treatment as between-subject factor and root zone as within-subject factor. The effect of irrigation treatment on the relationship between plant and soil variables was tested using ANCOVA. 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.). Regressions were fitted using data below a θ_{pot} of 2.5 g g^{-1} (below a soil matric potential of -1 kPa) in all figures to compare the different deficit irrigation treatments.

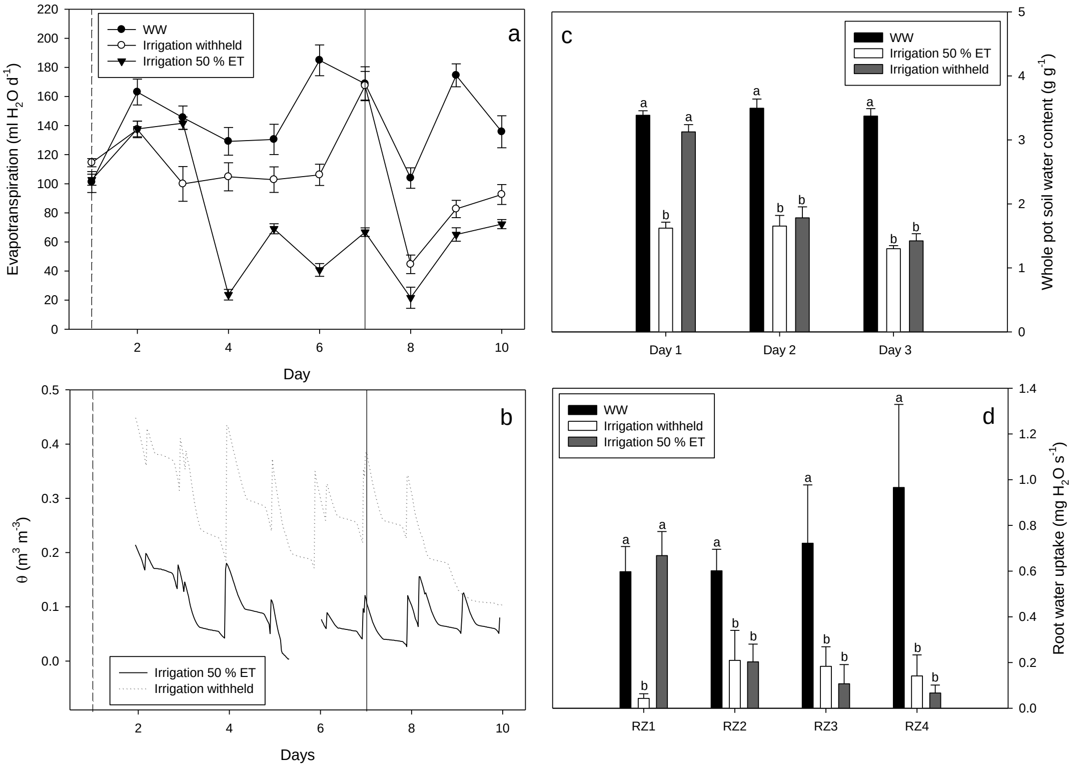


Figure. 4.3. Daily evapotranspiration (n=12) a) and volumetric soil moisture content (θ) b) over a 10 day treatment period; c) whole-pot gravimetric soil moisture content (θ_{pot}; n=8); d) root water uptake on Day 3 in each layer (RZ; n=6) of tomato plants where irrigation was either withheld or supplied at 50 % of daily evapotranspiration (ET), and in well-watered (WW) plants, over the 10 day treatment period. For daily ET, vertical lines indicate the start of each irrigation treatment (irrigation at 50 % ET, broken line; irrigation withheld, solid line). For θ, measurements were made every 30 mins, with missing data due to a power shortage in the GP1 data logger. Values are the average moisture content over the 4 layers of soil in each pot, with one plant per treatment measured. For RWU, RZ1 indicates the upper 5 cm of the soil profile, and RZ4 indicates the bottom 6 cm of the soil profile. Different letters on panels c) and d) indicate significant differences between treatments on each day according to a one-way ANOVA ($p<0.05$). Data are means ± SEM.

4.3 Results

Whole plant evapotranspiration remained constant over the experimental period in WW plants (Fig. 4.3a). Plants irrigated at 50 % ET showed a decrease in ET by Day 4 (coinciding with depleting soil moisture; Fig. 4.3b) which was maintained over the experimental period, whilst plants where irrigation was withheld showed a rapid decrease in ET within 24 h of the treatment being imposed. When measured during the 3 day experimental period, θ_{pot} remained constant in WW plants (3.4±0.1 g g^{-1}) and plants irrigated at 50 % ET (2.0±0.3 g g^{-1}), while withholding irrigation significantly decreased soil moisture by 43% within 24 h (Fig. 4.3c). Soil moisture content was also measured gravimetrically at each depth within the soil profile (Table. 4.1). When irrigation was withheld, soil moisture was significantly lower compared to WW plants in all layers, although moisture was evenly distributed throughout the pot. When plants were irrigated at 50 % ET, θ_{pot} was highest in the upper layers but then decreased in lower layers. No significant differences were found between irrigation treatments for total plant fresh weight (Table. 4.2), whilst root FW was lowest when plants were irrigated at 50 % ET (9.2±0.6 g), but not significantly different from plants where irrigation was withheld (10.7±0.7 g; Table. 4.2).

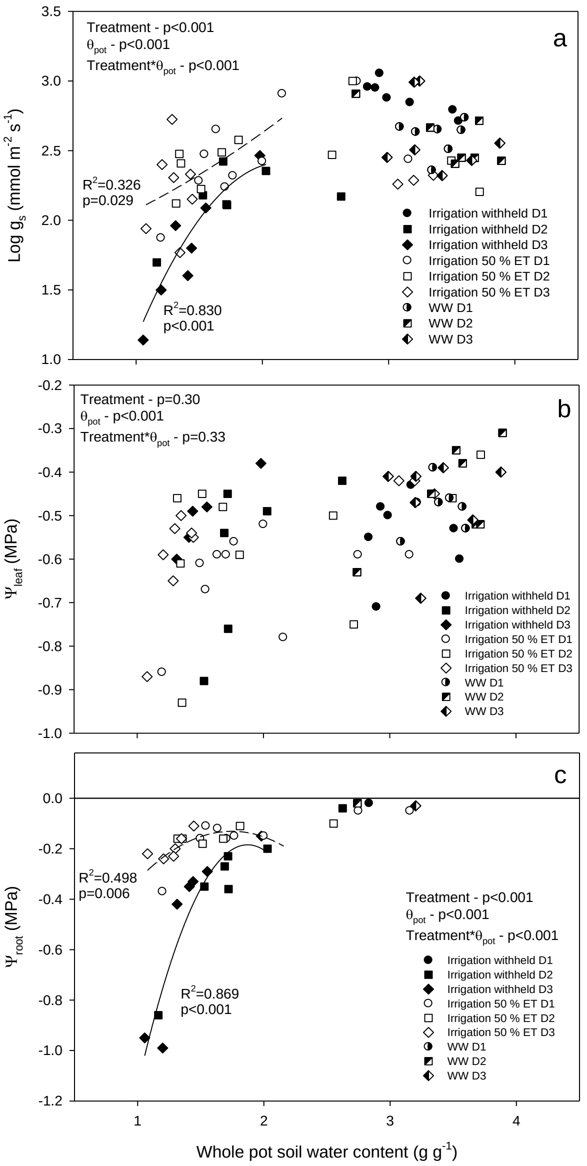


Figure. 4.4. a) Log stomatal conductance (g_S); b) leaf water potential (Ψ_{leaf}); c) root water potential (Ψ_{root}) of tomato where irrigation was either withheld or supplied at 50 % of daily evapotranspiration (ET), and in well-watered (WW) plants on Days 1-3 of sampling. Closed symbols indicate plants from which irrigation was withheld (n= 42); open symbols indicate plants from which irrigation was supplied at a fraction of daily ET (n= 51); half & half symbols indicate WW plants (n=21). Horizontal line on panel c) indicates where Ψ_{root} could not be measured due to root exudation upon de-topping the plant. Data points are paired individual samples, regressions line of data below 2.5 g g^{-1} are fitted where significant and P values from ANCOVA reported.

Measurements of RWU (Fig. 4.3d) revealed significant differences between WW plants and those under the two deficit irrigation treatments (significant treatment x layer interaction; $p=0.038$). This occurred in all layers, except in plants that were irrigated at 50 % ET, which maintained the same RWU in the upper 5 cm as in WW plants. In all other layers, there was no significant difference in RWU between the two deficit irrigation treatments, which were between 3- and 9- fold lower than in WW plants.

Treatment	Day	RZ1 θ_{pot}	RZ2 θ_{pot}	RZ3 θ_{pot}	RZ4 θ_{pot}	Shoot and root FW	Root FW
Well-watered	1	3.4±0.1a	3.3±0.0a	3.3±0.1a	3.6±0.1a	-	-
	2	3.4±0.1a	3.4±0.1a	3.5±0.1a	3.6±0.2a	-	-
	3	3.4±0.0a	3.4±0.1a	3.4±0.1a	3.3±0.3a	53.5±5.1a	12.6±0.4a
Irrigation 50 % ET	1	2.5±0.2b	2.0±0.1b	1.3±0.1bc	0.8±0.0c	-	-
	2	2.6±0.2b	1.9±0.3b	1.3±0.3bc	0.9±0.1bc	-	-
	3	2.3±0.1bcd	1.4±0.1b	1.0±0.0c	0.7±0.0c	36.0±3.5b	9.2±0.6b
Irrigation withheld	1	3.2±0.1a	3.2±0.1a	3.1±0.1a	2.9±0.3a	-	-
	2	1.8±0.2de	2.0±0.2b	1.8±0.2b	1.5±0.2b	-	-
	3	1.4±0.1e	1.6±0.1b	1.5±0.1bc	1.2±0.1bc	48.7±7.8ab	10.7±0.7ab

Table. 4.1. Gravimetric water content (θ_{pot} – g g^{-1}; n=8), Shoot and root fresh weight (FW - g; n=4) and root fresh weight (FW - g; n=9) of tomato plants which were well-watered (WW), or subject to either irrigation at 50 % evapotranspiration (ET) or irrigation withheld, sampled over a 3 day period. Measurements of θ_{pot} were from the upper 5cm layer (RZ1) and three subsequent 6cm layers down the soil profile (RZ2 - 4). Different letters within a column indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ± SEM of all measurements.

Treatment	Day	g_s	Ψ_{leaf}	Ψ_{root}	$[X\text{-}ABA]_{leaf}$	$[X\text{-}ABA]_{root}$	ABA delivery rate
Well-watered	1	412±39a	-0.48±0.02a	Not measurable	189±27c	11±4c	2.4±0.7b
	2	410±78a	-0.45±0.04a		161±36c	18±4c	2.9±0.7b
	3	488±131a	-0.47±0.04a		150±36c	17±3c	4.0±0.6b
Irrigation 50 % ET	1	374±94b	-0.64±0.03b	-0.15±0.03bcd	163±18c	26±8c	3.6±1.0b
	2	326±79bcd	-0.56±0.05ab	-0.10±0.03ab	168±38c	34±12c	4.1±1.1b
	3	207±41bc	-0.55±0.04ab	-0.13±0.04bc	234±40c	34±8bc	3.5±0.8b
Irrigation withheld	1	789±78a	-0.54±0.04ab	Not measurable	161±13bc	11±4c	3.1±0.9b
	2	157±26c	-0.59±0.08ab	-0.33±0.10cd	547±112ab	364±169ab	54.2±24.6a
	3	94±36d	-0.50±0.04ab	-0.50±0.13d	951±287a	1086±701a	50.7±21.7a

Table. 4.2. Stomatal conductance (g_s; mmol m^{-2} s^{-1}), leaf water potential (Ψ_{leaf}; MPa), root water potential (Ψ_{root}; MPa), leaf ($[X\text{-}ABA]_{leaf}$; nM) and root ($[X\text{-}ABA]_{root}$; nM) xylem sap ABA concentration, and ABA delivery rates (nmoles s^{-1}) of tomato plants which were well-watered (WW), or subject to either irrigation at 50 % evapotranspiration (ET) or irrigation withheld, sampled over a 3 day period (n=8). ABA delivery rate was calculated as concentration multiplied by sap flow rates according to Else et al. (1996). Different letters within a column indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ± SEM of all measurements.

Mean g_s of WW plants was 437±83 mmol m^{-2} s^{-1} over the sampling period. Withholding irrigation significantly reduced g_s by 62% within 24 h, and 81% within 48 h compared to WW plants. However, supplying irrigation at 50 % ET only decreased g_s by 21% and 58% compared to WW plants on the same sampling days (Table. 4.2). g_s decreased with decreasing θ_{pot} in both deficit irrigation treatments (Fig. 4.4a), although g_s was lower at the same θ_{pot} when irrigation was withheld (significant treatment x θ_{pot} interaction).

Compared to WW plants, both deficit irrigation treatments decreased Ψ_{leaf} by approximately -0.1 MPa over the entire sampling period (Table. 4.2). Both treatments decreased Ψ_{leaf} similarly as soil moisture was depleted (Fig. 4.4b; no significant treatment x θ_{pot} interaction). However, g_s was only weakly related to Ψ_{leaf} ($r^2 = 0.09$, $p=0.06$; Fig. 4.6a).

Whilst Ψ_{root} could not be measured in WW plants, as de-topping the shoot caused spontaneous root exudation, Ψ_{root} of plants irrigated at 50 % ET was -0.13±0.03 MPa. Withholding irrigation significantly decreased Ψ_{root} within 24 h (-0.33±0.10 MPa), which declined further as soil dried (Table. 4.2). Furthermore, Ψ_{root} decreased as soil moisture was depleted under both treatments (Fig. 4.4c), although this was more pronounced in plants from which irrigation was withheld (significant treatment x θ_{pot} interaction).

There was no significant change in $[X\text{-}ABA]_{leaf}$ and $[X\text{-}ABA]_{root}$ throughout the experiment in WW plants; mean values were 167±33 nM and 15±4 nM respectively. Plants showed significant increases in $[X\text{-}ABA]_{leaf}$ and $[X\text{-}ABA]_{root}$ within 24 h of irrigation being withheld (Table. 4.2). Plants irrigated at 50 % ET also showed higher $[X\text{-}ABA]_{leaf}$ and $[X\text{-}ABA]_{root}$ than WW plants by 13% and 104%

respectively, but significantly lower than when irrigation was withheld on Days 2 and 3 (Table. 4.2). These ABA concentrations correlated with decreasing θ_{pot}, although this response was attenuated in plants irrigated at 50 % ET (significant treatment x θ_{pot} interaction; Fig. 4.4 a & b respectively). Furthermore, increased [X-ABA]$_{root}$ was positively correlated with both [X-ABA]$_{leaf}$ (Fig. 4.5c) under both irrigation treatments (no significant treatment x ABA interactions).

Predicting [X-ABA]$_{leaf}$ based on θ_{pot} (Equation. 1) underestimated its value (by 20%) when irrigation was withheld and overestimated it (by 40%) when irrigation was supplied at 50 % ET (Table. 4.3). Including the fraction of root water uptake (Equation. 2) within each layer improved the accuracy of the model for both irrigation treatments (except for [X-ABA]$_{leaf}$ when irrigation was withheld, which did not change). Further, predicting [X-ABA]$_{root}$ based on θ_{pot} (Equation. 3) overestimated the value in both irrigation treatments, while accounting for the fraction of root water uptake (Equation. 4) made no improvement.

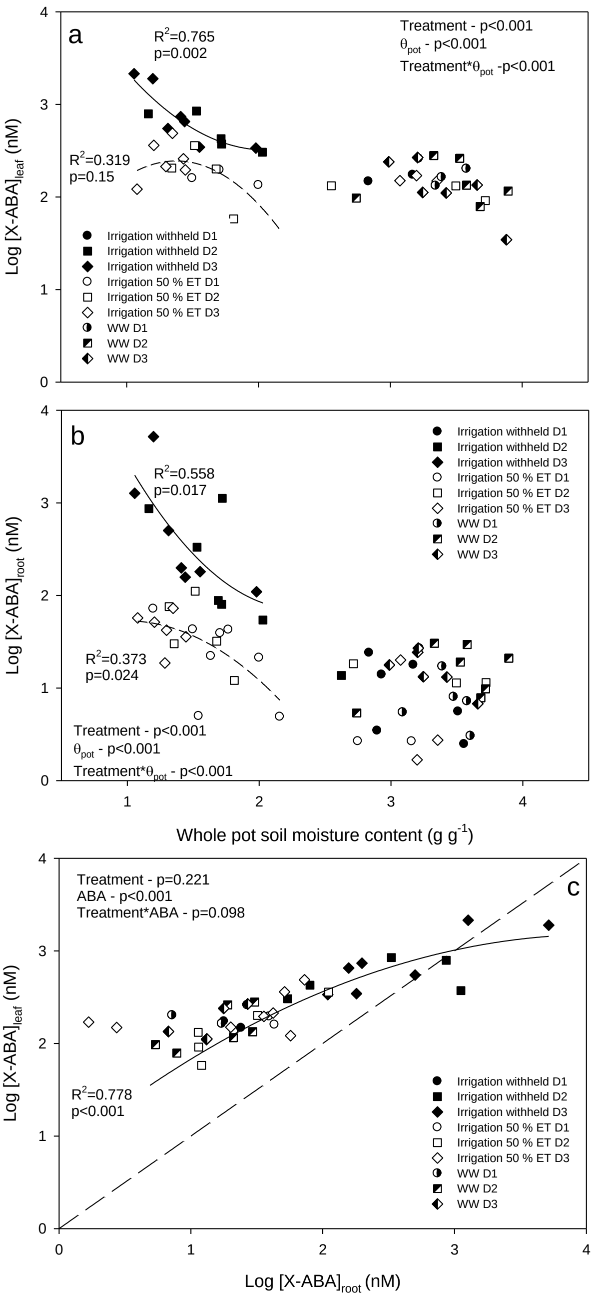


Figure. 4.5. Log a) leaf xylem ABA concentration ([X-ABA]$_{leaf}$); b) root xylem ABA concentration ([X-ABA]$_{root}$) of tomato in drying soil; c) log [X-ABA]$_{leaf}$ in response to log [X-ABA]$_{root}$ of tomato where irrigation was either withheld or supplied at 50 % of daily evapotranspiration (ET), and in well-watered (WW) plants on Days 1-3 of sampling. Closed symbols indicate plants from which irrigation was withheld (a) n=28; b) n=35; c) n=28); open symbols indicate plants from which irrigation was supplied at a fraction of daily ET (a) n=36; b) n=47; c) n=35); half & half symbols indicate WW plants (a) n=16; b) n=19; c) n=16). A 1:1 line is included in panel c). Data points are paired individual samples, regression line of data below 2.5 g g^{-1} are fitted where significant and P values from ANCOVA reported.

	$[X\text{-}ABA]_{leaf}$			$[X\text{-}ABA]_{root}$		
Treatment	Mean (equation 1)	Fractional (equation 2)	***P-value***	Mean (equation 3)	Fractional (equation 4)	***P-value***
Irrigation 50 % ET	1.7 (11)	1.5 (11)	***0.72***	12.2 (12)	7.0 (12)	***0.045***
Irrigation withheld	0.8 (7)	0.8 (7)	***0.93***	1.3 (10)	1.3 (10)	***0.97***
***P*-value**	***0.14***	***0.18***	**-**	***<0.001***	***<0.001***	**-**
Combined data	1.3 (18)	1.2 (18)	**-**	6.7 (22)	4.1 (22)	**-**

Table. 4.3. Model predictions of leaf ($[X\text{-}ABA]_{leaf}$) and root ($[X\text{-}ABA]_{root}$) xylem sap ABA concentration under irrigation at either 50 % of daily evapotranspiration (ET) or when irrigation was withheld. The different between predicted values from models (equations 1-4; see Section 4.2.8) is calculated as the ratio of $[X\text{-}ABA]_{model}/[X\text{-}ABA]_{leaf}$ or $[X\text{-}ABA]_{root}$ (Pérez-Pérez and Dodd, 2015). The model overestimates or underestimates [X-ABA] when the value reported is above or below 1 respectively. Numbers in brackets represent *n* values, and the number of plants used is reported in parenthesis. P values are reported for ANOVA between deficit irrigation treatments for each equation.

No relationship was found between Ψ_{leaf} and $[X\text{-}ABA]_{leaf}$ ($r^2=0.02$, $P=0.83$; Fig. 4.6b), and decreased g_s was only associated with increased $[X\text{-}ABA]_{leaf}$ when irrigation was withheld (significant treatment x ABA interaction; Fig. 4.7a). However, increased $[X\text{-}ABA]_{root}$ was negatively correlated with g_s (Fig. 4.7b) under both irrigation treatments (no significant treatment x ABA interactions). In addition, $[X\text{-}ABA]_{root}$ showed a similar increase as Ψ_{root} decreased under both irrigation treatments (no significant treatment x Ψ_{root} effect; Fig. 4.7c).

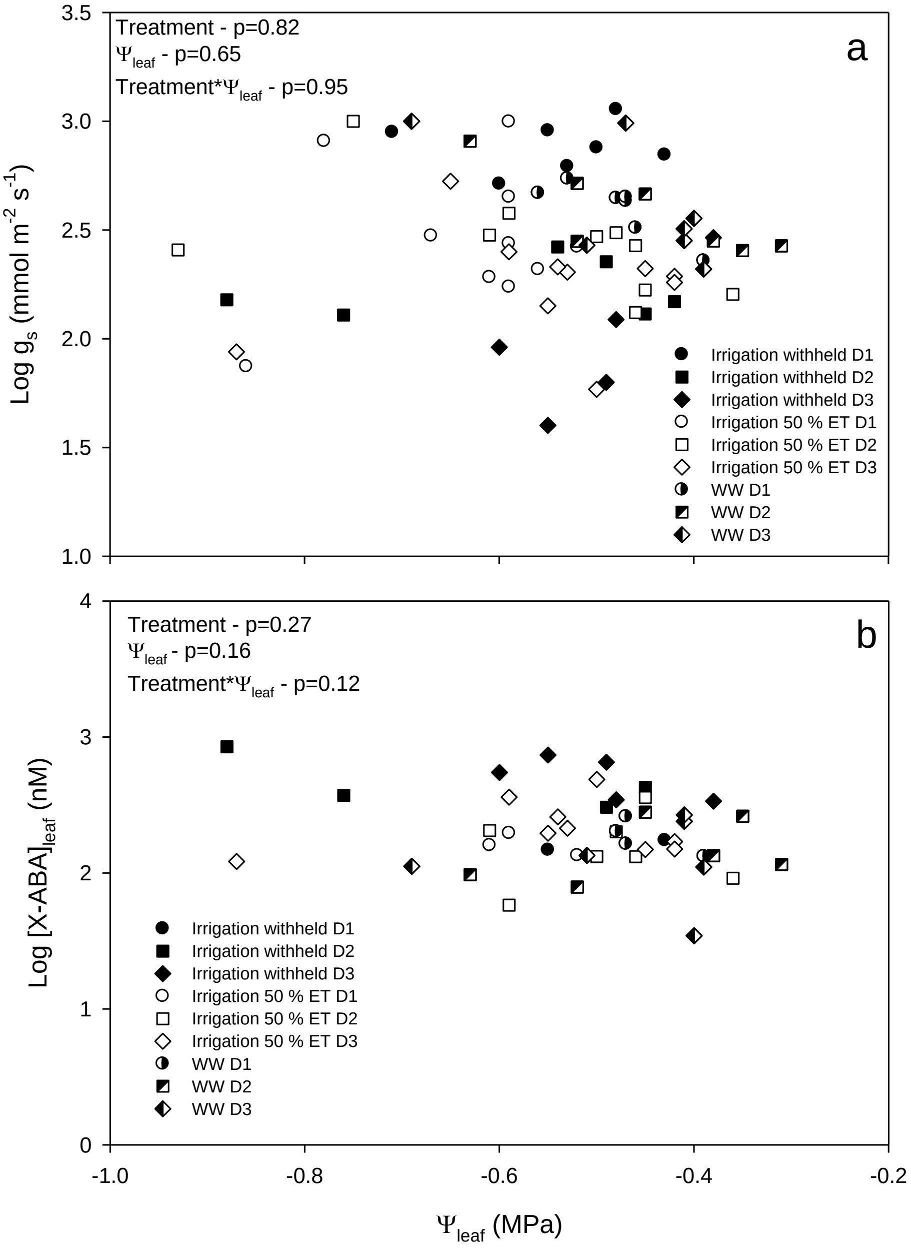


Figure. 4.6. Log a) stomatal conductance (g_s); b) leaf xylem ABA concentration ($[X\text{-}ABA]_{leaf}$) in response to leaf water potential (Ψ_{leaf}) of tomato where irrigation was either withheld or supplied at 50 % of daily evapotranspiration (ET), and in well-watered (WW) plants on Days 1-3 of sampling. Closed symbols indicate plants from which irrigation was withheld (a) n=42; b) n=28); open symbols indicate plants from which irrigation was supplied at a fraction of daily ET (a) n=51; b) n=35); half & half symbols indicate WW plants (a) n=21; b) n=16). Data points are paired individual samples, regression line of data below 2.5 g g^{-1} are fitted where significant and P values from ANCOVA reported.

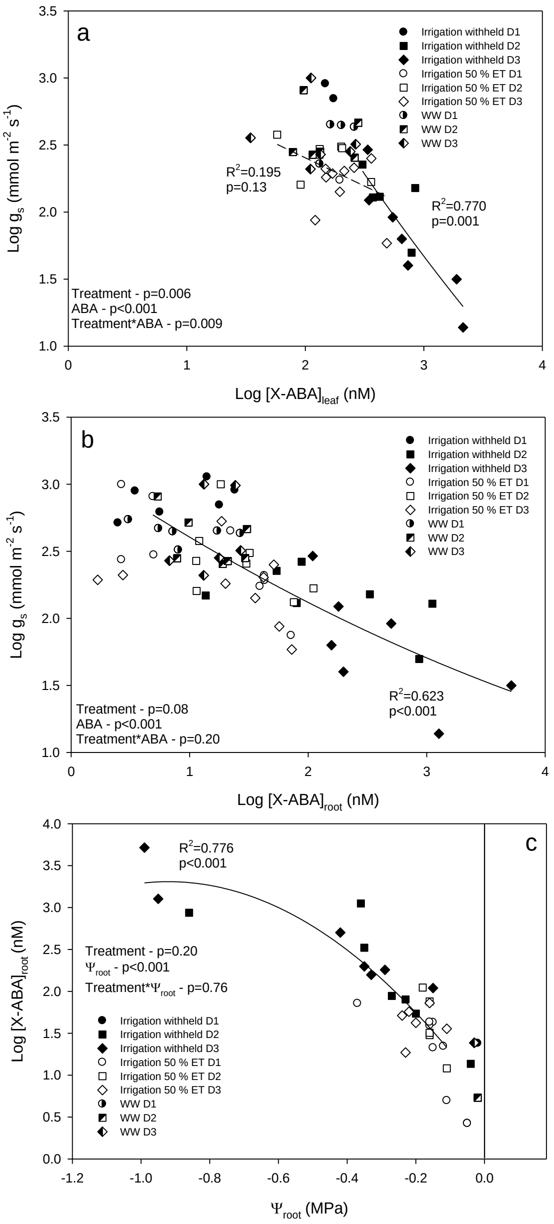


Figure. 4.7. Log stomatal conductance (g_S) in response to log a) leaf xylem ABA concentration ([X-ABA]$_{leaf}$); b) root xylem ABA concentration ([X-ABA]$_{root}$); c) log [X-ABA]$_{root}$ in response to root water potential (Ψ_{root}) of tomato where irrigation was either withheld or supplied at 50 % of daily evapotranspiration (ET), and in well-watered (WW) plants on Days 1-3 of sampling. Closed symbols indicate plants from which irrigation was withheld (a) n=28; b) n=35; c) n=36); open symbols indicate plants from which irrigation was supplied at a fraction of daily ET (a) n=36; b) n=47; c) n=47); half & half symbols indicate WW plants (a) n=16; b) n=19; c) n=19). Different sample numbers per treatment is due to lost samples. Vertical line on panel c) indicates data points where Ψ_{root} could not be measured due to a positive pressure upon de-topping the plant. Data points are paired individual samples, regression line of data below 2.5 g g^{-1} are fitted where significant and P values from ANCOVA reported.

Root tissue ABA was measured at 4 depths within the soil profile (Table. 4.4), and followed the trend observed in soil moisture distribution (Fig. 4.8). $[ABA]_{root}$ was consistently low in WW plants in all layers on all days. When plants were irrigated at 50 % ET, $[ABA]_{root}$ was greatest in the lower layers over the 3 days of sampling, but the upper two layers were comparable to WW plants, with little increase in $[ABA]_{root}$ as moisture decreased (Fig. 4.8). In contrast, plants from which irrigation was withheld showed significant increases in $[ABA]_{root}$ in all layers within 24 h. This is coincident with a difference in the relationship between total $[ABA]_{root}$ and θ_{pot} under the different irrigation treatments (Fig. 4.9a), which was higher at the lowest soil moisture content when irrigation was withheld (significant treatment x ABA interaction). Furthermore, higher mean $[ABA]_{root}$ showed a stronger correlation with $[X\text{-}ABA]_{root}$ when irrigation was withheld compared to irrigation at 50 % ET (significant treatment x ABA interaction; Fig. 4.9b).

Treatment	Day	RZ1 $[ABA]_{root}$	RZ2 $[ABA]_{root}$	RZ3 $[ABA]_{root}$	RZ4 $[ABA]_{root}$	Mean $[ABA]_{root}$
Well-watered	1	187±38bc	278±72b	178±48cd	238±30bc	220±47c
	2	180±37bc	161±45b	378±299c	209±46c	232±107c
	3	118±53c	196±33b	145±38c	213±47bc	168±43c
Irrigation 50 % ET	1	236±38bc	170±25b	294±58bc	354±86bc	263±52bc
	2	184±29bc	257±65b	362±110bc	472±103bc	320±77bc
	3	172±32bc	139±45b	456±124b	488±63bc	314±66bc
Irrigation withheld	1	297±42ab	271±53b	192±64cd	226±60bc	247±54.6bc
	2	1074±480a	631±268ab	564±209abd	924±218ab	798±294ab
	3	934±332a	971±332a	1138±487ab	1343±370a	1096±380a

Table. 4.4. Root abscisic acid concentration ($[ABA]_{root}$; ng g-1 DW) of tomato plants which were well-watered, or subject to either irrigation at 50 % evapotranspiration (ET) or irrigation withheld, sampled over a 3 day period (n=6). Measurements of $[ABA]_{root}$ were from the upper 5cm layer (RZ1) and the subsequent three, 6 cm layers down the soil profile (RZ2 - 4). Mean $[ABA]_{root}$ indicates average concentration over all four layers. Different letters within a column indicate significant differences according to a one-way ANOVA ($p<0.05$). Data are means ± SEM of all measurements.

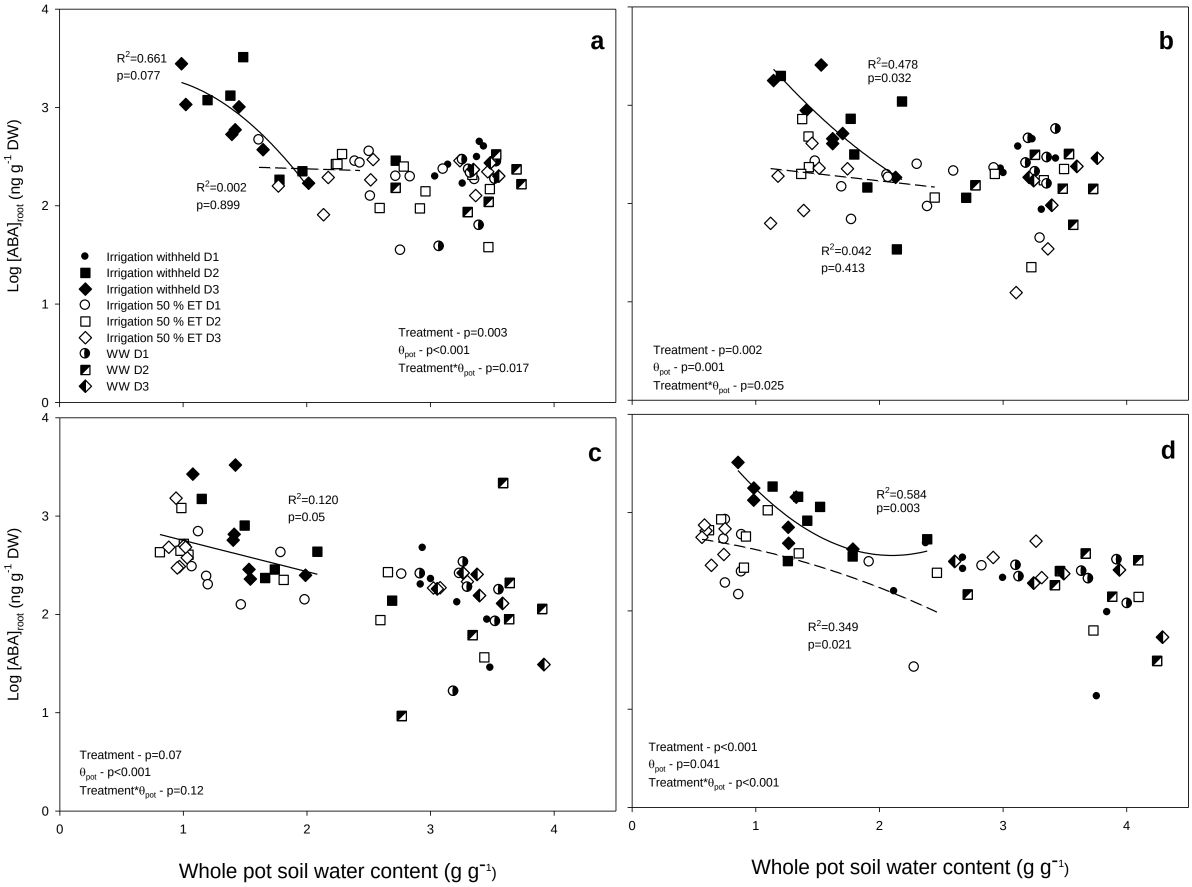


Figure. 4.8. Log root tissue ABA concentration ([ABA]$_{root}$) of tomato at different soil depths (a) upper 5 cm; b) middle upper 6 cm; c) middle lower 6 cm; d) bottom 6 cm) in drying soil where irrigation was either withheld or supplied at 50 % of daily evapotranspiration (ET), and in well-watered (WW) plants on Days 1-3 of sampling. Closed symbols indicate plants from which irrigation was withheld (n= 36); open symbols indicate plants from which irrigation was supplied at a fraction of daily ET (n= 44); half & half symbols indicate WW plants (n=16). Data points are paired individual samples, regression line of all data are fitted where significant and P values from ANCOVA reported.

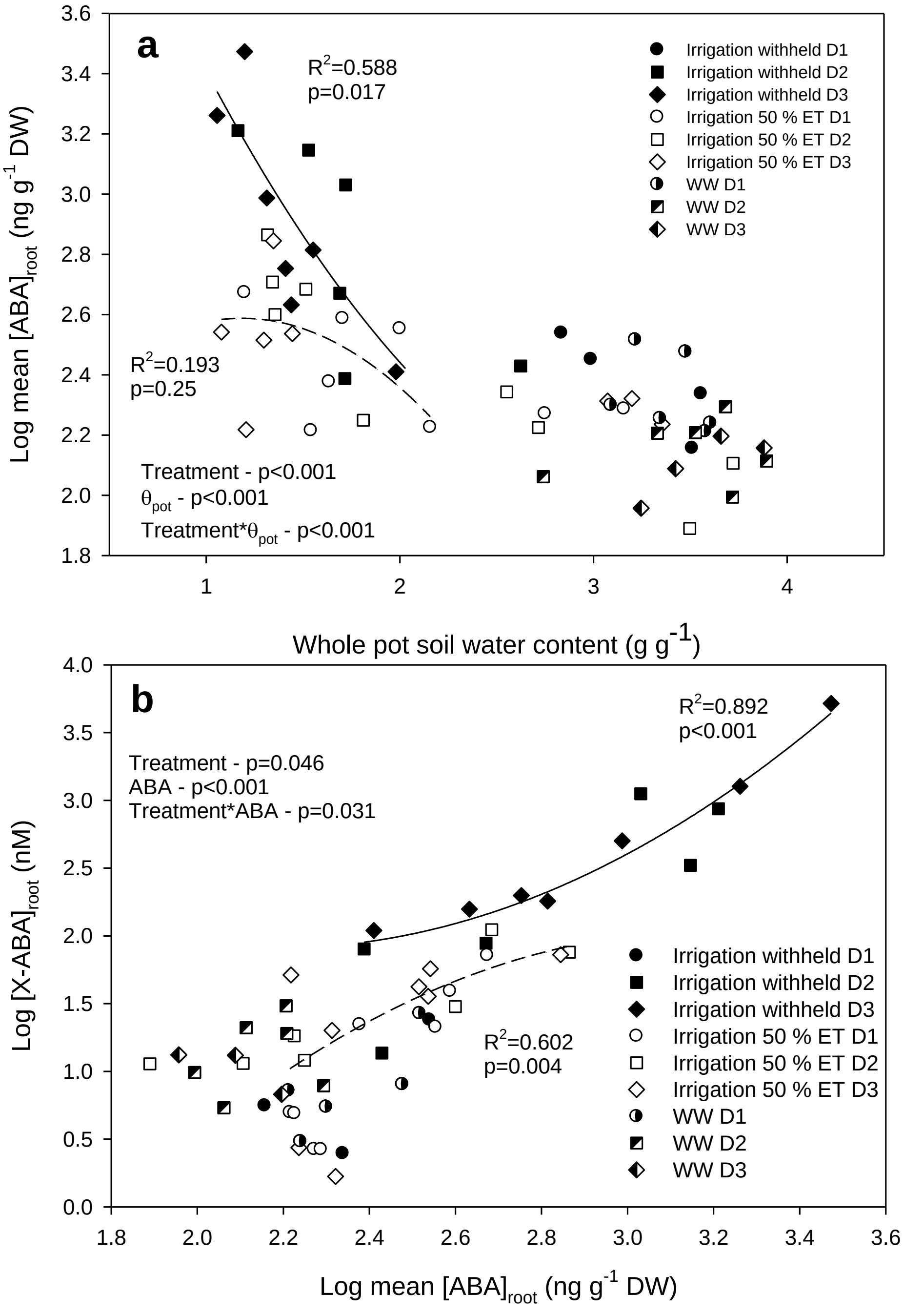


Figure. 4.9. a) Log mean root tissue ABA concentration ($[ABA]_{root}$) of tomato in drying soil; b) log root xylem ABA concentration ($[X\text{-}ABA]_{root}$) in response to mean $[ABA]_{root}$ of tomato where irrigation was either withheld or supplied at 50 % of daily evapotranspiration (ET), and in well-watered (WW) plants on Days 1-3 of sampling. Closed symbols indicate plants from which irrigation was withheld (n= 33); open symbols indicate plants from which irrigation was supplied at a fraction of daily ET (n= 41); half & half symbols indicate WW plants (n=16). Data points are paired individual samples, regression line of data below 2.5 g g^{-1} are fitted in both figures where significant and P values from ANCOVA reported.

4.4 Discussion

Both deficit irrigation treatments used in this study resulted in comparable whole-pot soil moisture content as the soil dried (Fig. 4.3c), although there were marked differences in moisture distribution within the soil profile (Table. 4.1). Withholding irrigation decreased soil moisture content rapidly within 24 h, and resulted in homogenous drying of the soil in each of the 4 layers measured. In contrast, irrigating plants at 50 % ET for 10 days resulted in a gradient of moisture from the top to the bottom of the soil profile, with the highest moisture content in the uppermost layer. Importantly, this soil moisture heterogeneity resulted in a higher stomatal conductance at the same whole pot water content as plants where irrigation was withheld (Fig. 4.4a), suggesting that differences in irrigation frequency may alter the mechanisms by which stomata respond to soil drying.

Changes in Ψ_{leaf} in response to soil drying can either cause (Buckley, 2005), or be a consequence (Sperry et al., 2002) of stomatal closure depending on the species. In tomato, the duration of soil drying affected the direction of the relationship between g_s and Ψ_{leaf} in plants exposed to different irrigation treatments, where initially g_s decreased as Ψ_{leaf} increased (2 days after PRD was imposed), whilst 2 days later no relationship was found (Kudoyarova et al., 2007). In *P.hortorum*, withholding irrigation caused concomitant decreases in both g_s and Ψ_{leaf}, while with daily irrigation at 50 % ET, stomatal closure was associated with higher Ψ_{leaf} (Boyle et al., 2015). In the current study conducted under comparable greenhouse conditions, Ψ_{leaf} decreased as θ_{pot} decreased regardless of irrigation treatment (Fig. 4.4b), suggesting an anisohydric response of tomato (Sobeih et al., 2004). The similar stomatal closure (albeit with greater sensitivity when irrigation was withheld) in both irrigation treatments (Fig. 4.4a), and the lack of any relationship between g_s and Ψ_{leaf} (Fig. 4.6a), suggests that Ψ_{leaf} is not the primary regulator of stomatal conductance in tomato (Dodd et al., 2006, Dodd, 2007).

ABA is classically accepted as having a key role in stomatal regulation under soil drying (Dodd, 2005, Schachtman and Goodger, 2008, Wilkinson and Davies, 2002). As previously shown in *P.hortorum* (Boyle et al., 2015), $[X\text{-}ABA]_{leaf}$ increased as θ_{pot} decreased, although this increase was significantly lower when irrigation was supplied twice daily at 50 % ET (Fig. 4.5a). This difference may explain why stomata are less sensitive to θ_{pot} under this treatment, particularly as $[X\text{-}ABA]_{leaf}$ was only correlated with g_s when irrigation was withheld (Fig. 4.7a). Consequently, it is essential to understand how different irrigation treatments regulate $[X\text{-}ABA]_{leaf}$.

There are several mechanisms by which $[X\text{-}ABA]_{leaf}$ might be affected by different irrigation treatments. Localised ABA synthesis (in response to leaf water deficits) can increase foliar ABA concentrations in the absence of a root-sourced ABA signal (Christmann et al., 2007). This would imply that leaf ABA concentrations influences $[X\text{-}ABA]_{leaf}$, although it has also been shown that in water stressed plants, AtNCED3, AtABA2 and AAO3 proteins were detected in vascular parenchyma cells, suggesting these cells may be the first site for ABA synthesis in response to drought (Endo et al., 2008). Decreased Ψ_{leaf} may also stimulate ABA biosynthesis in response to a decrease in leaf turgor (Pierce and Raschke, 1980). However, since $[X\text{-}ABA]_{leaf}$ was not correlated with Ψ_{leaf} under either irrigation treatment (Fig. 4.6a), an alternative explanation is required for the increased $[X\text{-}ABA]_{leaf}$.

Root-sourced ABA, transported via the xylem, can influence stomatal responses in drying soils (Zhang and Davies, 1991) and likely regulates $[X\text{-}ABA]_{leaf}$. In all irrigation treatments, $[X\text{-}ABA]_{root}$ and $[X\text{-}ABA]_{leaf}$ were strongly correlated (Fig. 4.5c). Indeed, the increase of $[X\text{-}ABA]_{root}$ with soil drying was limited by irrigation at 50 % ET (Fig. 4.5b), as was $[X\text{-}ABA]_{leaf}$ (Fig. 4.5a). Previous soil drying can reduce ABA metabolism in the roots (by doubling the half-life of artificially fed ^{3}H-ABA; Liang et al., 1997), and the daily cycle of drying and rewetting (when tomato was irrigated with 50 % ET) may attenuate root ABA accumulation. Indeed, plants grown under 50 % ET had a lower $[ABA]_{root}$ in the bottom soil layers compared to plants from which irrigation was withheld, even though θ_{pot} was comparable (Table. 4.4; Fig. 4.8). Increased $[X\text{-}ABA]_{root}$ correlated with decreased bulk Ψ_{root} under both irrigation treatments (Fig. 4.7c), consistent with previous reports in bean (Puértolas et al., 2013) and potato (Liu et al., 2006b) using vertical and horizontal PRD respectively. This suggests that Ψ_{root} regulates root ABA synthesis in tomato under soil drying, but further work is required to determine whether there is variation in localised Ψ_{root} (which was not measured in the current study due to the difficulties in measuring the fine roots of tomato in a psychrometer) that may explain the spatial variation in $[ABA]_{root}$.

$[X\text{-}ABA]_{root}$ and g_s exhibited a similar relationship across both irrigation treatments (Fig. 4.7a). Elevated ABA concentrations may be a consequence of reduced g_s rather than causative, particularly as reduced transpiration under soil drying may lead to accumulation of ABA (Jackson et al., 2003). However, root ABA export (ABA delivery) significantly increased within 24 h of irrigation being withheld, compared to both WW plants and those irrigated at 50 % ET (Table. 4.2). This suggests that ABA is still being transported from the roots despite the reduction in soil moisture (particularly as root xylem sap was collected at flow rates matching whole plant transpiration), and is thus influencing $[X\text{-}ABA]_{leaf}$ and in turn g_s. When plants were irrigated at 50 % ET, stomatal closure in the absence of an increase in ABA delivery may suggest that delivery rate has a less important role than actual concentration (Trejo et al., 1995), of perhaps an earlier signal (such as an increase in pH) occurs in tomato (Wilkinson et al., 1998). Ultimately, these findings support the hypothesis that the difference in ABA-induced stomatal closure between the irrigation treatments in this study is a function of a root derived ABA signal.

Given the differences in soil moisture distribution between the two deficit irrigation treatments (Table. 4.1), it was therefore important to establish whether the attenuated ABA concentrations in plants exposed to 50 % ET was due to limited transport of ABA out of the roots. Diminished water uptake from roots in dry soil (Table. 4.1) may be analogous to the diminished sap flow from the non-irrigated roots of plants exposed to PRD which limited $[X\text{-}ABA]_{leaf}$ (Dodd et al., 2008, Puértolas et al., 2014). Due to spatial variation in RWU in plants irrigated at 50 % ET (Fig. 4.3d), export of ABA was dominated by roots in the upper layer, which had lower $[ABA]_{root}$ (Table. 4.4, Fig. 4.8a), thereby explaining the lower $[X\text{-}ABA]_{root}$ and $[X\text{-}ABA]_{leaf}$ compared to plants from which irrigation was withheld (Dodd et al., 2008).

Developing models to predict ABA concentration can be valuable to demonstrate the importance of different plant and soil variables. When irrigation was withheld, both models showed similar accuracy whether the fraction of root water uptake (RWUF) was accounted for or not, suggesting localised soil water content accurately reflected root activity. When plants were irrigated at 50 % ET, accounting for the RWUF from individual soil layers improved the prediction of $[X\text{-}ABA]_{leaf}$ and $[X\text{-}ABA]_{root}$. However, compared to previous studies modelling xylem ABA concentrations (Dodd et al., 2008, Pérez-Pérez and Dodd, 2015), $[X\text{-}ABA]_{root}$ of plants irrigated at 50 % ET was greatly overestimated, possibly as a consequence of the large heterogeneity in ABA within the roots (Table. 4.4), implying that soil moisture content and RWU alone cannot fully explain the increase in ABA under this irrigation treatment. Accounting for localised differences in localised Ψ_{root} in future work may improve the accuracy of predicting $[X\text{-}ABA]_{root}$, particularly under 50 % ET.

While the relative water fluxes from different parts of the root system can clearly influence long-distance ABA signalling when plants are exposed to heterogeneous soil moisture (Table. 4.1; Dodd et al., 2008, Dodd et al., 2010, Pérez-Pérez and Dodd, 2015), differences in the root mass exposed to drying soil (Martin-Vertedor and Dodd, 2011) and their ABA concentration may also be important. $[ABA]_{root}$ increased as θ_{pot} decreased within all soil layers (Fig. 4.8; Table. 4.4). Plants irrigated at 50 % ET had lower $[ABA]_{root}$ throughout the soil profile, independent of whether soil moisture was higher (upper layers) or equivalent (lower layers), compared to when irrigation was withheld (Table. 4.4). Furthermore, $[ABA]_{root}$ was consistently higher on Days 2 and 3 in all layers of the soil in plants where irrigation was withheld. Although root ABA accumulation during soil drying occurs primarily in younger roots and root tips, the total mass of mature roots may have a greater contribution to the overall flux of ABA from root to shoot (Zhang and Tardieu, 1996). Thus it seems likely that whilst a proportion of the roots are experiencing soil drying (particularly younger roots) when irrigation is applied at 50 % of daily ET, the highest density of mature roots is found in the upper layer of the soil which is maintained at a higher moisture content (Table. 4.1).

4.5 Conclusion

In summary, this work provides evidence that the differences in $[X\text{-}ABA]_{leaf}$ under different irrigation treatments could be accounted for by differences in root ABA concentrations and hence ABA delivery to the shoot. $[X\text{-}ABA]_{root}$ increased under both irrigation treatments, although to a lesser extent when irrigation was supplied at 50 % ET. Despite this, $[X\text{-}ABA]_{root}$ was similarly correlated with increased $[X\text{-}ABA]_{leaf}$ under both irrigation treatments. Furthermore, increased $[X\text{-}ABA]_{root}$ could be better predicted when RWUF was accounted for (as opposed to whole pot soil moisture content), although this was still over-estimated in the 50 % ET irrigation treatment. This can however be explained by considerable vertical variation in $[ABA]_{root}$, and the significantly higher water uptake in the upper layer of the soil profile. Therefore, maintaining high soil moisture within the upper layers of containerised plants (by frequent irrigation) may significantly improve crop water use efficiency (Fig. 2.7) by attenuating root-to-shoot signalling of ABA and stomatal limitation of photosynthesis. Certainly, the ABA concentrations detected in this study (Table. 4.2, Fig. 4.5) were sufficient to elicit substantial stomatal closure when fed to detached leaves via the transpiration stream (Else et al., 2006), indicating that differences in $[X\text{-}ABA]_{leaf}$ between the two deficit irrigation treatments could explain the different physiological responses.

Chapter 5 - General discussion

"*Freshwater availability is relevant to almost all socioeconomic and environmental impacts of climate and demographic change and their implications for sustainability*" (Elliott et al., 2014).

The sustainable management of water resources presents a huge challenge for agriculture. Irrigation accounts for 70% of available fresh water (IFAD, 2013) and irrigation withdrawals are often applied inefficiently and exceeding plant water demands (Frenken and Gillet, 2012), and leaching of fertilisers occurs below the rootzone. This can be particularly damaging for the environment when groundwater withdrawal from aquifers unnecessarily exceeds natural replenishment (Gleeson et al., 2012). With an expanding world population, agricultural land at capacity, and a changing climate, attention is increasingly focused on improving the sustainability of irrigation practices and maximising resources available. One of the most suitable approaches to this may be to improve grower knowledge as to how their irrigation scheduling can be adapted to the specific water requirements of the plant (as opposed to traditional methods such as assessing soil moisture content).

Adapting irrigation practices to crop water requirements may be a challenge however due to the difficulties in creating effective knowledge transfer between research and industry. Thus, any findings should be easily simplified for a non-specialist audience, and techniques should be feasible for growers to adopt on a large scale. With this in mind, the initial aim set out in Chapter 2 was to investigate whether irrigation frequency could be adapted to plant water requirements, but subsequently to positively manage the production and water use of an ornamental bedding plant species, *Pelargonium* x *hortorum*. Whilst these are all essential aspects for ornamental plant production, physiological assessments were intended to provide a valuable baseline which could be built upon in the future to inform growers how to best monitor, and thus modify their irrigation scheduling. This is particularly important as the ornamental industry, although extremely valuable both in the UK and worldwide, often utilises inefficient irrigation management techniques such as overhead sprinkler systems (Briercliffe et al., 2000).

In order to evaluate plant physiological status when irrigation was applied at different frequencies, paired measurements of θ_{pot}, g_s and Ψ_{leaf} were carried out at the same time every 2 day (1100 h-1300 h) over 24 days. Daily sampling was impractical due to growth space limitations, but sampling every second day provided a representative indication of the plant’s physiological status. Measurements were always carried out at the same time each day to minimise diurnal variation between days (Correia et al., 1997). IDI and FDI both showed lower ET than WW plants, but IDI showed a series of increases in ET after re-watering (typically within 24 h), followed by a decrease over the subsequent 24 h (Fig. 2.3 c). Decreases in g_s were similar under both deficit irrigation treatments (Fig. 2.4a), albeit earlier under IDI (Fig. 2.5a) and coinciding with decreased Ψ_{leaf}, whilst FDI plants showed an increase in Ψ_{leaf} (Fig. 2.4 d & 2.5 d). FDI may maintain a more positive Ψ_{leaf} through decreased g_s.

These simple measurements inform growers of the intensity of stress the plant is experiencing (indicated by ET, Ψ_{leaf} and g_s), which can impact upon plant water use and photosynthetic activity (e.g. Fig. 5.1), and in turn growth and development (Blum, 2005, Chaves and Oliveira, 2004). However, purchasing the equipment required for the above will incur additional expenditure. Ultimately, this will enable growers to increase their irrigation frequency if plants are showing high stress (decreased ET, Ψ_{leaf} and/or g_s), and reduce the frequency upon recovery of Ψ_{leaf} and g_s. This is aligned to current knowledge transfer efforts between research and industry within the ornamental sector, where DEFRA's "Water Link" project (supported by research councils such as AHDB and HTA, and supplemented with Knowledge Transfer Partnerships) aims to develop technology and advice that can be supplied to growers, such as the use of thermal imaging (as a surrogate measure of g_s) to schedule irrigation (HTA, 2011).

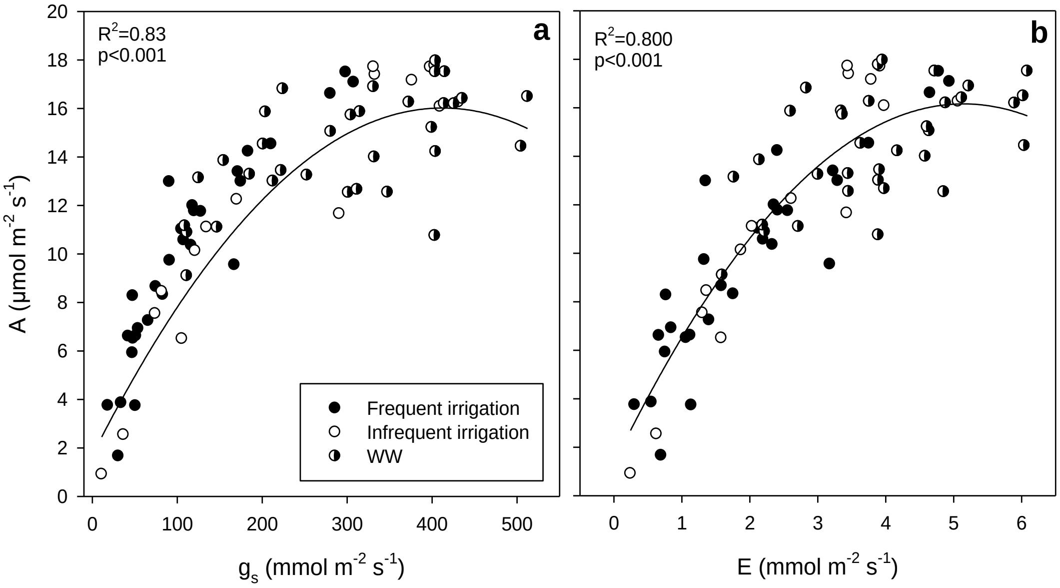


Fig. 5.1. Relationship between a) stomatal conductance (g_s); and b) transpiration (*E*) with photosynthesis (A) of *P.hortorum* subject to frequent (closed symbols; n=44) or infrequent (open symbols; n=34) deficit irrigation. Data points are paired individual samples, regression line is fitted and P value is reported.

Accurate irrigation management can also benefit plant production. Both IDI and FDI resulted in ~ 30% reductions in water input (Fig. 2.3), with a concomitant reduction in canopy expansion (Fig. 2.6), but without reducing foliar pigment composition (Fig. 2.8). Ornamental plants are grown to be aesthetically favourable (Cameron et al., 2008), and as such, any irrigation strategies must be implemented without decreasing plant economic value. In *P.hortorum*, plant quality (and thus value) was in fact increased due to the increased canopy compactness (a desirable ornamental trait) with no loss in foliar quality. Flowering quality under the different deficit irrigation frequency treatments was not assessed due to the long growth period of *P.hortorum* (2-3 months), although this is an essential area of interest for future work. Considering irrigation frequency from a broader horticultural perspective, it was found that decreasing irrigation frequency could increase WUE short-term by decreasing water use (during the first 2 weeks after treatments were imposed), although this effect on WUE was not maintained due to later growth reduction (Fig. 2.7). Growers can therefore adapt irrigation frequency to affect different outcomes, including reduced water use, increased WUE and increased plant quality.

The key challenge facing any grower with novel crop management techniques is how best to integrate them into/replace their current scheduling regimes. Current industry recommendations are vague, and fail to account for the variation in irrigation techniques and technologies, substrates, and species of plants grown (Knox et al., 2008). Further, industry-driven studies show that many growers and farmers are presented with a huge number of challenges that can lead to reluctance to adopt new strategies, particularly given the interaction of pressures from stakeholders (such as supermarkets and garden centres, as well as investors and environmental bodies), along with changing laws and regulations regarding water management (see Fig. 5.2). However, there are considerable benefits of sustainable irrigation management, particularly in terms of reduced economic outputs associated with water prices, labour and general crop management (Fig. 5.2).

More appropriate recommendations need to be established that encompass an understanding of the potential risks and drivers for change, and will effect positive outcomes. Knox et al. (2009) put forward a framework for improving water management, which includes establishing baseline knowledge, improving communications and partnerships between all the invested partners, and improving the efficiency of irrigation practices. The work in Chapter 2 has attempted to fulfil all of these criteria through the benefits described previously, but also as a result of this research being carried out alongside a commercial nursery and with support of the Agriculture and Horticulture Development Board which provides feedback to the wider industry. Perhaps the biggest challenge remaining is to investigate whether these irrigation strategies can be implemented on a commercial scale. This may be achieved through the use of an ebb and flow system (Dole et al., 1994) to ensure even distribution of irrigation, as well as the use of sensors to accurately monitor and regulate irrigation inputs to match the requirements of the plants on a larger scale.

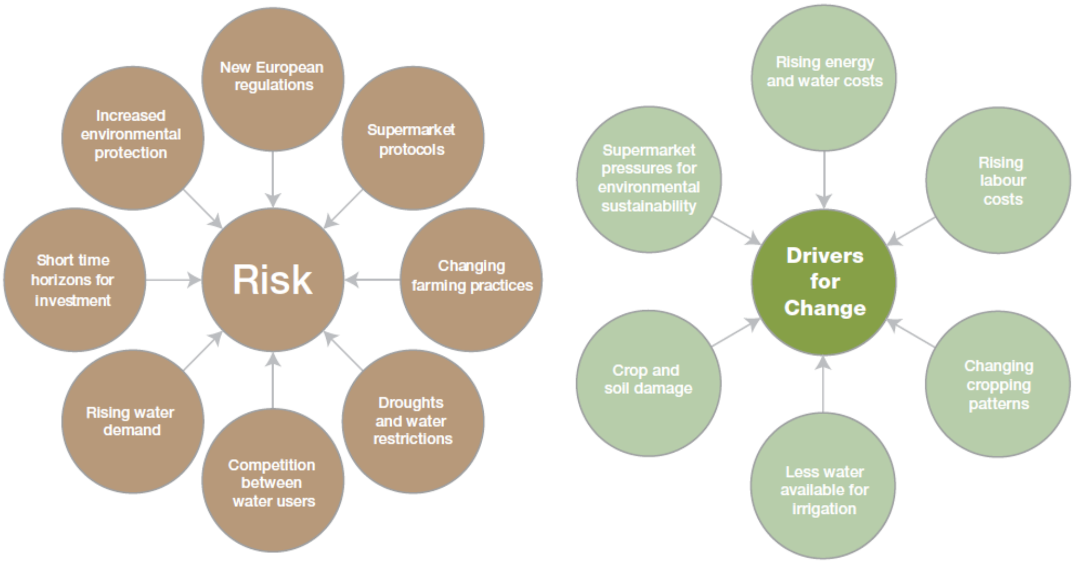


Fig. 5.2. Potential risks and drivers for change facing the irrigated agriculture industry as compiled by the UK Irrigation Association (UKIA). “Risks” adapted from Knox et al. (2007) and “Drivers for Change” adapted from Knox et al. (2008).

Effective implementation of novel irrigation techniques will require growers to have access to tools to evaluate the physiological status of the plants (or alternatively to measure the effects on canopy growth/phenological progression). The conventional understanding from the literature suggests that stomata will close when Ψ_{leaf} decreases (Brodribb and Holbrook, 2003). However, as shown in Chapter 2, whilst that relationship may explain the response under IDI, it fails to account for the observed decrease in g_s in FDI, where stomatal closure occurred without a decrease in Ψ_{leaf}. Thus a consistent response may not always be observed in a single species, supporting previous findings that some species can display both isohydric and anisohydric responses, for example poplar (Almeida-Rodriguez et al., 2010) and grapevine (Sade et al., 2012, Schultz, 2003). By modifying the irrigation regime, it appeared that another signal may have a more central, unifying role in regulating g_s. Since a hydraulic signal was excluded (consistent with previous literature (Sobeih et al., 2004, Bates and Hall, 1981)), it seemed likely that chemical signals, such as hormones and/or ionic components of the xylem sap, may have acted either locally, or as a long distance signal transported in the xylem sap (Dodd, 2005).

The irrigation treatments applied in Chapter 2 were subsequently adapted in Chapter 3 (Fig. 3.2) to allow different signalling responses to be measured over a single drying cycle, and at comparable soil moisture levels (Fig. 3.1, Table. 3.1). Consistent with data from Chapter 2, g_s decreased at a similar rate as soil moisture decreased under both deficit irrigation treatments (Fig. 3.4), although Ψ_{leaf} only decreased when irrigation was withheld (Fig. 3.6). The lack of a consistent response across both irrigation treatments (over two different studies) further supports the argument that Ψ_{leaf} is not a central regulator of stomata under soil drying in *P.hortorum* (Fig. 3.7). It was therefore hypothesised that a chemical signal may have a more important role in causing stomatal closure. However, no differences in xylem sap pH, or NO_3- and Ca^{2+} concentrations, all of which have previously been shown to regulate stomata (Wilkinson et al., 2007, Ruiz et al., 1993), were observed between irrigation treatments (Table. 3.2), suggesting the need for alternate regulatory mechanisms under these conditions.

It has been proposed that ABA, a potent antitranspirant, regulates stomata under soil drying (Zhang et al., 1987). Indeed, xylem ABA concentration did increase under both irrigation treatments as the soil dried (Fig. 3.5), although this response was attenuated when irrigation was supplied at a fraction of crop ET, similar to FDI in Chapter 2). However, increased $[X\text{-}ABA]_{leaf}$ and decreased g_s were strongly correlated across both deficit irrigation treatments (Fig. 3.9), but it was not clear whether this relationship was correlative or causative. In support of the latter, causative argument, supplying detached leaves with synthetic ABA via the transpiration stream generated a similar relationship between g_s and ABA as occurred *in vivo* when plants were subject to different irrigation treatments (Fig. 3.10). By duplicating the correlation between g_s and ABA in an isolated leaf fed synthetic ABA in the transpiration stream, these experiments fulfilled the criteria of plant hormone action regulating physiological processes (in this case stomatal closure) (Jacobs, 1959, Jackson, 1987).

While it was concluded that ABA was the central regulator of stomata in *P.hortorum* independent of changes in Ψ_{leaf}, the exact mechanism(s) behind the attenuated ABA response under 50 % ET remained unanswered. Possible explanations included decreased root ABA synthesis (Zhang and Tardieu, 1996), reduced root flux of ABA (Jokhan et al., 1996), and spatial variation in soil moisture availability (Puértolas et al., 2013) between the different irrigation treatments.

Understanding this response is essential for developing alternative irrigation strategies, particularly giving the importance of ABA in regulating stomata (and thus photosynthesis) (Wilkinson et al., 2012).

Previous work investigating vertical soil moisture gradients showed both species- and gradient-specific ABA responses (discussed in Chapter 4; see also Puértolas et al., 2013, Puértolas et al., 2014, Trejo and Davies, 1991, Gallardo et al., 1994). As the irrigation treatments imposed in Chapter 3 resulted in differences in soil moisture gradients (heterogeneous *vs* homogenous soil drying at the same soil moisture availability, in FDI and IDI respectively), it was hypothesised that irrigation placement was regulating Ψ_{root} and $[ABA]_{root}$. This in turn could explain the attenuated $[X\text{-}ABA]_{leaf}$ (and consequent effects on shoot physiology; see Chapter 4). Tomato (*Solanum lycopersicum*) was selected as a more appropriate species than *P.hortorum* as it facilitated sampling of root tissue and xylem sap. To collect xylem sap from the roots, plants had to be grown in cylindrical pots and placed in a pressure chamber, which was unsuitable in *P.hortorum* as the canopy occurred at soil level, without a prominent stem. Irrigation treatments adapted from those used in Chapter 3 (Fig. 4.3), albeit for a reduced duration due to the high water use and susceptibility of tomato to soil drying, and to allow daily sampling of all treatments.

Soil moisture distribution patterns were similar in both tomato and *P.hortorum* (Table. 3.1 & 4.1). Soil drying decreased g_s in both irrigation treatments (Fig. 4.4a), and a greater reduction in g_s was observed when irrigation was withheld. Ψ_{leaf} decreased as soil moisture decreased (Fig. 4.4b) although there was no treatment effect, and Ψ_{root} decreased under both deficit irrigation treatments (Fig. 4.4c), with a greater reduction when irrigation was withheld. $[X\text{-}ABA]_{root}$ increased as soil moisture decreased under both irrigation treatments (Fig. 4.5a), although $[X\text{-}ABA]_{leaf}$

only increased when irrigation was withheld (Fig. 4.5b), which may have been a result of reduced ABA synthesis in the leaves, or a result of reduced ABA transport from the roots (particularly as there was a strong correlation between $[X\text{-}ABA]_{root}$ and $[X\text{-}ABA]_{leaf}$ under both irrigation treatments (Fig. 4.5c)).

This corresponded with a consistent relationship between $[X\text{-}ABA]_{root}$ and g_s under both deficit irrigation treatments (Fig. 4.6b). However, $[X\text{-}ABA]_{leaf}$ only correlated with g_s when irrigation was withheld (Fig. 4.6a). Ψ_{root} also showed a consistent correlation with $[X\text{-}ABA]_{root}$ over both irrigation treatments. These results show a similar response to previous work in tomato (Dodd, 2007), and that observed in *P.hortorum*, providing further support for the role of Ψ_{root} in regulating foliar ABA concentrations, which can in turn influence shoot physiology.

Previous research has shown that ABA can be synthesised in both the roots and the leaves (Kim et al., 2010, Merilo et al., 2014). Therefore, one of the challenges of this work was establishing the site of ABA production, especially as previous work has down-played the role of the root system in ABA synthesis (Holbrook et al., 2002). However, reduced g_s was correlated with increased $[X\text{-}ABA]_{root}$ under both irrigation treatments (Fig. 4.7a), and withholding irrigation showed significant increases in root ABA export compared to WW plants and those irrigated at 50 % ET (Table. 4.2). As $[X\text{-}ABA]_{root}$ samples were collected at flow rates matching plant transpiration, this highlights that ABA is still being transported from the roots, explaining the significant increase in $[X\text{-}ABA]_{leaf}$ when irrigation was withheld, and the attenuated response when irrigation was applied at 50 % ET that influenced shoot physiology. Consequently, it was concluded that increased ABA is likely a root-derived response in tomato under the different treatments that regulated stomata.

A number of models were developed to determine whether root water uptake and soil moisture content could further explain the observed differences in $[\text{X-ABA}]_{\text{leaf}}$ and $[\text{X-ABA}]_{\text{root}}$ (Table. 4.3). When irrigation was withheld, there was little difference in predicting $[\text{X-ABA}]_{\text{leaf}}$ and $[\text{X-ABA}]_{\text{root}}$ irrespective of whether the fraction of root water uptake (RWUF) was included or not in the model. When plants were irrigated at 50 % ET, predictions of $[\text{X-ABA}]_{\text{leaf}}$ and $[\text{X-ABA}]_{\text{root}}$ improved when RWUF was included (although $[\text{X-ABA}]_{\text{root}}$ was still significantly overestimated). ABA modelling in Chapter 4 was essential due to the complexity of the initial ABA response observed between the treatments as it allowed additional plant and soil variables relations to be included, as well as highlighting other variables that may not have been considered. For instance, localised variation in Ψ_{root} may be significant in determining $[\text{X-ABA}]_{\text{root}}$ under both treatments (Puértolas et al., 2013), particularly under the heterogeneous soil drying when irrigation was applied at 50 % ET, and should thus be the subject of future work in this area.

Despite the key results above, the limited transport of ABA from the roots when plants were irrigated at 50 % ET requires further explanation. To address this, $[\text{ABA}]_{\text{root}}$ was measured at four depths within the soil profile. $[\text{ABA}]_{\text{root}}$ increased with diminishing soil water availability in all layers (Fig. 4.8; Table. 4.4), although it was lower in plants irrigated at 50 % ET, compared to when irrigation was withheld, throughout the soil profile, even at comparable soil moisture availability (Table. 4.4). It was hypothesised that as the higher density of mature roots in the upper layers of the soil profile were exposed to a higher soil moisture content when irrigation was applied at 50 % ET, this reduced the overall synthesis and thus the flux of ABA from the roots to the shoots.

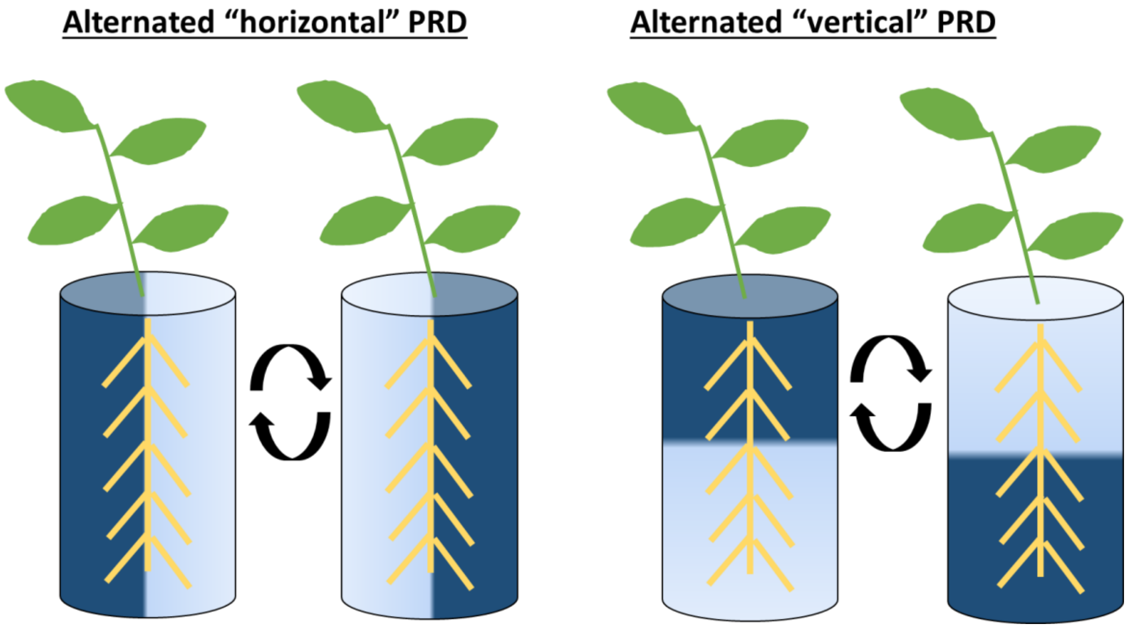
In summary, Chapter 4 provides a root-based explanation for the different [X-ABA]$_{leaf}$ response first observed in *P.hortorum* in Chapter 3, and then in tomato under different irrigation treatments. This advances our understanding of ABA signalling by showing that spatial variation in soil moisture can alter long distance, root-to-shoot ABA signalling, even in the same species at similar whole-pot soil moisture availability, thereby affecting shoot physiological processes. Thus, any future work investigating long-distance ABA signalling should take account of the effects of irrigation treatments on soil moisture gradients as this may have significant impacts upon shoot physiological changes.

5.1 Future work

This present study highlights two distinct areas for future work. First, it is essential that further research continues to improve the understanding of plant physiological responses to irrigation frequency in a commercial context. Whilst baseline data have been established for an ornamental species, arguably the biggest constraint to implementing more accurate irrigation scheduling is the need for communication with growers in order to scale up and adapt the knowledge and findings to match their requirements. In addition, whilst the work in this thesis used a peat-based substrate, the horticultural industry is intensifying its efforts to utilise alternative, non-peat substrates. Therefore, it may be appropriate for work to be carried out investigating the impact of alternative irrigation techniques in more sustainable substrates (such as coir or green composts).

Secondly, additional work could focus further on plant physiological responses to altered soil moisture distribution (through accurate irrigation placement). Measurements of localised Ψ_{root} under the different irrigation treatments described in

Chapter 4 may provide additional insights into the mechanistic differences in localised $[ABA]_{root}$. Furthermore, adapting this experimental system for commercial alternative irrigation systems (such as vertical "alternated-PRD" (Fig. 5.3), which may be achieved by a combination of an ebb-and-flow system and overhead irrigation, or accurate irrigation placement in the field using buried drippers) may be advantageous for growers, and needs to be assessed further. Implementing alternated vertical PRD may allow growers to maintain Ψ_{leaf} whilst tightly regulating transport and synthesis of ABA, which would potentially increase WUE by maintaining carbon assimilation (and growth) whilst also limiting water loss.


Alternated "horizontal" PRD

Alternated "vertical" PRD

Figure. 5.3. Diagrammatic representation of conventional alternated "horizontal" partial root zone drying (PRD); and proposed approach for alternated "vertical" PRD. In both irrigated regimes, only half of the pot is irrigated (dark blue) whilst the other half of the soil is allowed to dry (light blue), which is alternated between each half of the pot.

5.2 Conclusions

In conclusion, this thesis has established that irrigation management of the ornamental bedding plant species *Pelargonium* x *hortorum* can be improved by manipulating irrigation frequency based upon plant water requirements. Benefits included reduced water inputs, enhanced ornamental characteristics (and thus plant economic value), and also increased water productivity. Perhaps the two major barriers to accurately implementing this are firstly improving grower's knowledge about irrigation scheduling, but also scaling it to a commercial grower. This thesis focused on elucidating the physiological differences observed between different irrigation treatments. The research has firstly addressed the leaf-level response and the ABA-mediated regulation of stomata. Secondly, tomato has been used as a model species to determine whether the response observed in P.*hortorum* can be explained by differences in ABA signalling in the roots, and the mechanisms which may have been regulating this. Taken together, this research provides both important information from an agronomic perspective, and also improves our fundamental understanding of long distance root-to-shoot ABA signalling, which may be informative in developing novel irrigation techniques in the future.

References

Abalos, D., Sanchez-Martin, L., Garcia-Torres, L., Van Groenigen, J. W. & Vallejo, A. 2014. Management of irrigation frequency and nitrogen fertilization to mitigate GHG and NO emissions from drip-fertigated crops. *Science of The Total Environment,* 490**,** 880-888.

Acharya, B. & Assmann, S. 2009. Hormone interactions in stomatal function. *Plant Molecular Biology,* 69**,** 451-462.

Ali, M. H., Hoque, M. R., Hassan, A. A. & Khair, A. 2007. Effects of deficit irrigation on yield, water productivity, and economic returns of wheat. *Agricultural Water Management,* 92**,** 151-161.

Almedia-Rodriguez, A. M., Cooke, J. E. K., Yeh, F. & Zwiazek, J. J. 2010. Functional characterization of drought-responsive aquaporins in *Populus balsamifera* and *Populus simonii* x *balsamifera* clones with different drought resistance strategies. *Physiologia Plantarum,* 140**,** 321-333.

Álvarez, S., Bañón, S. & Sánchez-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.

Álvarez, S., Gómez-Bellot, M. J., Castillo, M., Bañón, S. & Sánchez-Blanco, M. J. 2012. Osmotic and saline effect on growth, water relations, and ion uptake and translocation in *Phlomis purpurea* plants. *Environmental and Experimental Botany,* 78**,** 138-145.

Álvarez, S., Navarro, A., Bañón, S. & Sánchez-Blanco, M. J. 2009. Regulated deficit irrigation in potted Dianthus plants: Effects of severe and moderate water stress on growth and physiological responses. *Scientia Horticulturae,* 122**,** 579-585.

Álvarez, S. & Sánchez-Blanco, M. J. 2013. Changes in growth rate, root morphology and water use efficiency of potted *Callistemon citrinus* plants in response to different levels of water deficit. *Scientia Horticulturae,* 156**,** 54-62.

Anderson, J. P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., Maclean, D. J., Ebert, P. R. & Kazan, K. 2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell,* 16**,** 3460-3479.

Andersson, N. E. 2001. Weight controlled irrigation of potted plants. *In:* Fernandez, J. A., Mertinez, P. F. & Castilla, N. (eds.) *Proceedings of the Fifth International Symposium on Protected Cultivation in Mild Winter Climates: Current Trends for Sustainable Technologies, Vols I and Ii.*

Andersson, N. E. 2011. The influence of water stress and air velocity on growth of *Impatiens walleriana* and *Petunia* x hybrid. *Scientia Horticulturae,* 128**,** 146-151.

Arora, R., Pitchay, D. S. & Bearce, B. C. 1998. Water-stress-induced heat tolerance in geranium leaf tissues: A possible linkage through stress proteins? *Physiologia Plantarum,* 103**,** 24-34.

Ashraf, M. & Harris, P. J. C. 2013. Photosynthesis under stressful environments: An overview. *Photosynthetica,* 51**,** 163-190.

Askri, B., Ahmed, A. T., Abichou, T. & Bouhlila, R. 2014. Effects of shallow water table, salinity and frequency of irrigation water on the date palm water use. *Journal of Hydrology,* 513**,** 81-90.

Assmann, S. M. & Shimazaki, K. 1999. The multisensory guard cell. Stomatal responses to blue light and abscisic acid. *Plant Physiology,* 119**,** 809-815.

Assouline, S., Möller, M., Cohen, S., Ben-Hur, M., Grava, A., Narkis, K. & Silber, A. 2006. Soil-plant system response to pulsed drip irrigation and salinity: Bell pepper case study. *Soil Science Society of America Journal,* 70**,** 1556-1568.

Atkinson, C. J., Mansfield, T. A. & Davies, W. J. 1990. Does calcium in xylem sap regulate stomatal conductance. *New Phytologist,* 116**,** 19-27.

Augé, R. M., Stodola, A. J. W., Moore, J. L., Klingeman, W. E. & Duan, X. R. 2003. Comparative dehydration tolerance of foliage of several ornamental crops. *Scientia Horticulturae,* 98**,** 511-516.

Ávila, M. B., Lúcio, J. A. G. D., Mendoza, N. V., González, C. V., Arciniega, M. D. L. O. & Vargas, G. A. 2013. Geranium Species as Antioxidants, Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants, Dr. Jose Antonio Morales-Gonzalez (Ed.), ISBN: 978-953-51-1123-8, InTech, DOI: 10.5772/52002. Available from: http://www.intechopen.com/books/oxidative-stress-and-chronic-degenerative-diseases-a-role-for-antioxidants/geranium-species-as-antioxidants

Bacon, M., Wilkinson, S., & Davies, W. J. 1998. pH-regulated cell expansion is ABA-dependent. *Plant Physiology,* 118, 1507-1515.

Bahrun, A., Jensen, C. R., Asch, F. & Mogensen, V. O. 2002. Drought-induced changes in xylem pH, ionic composition, and ABA concentration act as early signals in field-grown maize (*Zea mays* L.). *Journal of Experimental Botany,* 53**,** 251-263.

Barigah, T. S., Charrier, O., Douris, M., Bonhomme, M., Herbette, S., Améglio, T., Fichot, R., Brignolas, F. & Cochard, H. 2013. Water stress-induced xylem hydraulic failure is a causal factor of tree mortality in beech and poplar. *Annals of Botany*.

Bates, L. M. & Hall, A. E. 1981. Stomatal closure with soil-water depletion not associated with changes in bulk leaf water status. *Oecologia,* 50**,** 62-65.

Beeson, R. C. 1992. Restricting Overhead Irrigation to Dawn Limits Growth in Container-grown Woody Ornamentals. *HortScience*, 27, 996-999.

Beeson, R. C. 2006. Relationship of plant growth and actual evapotranspiration to irrigation frequency based on management allowed deficits for container nursery stock. *Journal of the American Society for Horticultural Science,* 131**,** 140-148.

Blanusa, T., Vysini, E. & Cameron, R. W. F. 2009. Growth and Flowering of Petunia and Impatiens: Effects of Competition and Reduced Water Content Within a Container. *Hortscience,* 44**,** 1302-1307.

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research,* 56**,** 1159-1168.

Blum, A. 2011. *Plant water relations, plant stress and plant production*, Springer.

Bolla, A., Voyiatzis, D., Koukourikou-Petridou, M. & Chimonidou, D. 2010. Photosynthetic parameters and cut-flower yield of rose 'Eurored' (HT) are adversely affected by mild water stress irrespective of substrate composition. *Scientia Horticulturae,* 126**,** 390-394.

Borel, C., Frey, A., Marion-Poll, A., Tardieu, F. & Simonneau, T. 2001. Does engineering abscisic acid biosynthesis in *Nicotiana plumbaginifolia* modify stomatal response to drought? *Plant, Cell & Environment,* 24**,** 477-489.

Borel, C. & Simonneau, T. 2002. Is the ABA concentration in the sap collected by pressurizing leaves relevant for analysing drought effects on stomata? Evidence from ABA-fed leaves of transgenic plants with modified capacities to synthesize ABA. *Journal of Experimental Botany,* 53**,** 287-296.

Boyle, R. K. A., McAinsh, M. & Dodd, I. C. 2015. Stomatal closure of *Pelargonium* × *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.

Briercliffe, T., Hewson, A. & Brough, W. 2000. Independent water audits for container grown nursery stock producers. *ADAS Horticulture*.

Brodribb, T. J. & Holbrook, N. M. 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology,* 132**,** 2166-2173.

Brodribb, T. J., Holbrook, N. M., Edwards, E. J. & Gutierrez, M. V. 2003. Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant Cell and Environment,* 26**,** 443-450.

Buckley, T. N. 2005. The control of stomata by water balance. *New Phytologist,* 168**,** 275-291.

Bunce, J. A. 2006. How do leaf hydraulics limit stomatal conductance at high water vapour pressure deficits? *Plant, Cell & Environment,* 29**,** 1644-1650.

Caldeira, C. F., Bosio, M., Parent, B., Jeanguenin, L., Chaumont, F. & Tardieu, F. 2014. A Hydraulic Model Is Compatible with Rapid Changes in Leaf

Elongation under Fluctuating Evaporative Demand and Soil Water Status. *Plant Physiology,* 164**,** 1718-1730.

Cameron, R., Harrison-Murray, R., Fordham, M., Wilkinson, S., Davies, W., Atkinson, C. & Else, M. 2008. Regulated irrigation of woody ornamentals to improve plant quality and precondition against drought stress. *Annals of Applied Biology,* 153**,** 49-61.

Cameron, R. W. F., Harrison-Murray, R. S., Atkinson, C. J. & Judd, H. L. 2006. Regulated deficit irrigation - a means to control growth in woody ornamentals. *Journal of Horticultural Science & Biotechnology,* 81**,** 435-443.

Cameron, R. W. F., Harrison-Murray, R. S. & Scott, M. A. 1999. The use of controlled water stress to manipulate growth of container-grown *Rhododendron* cv. Hoppy. *Journal of Horticultural Science & Biotechnology,* 74**,** 161-169.

Čerekovic, N., Pagter, M., Kristensen, H. L., Pederesen, H. L., Brennan, R. & Petersen, K. K. 2013. Effects of drought stress during flowering of two pot-grown blackcurrant (*Ribes nigrum* L.) cultivars. *Scientia Horticulturae,* 162**,** 365-373.

Chalker-Scott, L. 1999. Environmental Significance of Anthocyanins in Plant Stress Responses. *Photochemistry and Photobiology,* 70**,** 1-9.

Chalmers, D. J., Mitchell, P. D. & Vanheek, L. 1981. Control of peach-tree growth and productivity by regulated water-supply, tree density and summer pruning. *Journal of the American Society for Horticultural Science,* 106**,** 307-312.

Chanming, L., Jingjie, Y. & Kendy, E. 2001. Groundwater Exploitation and Its Impact on the Environment in the North China Plain. *Water International,* 26**,** 265-272.

Chasan, R. 1995. New openings into stomata. *Plant Cell,* 7**,** 1113-1115.

Chavea, M. M. & Oliveira, M. M. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *Journal of Experimental Botany,* 55**,** 2365-2384.

Chen, C., Xiao, Y.-G., Li, X. & Ni, M. 2012. Light-Regulated Stomatal Aperture in Arabidopsis. *Molecular Plant,* 5**,** 566-572.

Chen, L., Dodd, I. C., Davies, W. J. & Wilkinson, S. 2013. Ethylene limits abscisic acid- or soil drying-induced stomatal closure in aged wheat leaves. *Plant Cell and Environment,* 36**,** 1850-1859.

Christmann, A., Grill, E. & Huang, J. 2013. Hydraulic signals in long-distance signaling. *Current Opinion in Plant Biology,* 16**,** 293-300.

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.

Chyliński, W. K., Łukazewska, A. J. & Kutnik, K. 2007. Drought response of two bedding plants. *Acta Physiologiae Plantarum,* 29**,** 399-406.

Cifre, J., Bota, J., Escalona, J. M., Medrano, H. & Flexas, J. 2005. Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.): An open gate to improve water-use efficiency? *Agriculture, Ecosystems & Environment,* 106**,** 159-170.

Cirillo, C., Rouphael, Y., Caputo, R., Raimondi, G. & De Pascale, S. 2014. The Influence of Deficit Irrigation on Growth, Ornamental Quality, and Water

Use Efficiency of Three Potted *Bougainvillea* Genotypes Grown in Two Shapes. *Hortscience,* 49**,** 1284-1291.

Clifford, S. C., Runkle, E. S., Langton, F. A., Mead, A., Foster, S. A., Pearson, S. & Heins, R. D. 2004. Height control of poinsettia using photoselective filters. *Hortscience,* 39**,** 383-387.

Clifton-Brown, J. C. & Jones, M. B. 1999. Alteration of transpiration rate, by changing air vapour pressure deficit, influences leaf extension rate transiently in Miscanthus. *Journal of Experimental Botany,* 50**,** 1393-1401.

Cochard, H. 2002. Xylem embolism and drought-induced stomatal closure in maize. *Planta,* 215**,** 466-471.

Collatz, G. J., Ball, J. T., Grivet, C. & Berry, J. A. 1991. Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. *Agricultural and Forest Meteorology,* 54**,** 107-136.

Comstock, J. & Mencuccini, M. 1998. Control of stomatal conductance by leaf water potential in *Hymenoclea salsola* (T. & G.), a desert subshrub. *Plant, Cell & Environment,* 21**,** 1029-1038.

Comstock, J. P. 2002. Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. *Journal of Experimental Botany,* 53**,** 195-200.

Conway, G. R. & Barbier, E. B. 2009. *After the green revolution: sustainable agriculture for development*, Earthscan.

Correia, M. J. & Pereira, J. S. 1995. The control of leaf conductance of white lupin by xylem ABA concentration decreases with the severity of water deficits. *Journal of Experimental Botany,* 46**,** 101-110.

Correia, M. J., Rodrigues, M. L., Ferreira, M. I. & Pereira, J. S. 1997. Diurnal change in the relationship between stomatal conductance and abscisic acid in the xylem sap of field-grown peach trees. *Journal of Experimental Botany,* 48**,** 1727-1736.

Cutler, A. J. & Krochko, J. E. 1999. Formation and breakdown of ABA. *Trends in Plant Science,* 4**,** 472-478.

Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R., & Abrams, S. R. 2010. Abscisic acid: emergence of a core signaling network. *Annual Reviews Plant Biology,* 61, 651-679.

Daie, J. & Wyse, R. 1983. ABA Uptake in Source and Sink Tissues of Sugar Beet. *Plant Physiology,* 72**,** 430-433.

de Matos Pires, R. C., Furlani, P. R., Ribeiro, R. V., Bodine Junior, D., Sakai, E., Lourenção, A. L. & Torre Neto, A. 2011. Irrigation frequency and substrate volume effects in the growth and yield of tomato plants under greenhouse conditions. *Scientia Agricola,* 68**,** 400-405.

DEFRA 2010. Monitoring the horticultural use of peat and progress towards the UK Biodiversity Action Plan target (SP08020).

Demotes-Mainard, S., Boumaza, R., Meyer, S. & Cerovic, Z. G. 2008. Indicators of nitrogen status for ornamental woody plants based on optical measurements of leaf epidermal polyphenol and chlorophyll contents. *Scientia Horticulturae,* 115**,** 377-385.

Dennehy, K. F., Litke, D. W. & McMahon, P. B. 2002. The High Plains Aquifer, USA: groundwater development and sustainability. *Geological Society, London, Special Publications,* 193**,** 99-119.

Denny, D. & Dimmock, J. 2014. UK ornamentals - growing your market. Horticultural Development Company.

Dodd, I. C. 2003. Hormonal interactions and stomatal responses. *Journal of Plant Growth Regulation,* 22**,** 32-46.

Dodd, I. C. 2005. Root-to-shoot signalling: Assessing the roles of 'up' in the up and down world of long-distance signalling in planta. *Plant and Soil,* 274**,** 251-270.

Dodd, I. C. 2007. Soil moisture heterogeneity during deficit irrigation alters root-to-shoot signalling of abscisic acid. *Functional Plant Biology,* 34**,** 439-448.

Dodd, I. C. 2009. Rhizosphere manipulations to maximize 'crop per drop' during deficit irrigation. *Journal of Experimental Botany,* 60**,** 2454-2459.

Dodd, I. C., Egea, G. & Davies, W. J. 2008. Abscisic acid signalling when soil moisture is heterogeneous: decreased photoperiod sap flow from drying roots limits abscisic acid export to the shoots. *Plant, Cell & Environment,* 31**,** 1263-1274.

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.

Dodd, I. C., Tan, L. P. & He, J. 2003. Do increases in xylem sap pH and/or ABA concentration mediate stomatal closure following nitrate deprivation? *Journal of Experimental Botany,* 54**,** 1281-1288.

Dodd, I. C., Theobald, J. C., Bacon, M. A. & Davies, W. J. 2006. Alternation of wet and dry sides during partial rootzone drying irrigation alters root-to-shoot signalling of abscisic acid. *Functional Plant Biology,* 33**,** 1081-1089.

Dodd, I. C., Theobald, J. C., Richer, S. K. & Davies, W. J. 2009. Partial phenotypic reversion of ABA-deficient flacca tomato (*Solanum lycopersicum*) scions by a wild-type rootstock: normalizing shoot ethylene relations promotes leaf area but does not diminish whole plant transpiration rate. *Journal of Experimental Botany,* 60**,** 4029-4039.

Dole, J. M., Cole, J. C. & von Broembsen, S. L. 1994. Growth of Poinsettias, Nutrient Leaching, and Water-use Efficiency Respond to Irrigation Methods. *Hortscience,* 29**,** 858-864.

dos Santos, T. P., Lopes, C. M., Lucília Rodrigues, M., de Souza, C. R., Ricardo-da-Silva, J. M., Maroco, J. P., Pereira, J. S. & Mnauela Chaves, M. 2007. Effects of deficit irrigation strategies on cluster microclimate for improving fruit composition of Moscatel field-grown grapevines. *Scientia Horticulturae,* 112**,** 321-330.

Easlon, H. M., Nemali, K. S., Richards, J. H., Hanson, D. T., Juenger, T. E. & McKay, J. K. 2014. The physiological basis for genetic variation in water use efficiency and carbon isotope composition in *Arabidopsis thaliana*. *Photosynthesis Research,* 119**,** 119-129.

Ehlert, C., Maurel, C., Tardieu, F., & Simonneau, T. 2009. Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiology,* 150, 1093-1104.

Eiasu, B. K., Steyn, J. M. & Soundy, P. 2012. Physiomorphological response of rose-scented geranium (*Pelargonium* spp.) to irrigation frequency. *South African Journal of Botany,* 78**,** 96-103.

El-Hendawy, S. E. & Schmidhalter, U. 2010. Optimal coupling combinations between irrigation frequency and rate for drip-irrigated maize grown on sandy soil. *Agricultural Water Management,* 97**,** 439-448.

Elliot, J., Deryng, D., Müller, C., Frieler, K., Konzmann, M., Gerten, D., Glotter, M., Flörke, M., Wada, Y., Best, N., Eisner, S., Fekete, B. M., Folberth, C., Foster, I., Gosling, S. N., Haddeland, I., Khabarov, N., Ludwig, F., Masaki, Y., Olin, S., Rosenzweig, C., Ruane, A. C., Satoh, Y., Schmid, E., Stacke, T., Tang, Q. & Wisser, D. 2014. Constraints and potentials of future irrigation water availability on agricultural production under climate change. *Proceedings of the National Academy of Sciences,* 111**,** 3239-3244.

Else, M. A., Taylor, J. M., & Atkinson, C. J. 2006. Anti-transpirant activity in xylem sap from flooded tomato (*Lycopersicon esculentum* Mill.) plants is not due to pH-mediated redistributions of root- or shoot-sourced ABA. *Journal of Experimental Botany*, 57, 3349-3357.

Else, M. A., Tiekstra, A. E., Croker, S. J., Davies, W. J. & Jackson, M. B. 1996. Stomatal Closure in Flooded Tomato Plants Involves Abscisic Acid and a Chemically Unidentified Anti-Transpirant in Xylem Sap. *Plant Physiology,* 112**,** 239-247.

Endo, A., Sawada, Y., Takahashi, H., Okamoto, M., Ikegami, K., Koiwai, H., Seo, M., Toyomasu, T., Mitsuhashi, W., Shinozaki, K., Nakazono, M., Kamiya, Y., Koshiba, T., & Nambara, E. 2008. Drought Induction of Arabidopsis 9-cis-Epoxycarotenoid Dioxygenase Occurs in Vascular Parenchyma Cells. *Plant Physiology*, 147, 1984-1993.

Ernst, L., Goodger, J. Q. D., Alvarez, S., Marsh, E. L., Berla, B., Lockhart, E., Jung, J., Li, P., Bohnert, H. J. & Schachtman, D. P. 2010. Sulphate as a xylem-borne chemical signal precedes the expression of ABA biosynthetic genes in maize roots. *Journal of Experimental Botany,* 61**,** 3395-3405.

Ertek, A., Şensoy, S., Küçükyumuk, C. & Gedik, I. 2004. Irrigation frequency and amount affect yield components of summer squash (*Cucurbita pepo* L.). *Agricultural Water Management,* 67**,** 63-76.

Fambrini, M., Vernieri, P., Toncelli, M. L., Rossi, V. D. & Pugliesi, C. 1995. Characterization of a wilty sunflower (*Helianthus annus* L.) mutant. 3. Phenotypic interaction in reciprocal grafts from wilty mutant and wild-type plants. *Journal of Experimental Botany,* 46**,** 525-530.

Fereres, E. & Soriano, M. A. 2007. Deficit irrigation for reducing agricultural water use. *Journal of Experimental Botany,* 58**,** 147-159.

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.

Fiebig, A., & Dodd, I. C. Inhibition of tomato shoot growth by over-irrigation is linked to nitrogen deficiency and ethylene. *Physiologia Plantarum*, DOI: 10.111/ppl. 12343.

Flexas, J., & Medrano, H. 2002. Drought-inhibition of Photosynthesis in C_3 Plants: Stomatal and Non-Stomatal Limitations Revisited. *Annals of Botany,* 89, 183-189.

Foster, K., Lambers, H., Real, D., Ramankutty, P., Cawthray, G. R. & Ryan, M. H. 2015. Drought resistance and recovery in mature *Bituminaria bituminosa* var. *albomarginata*. *Annals of Applied Biology,* 166**,** 154-169.

Franco, J. A., Martínez-Sánchez, J. J., Fernández, J. A. & Bañón, S. 2006. Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. *Journal of Horticultural Science & Biotechnology,* 81**,** 3-17.

Franks, P. J. 2013. Passive and active stomatal control: either or both? *New Phytologist*, 198**,** 325-327.

Frenken, K. & Gillet, V. 2012. Irrigation water requirement and water withdrawal by country. *In:* AQUASTAT (ed.). Food and Agriculture Organization of the United Nations.

Fu, J. & Dernoeden, P. H. 2009. Creeping Bentgrass Putting Green Turf Responses to Two Summer Irrigation Practices: Rooting and Soil Temperature. *Crop Science*, 49**,** 1063-1070.

Fuchs, E. E. & Livingston, N. J. 1996. Hydraulic control of stomatal conductance in Douglas fir *Pseudotsuga menziesi*i (Mirb) Franco and alder *Alnus rubra* (Bong) seedlings. *Plant Cell and Environment,* 19**,** 1091-1098.

Fustec, J. & Beaujard, F. 2000. Effect of photoperiod and nitrogen supply on basal shoots development in *Rhododendron catawbiense*. *Biologia Plantarum,* 43**,** 511-515.

Gallardo, M., Turner, N. C. & Ludwig, C. 1994. Water relations, gas exchange and abscisic acid content of Lupinus cosentinii leaves in response to drying different proportions of the root system. *Journal of Experimental Botany,* 45**,** 909-918.

Gallé, A., Csiszár, J., Benyó, D., Laskay, G., Leviczky, T., Erdei L. & Tari, I. 2013. Isohydric and anisohydric strategies of wheat genotypes under osmotic stress: Biosynthesis and function of ABA in stress responses. *Journal of Plant Physiology,* 170**,** 1389-1399.

Garcia-Navarro, M. C., Evans, R. Y. & Montserrat, R. S. 2004. Estimation of relative water use among ornamental landscape species. *Scientia Horticulturae,* 99**,** 163-174.

García-Sogo, B., Pineda, B., Roque, E., Antón, T., Atarés, A., Borja, M., Pío Beltrán, J., Moreno, V. & Antonio Cañas, L. 2012. Production of engineered long-life and male sterile *Pelargonium* plants. *Bmc Plant Biology,* 12.

Geerts, S. & Raes, D. 2009. Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. *Agricultural Water Management,* 96**,** 1275-1284.

Gheysari, M., Mirlatifi, S. M., Homaee, M., Asadi, M. E. & Hoogenboom, G. 2009. Nitrate leaching in a silage maize field under different irrigation and nitrogen fertilizer rates. *Agricultural Water Management,* 96**,** 946-954.

Gilroy, S., Fricker, M. D., Read, N. D. & Trewayas, A. J. 1991. Role of Calcium in Signal Transduction of Commelina Guard Cells. *Plant Cell,* 3**,** 333-344.

Gleeson, T., Wada, Y., Bierkens, M. F. P. & van Beek, L. P. H. 2012. Water balance of global aquifers revealed by groundwater footprint. *Nature,* 488**,** 197-200.

Goldhamer, D. A. & Beede, R. H. 2004. Regulated deficit irrigation effects on yield, nut quality and water-use efficiency of mature pistachio trees quality and. *Journal of Horticultural Science & Biotechnology,* 79**,** 538-545.

Gollan, T., Passioura, J. B. & Munns, R. 1986. Soil-water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. *Australian Journal of Plant Physiology,* 13**,** 459-464.

Gollan, T., Schurr, U. & Schulze, E. D. 1992. Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus.* I.

The concentration of cations, anions, amino acids in, and pH of, the xylem sap. *Plant, Cell & Environment,* 15**,** 551-559.

Goodger, J. Q. D. & Schachtman, D. P. 2010. Re-examining the role of ABA as the primary long-distance signal produced by water-stressed roots. *Plant signaling & behavior,* 5**,** 1298-301.

Goodger, J. Q. D., Sharp, R. E., Marsh, E. L. & Schachtman, D. P. 2005. Relationships between xylem sap constituents and leaf conductance of well-watered and water-stressed maize across three xylem sap sampling techniques. *Journal of Experimental Botany,* 56**,** 2389-2400.

Goodwin, P. B., Murphy, M., Melville, P. & Yiasoumi, W. 2003. Efficiency of water and nutrient use in containerised plants irrigated by overhead, drip or capillary irrigation. *Australian Journal of Experimental Agriculture,* 43**,** 189-194.

Gowing, D. J. G., Davies, W. J. & Jones, H. G. 1990. A Positive Root-sourced Signal as an Indicator of Soil Drying in Apple, *Malus* x *domestica* Borkh. *Journal of Experimental Botany,* 41**,** 1535-1540.

Grange, R. I. & Hand, D. W. 1987. A review of the effects of atmospheric humidity on the growth of horticultural crops. *Journal of Horticultural Science,* 62**,** 125-134.

Grant, O. M., Davies, M. J., Longbottom, H., Harrison-Murray, R. & Herrero, A. 2011. Application of Deficit Irrigation to Controlling Growth of Hardy Nursery Stock. *In:* ORTEGAFARIAS, S. & SELLES, G. (eds.) *Vi International Symposium on Irrigation of Horticultural Crops.*

Guo, J., Yang, X., Weston, D. J. & Chen, J.-G. 2011. Abscisic Acid Receptors: Past, Present and FutureF. *Journal of Integrative Plant Biology,* 53**,** 469-479.

Hamanishi, E. T., Thomas, B. R. & Campbell, M. M. 2012. Drought induces alterations in the stomatal development program in Populus. *Journal of Experimental Botany,* 63**,** 4959-4971.

Hamdy, A., Ragab, R. & Scarascia-Mugnozza, E. 2003. Coping with water scarcity: water saving and increasing water productivity. *Irrigation and Drainage,* 52**,** 3-20.

Hansen, H., & Dörffling, K. 2003. Root-derived trans-zeatin riboside and abscisic acid in drought-stressed and rewatered sunflower plants: interactions in the control of leaf diffusive resistance? *Funct. Plant Biol*, 20, 365-375.

Hartung, W., Sauter, A. & Hose, E. 2002. Abscisic acid in the xylem: where does it come from, where does it go to? *Journal of Experimental Botany,* 53**,** 27-32.

Hatfield, J. L., Sauer, T. J. & Prueger, J. H. 2001. Managing soils to achieve greater water use efficiency: A review. *Agronomy Journal,* 93**,** 271-280.

Hirayama, T. & Shinozaki, K. 2010. Research on plant abiotic stress responses in the post-genome era: past, present and future. *Plant Journal,* 61**,** 1041-1052.

Holbrook, N. M., Shashidhar, V. R., James, R. A. & Munns, R. 2002. Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. *Journal of Experimental Botany,* 53**,** 1503-1514.

Holland, T. C., Bowen, P., Bogdanoff, C. & Hart, M. 2014. Arbuscular Mycorrhizal Fungal Communities Associated with *Vitis vinifera* Vines under Different Frequencies of Irrigation. *American Journal of Enology and Viticulture,* 65**,** 222-229.

Howell, T. A., Schneider, A. D. & Evett, S. R. 1997. Subsurface and surface microirrigation corn - Southern High Plains. *Transactions of the Asae,* 40**,** 635-641.

HTA 2007. Technical Strategy for UK Producers of Ornamental Crops. The Horticultural Trades Association.

HTA 2011. Technical Strategy for UK Producers of Ornamental Crops. The Horticultural Trades Association.

HTA 2012. HTA Strategy for Growers of Ornamental Crops 2012-2014. The Horticultural Trades Association.

IFAD. 2013. *Water facts and figures* [Online]. www.ifad.org/english/water/key.html. [Accessed 06/07/2015 2015].

Ikegami, K., Okamoto, M., Seo, M. & Koshiba, T. 2009. Activation of abscisic acid biosynthesis in the leaves of *Arabidopsis thaliana* in response to water deficit. *Journal of Plant Research,* 122**,** 235-243.

Jackson, M. B. 1987. A structured evaluation of the involvement of ethylene and abscisic acid in plant responses to aeration stress. *In:* Hoad, G. V., Lenton, J. R. & Jackson, M. B. (eds.) *Hormone action in plant development – a critical appraisal.*: Butterworths.

Jackson, M. B., Saker, L. R., Crisp, C. M., Else, M. A. & Janowiak, F. 2003. Ionic and pH signalling from roots to shoots of flooded tomato plants in relation to stomatal closure. *Plant and Soil,* 253**,** 103-113.

Jacobs, W. P. 1959. What substance normally controls a given biological process? I. Formulation of some rules. *Developmental Biology,* 1**,** 527-533.

Jaleel, C. A., Gopi, R., Sanklar, B., Gomathinayagam, M. & Panneerselvam, R. 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *Comptes Rendus Biologies,* 331**,** 42-47.

Jarvis, A. J. & Davies, W. J. 1997. Whole-plant ABA flux and the regulation of water loss in *Cedrella odorata*. *Plant Cell and Environment,* 20**,** 521-527.

Jensen, C. R., Battilani, A., Plauborg, F., Psarras, G., Chartzoulakis, K., Janowiak, F., Stikic, R., Jovanovic, Z., Li, G., Qi, X., Liu, F., Jacobsen, S-E. & Andersen, M. N. 2010. Deficit irrigation based on drought tolerance and root signalling in potatoes and tomatoes. *Agricultural Water Management,* 98**,** 403-413.

Jia, W., & Zhang, J. 2008. Stomatal movements and long-distance signaling in plants. *Plant Signaling & Behaviour,* 3, 772-777.

Jokhan, A. D., Else, M. A. & Jackson, M. B. 1996. Delivery rates of abscisic acid in xylem sap of *Ricinus communis* L plants subjected to part-drying of the soil. *Journal of Experimental Botany,* 47**,** 1595-1599.

Jones, H. G. 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. *Journal of Experimental Botany,* 55**,** 2427-2436.

Jones, H. G., Sharp, C. S. & Higgs, K. H. 1987. Growth and water relations of wilty mutants of tomato (*Lycopersicon esculentum* Mill). *Journal of Experimental Botany,* 38**,** 1848-1856.

Jones, M. L., Kim, E. S. & Newman, S. E. 2001. Role of ethylene and 1-MCP in flower development and petal abscission in zonal geraniums. *Hortscience,* 36**,** 1305-1309.

Kang, S., Shi, W. & Zhang, J. 2000. An improved water-use efficiency for maize grown under regulated deficit irrigation. *Field Crops Research,* 67**,** 207-214.

Karam, F., Lahoud, R., Masaad, R., Kabalan, R., Breidi, J., Chalita, C. & Rouphael, Y. 2007. Evapotranspiration, seed yield and water use efficiency of drip irrigated sunflower under full and deficit irrigation conditions. *Agricultural Water Management,* 90**,** 213-223.

Katsoulas, N., Kittas, C., Dimokas, G. & Lykas, C. 2006. Effect of irrigation frequency on rose flower production and quality. *Biosystems Engineering,* 93**,** 237-244.

Khachatryan, H. & Choi, H. J. 2014. Factors Affecting Consumer Preferences and Demand for Ornamental Plants. *UF/IFAS Extension.* The Institute of Food and Agricultural Sciences University of Florida.

Khalil, A. A. M. & Grace, J. 1993. Does Xylem Sap ABA Control the Stomatal Behaviour of Water-Stressed Sycamore (*Acer pseudoplatanus* L.) Seedlings? *Journal of Experimental Botany,* 44**,** 1127-1134.

Kim, T. H., Bohmer, M., Hu, H. H., Nishimura, N. & Schroeder, J. I. 2010. Guard Cell Signal Transduction Network: Advances in Understanding Abscisic Acid, CO_2, and Ca^{2+} Signaling. *Annual Review of Plant Biology, Vol 61,* 61**,** 561-591.

Kirda, C., Cetin, M., Dasgan, Y., Topcu, S., Kaman, H., Ekici, B., Derici, M. R. & Ozguven, A. I. 2004. Yield response of greenhouse grown tomato to partial root drying and conventional deficit irrigation. *Agricultural Water Management,* 69**,** 191-201.

Knox, J., Kay, M. & Hammett, P. 2007. Working together to protect water rights. *In:* UKIA (ed.). www.ukia.org.

Knox, J., Kay, M. & Weatherhead, K. 2008. Switching irrigation technologies. *In:* UKIA (ed.). www.ukia.org: Centre for Water Science, Cranfield University & Natural England.

Knox, J., Weatherhead, K., Diaz, J. R. & Kay, M. 2009. Developing a strategy to improve irrigation efficiency in a temperate climate A case study in England. *Outlook on Agriculture,* 38**,** 303-309.

Kozlowski, T. T. & Pallardy, S. G. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. *Botanical Review,* 68**,** 270-334.

Kramer, P. J. 1988. Changing concepts regarding plant water relations. *Plant, Cell & Environment,* 11**,** 565-568.

Kudoyarova, G. R., Vysotskaya, L. B., Cherkozyanova, A. & Dodd, I. C. 2007. Effect of partial rootzone drying on the concentration of zeatin-type cytokinins in tomato (*Solanum lycopersicum* L.) xylem sap and leaves. *Journal of Experimental Botany,* 58**,** 161-168.

Lee, K. H., Piao, H. L., Kim, H.-Y., Choi, S. M., Jiang, F., Hartung, W., Hwang, I., Kwak, J. M., Lee, I.-J. & Hwang, I. 2006. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. *Cell,* 126**,** 1109-1120.

Leonardi, C., Guichard, S. & Bertin, N. 2000. High vapour pressure deficit influences growth, transpiration and quality of tomato fruits. *Scientia Horticulturae,* 84**,** 285-296.

Liakopoulos, G. & Spanorigas, I. 2012. Foliar anthocyanins in *Pelargonium* x *hortorum* are unable to alleviate light stress under photoinhibitory conditions. *Photosynthetica,* 50**,** 254-262.

Liang, J. S., Zhang, J. H. & Wong, M. H. 1997. How do roots control xylem sap ABA concentration in response to soil drying? *Plant and Cell Physiology,* 38**,** 10-16.

Liu, F., Shahnazari, A., Andersen, M. N., Jacobsen, S-E. & Jensen, C. R. 2006a. Effects of deficit irrigation (DI) and partial root drying (PRD) on gas exchange, biomass partitioning, and water use efficiency in potato. *Scientia Horticulturae,* 109**,** 113-117.

Liu, F. L., Shahnazari, A., Andersen, M. N., Jacobsen, S. E. & Jensen, C. R. 2006b. Physiological responses of potato (*Solanum tuberosum* L.) to partial root-zone drying: ABA signalling, leaf gas exchange, and water use efficiency. *Journal of Experimental Botany,* 57**,** 3727-3735.

Liu, X. G., Yue, Y. L., Li, B., Nie, Y. L., Li, W., Wu, W. H. & Ma, L. G. 2007. A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. *Science,* 315**,** 1712-1716.

Loveys, B. R. 1977. The Intracellular Location of Abscisic Acid in Stressed and Non-Stressed Leaf Tissue. *Physiologia Plantarum,* 40**,** 6-10.

Loveys, B. R. 1984. Diurnal changes in water relations and abscisic-acid in field-grown *Vitis vinifera* cultivars. 3. The inluence of xylem-derived abscisic-acid on leaf gas-exchange. *New Phytologist,* 98**,** 563-573.

Loveys, B. R., Robinson, S. P. & Downton, W. J. S. 1987. Season and diurnal changes in abscisic-acid and water relations of apricot leaves (*Prunus armeniaca* L.). *New Phytologist,* 107**,** 15-27.

Lütken, H., Clarke, J. L. & Müller, R. 2012. Genetic engineering and sustainable production of ornamentals: current status and future directions. *Plant Cell Reports,* 31**,** 1141-1157.

MacFarlane, C., Hansen, L. D., Edwards, J., White, D. A. & Adams, M. A. 2005. Growth efficiency increases as relative growth rate increases in shoots and roots of *Eucalyptus globulus* deprived of nitrogen or treated with salt. *Tree Physiology,* 25**,** 571-582.

MacRobbie, E. 1992. Calcium and ABA-induced stomatal closure. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 338, 5-18.

Manetas, Y. 2006. Why some leaves are anthocyanic and why most anthocyanic leaves are red? *Flora - Morphology, Distribution, Functional Ecology of Plants,* 201**,** 163-177.

Martin-Vertedor, A. I. & Dodd, I. C. 2011. Root-to-shoot signalling when soil moisture is heterogeneous: increasing the proportion of root biomass in drying soil inhibits leaf growth and increases leaf abscisic acid concentration. *Plant, Cell & Environment,* 34**,** 1164-1175.

Mbagwu, J. S. C. & Osuigwe, J. O. 1985. Effects of varying levels and frequencies of irrigation on growth, yield, nutrient-uptake and water-use efficiency on maize and cowpeas on a sandy loam ultisol. *Plant and Soil,* 84**,** 181-192.

McAinsh, M. R., Brownlee, C. & Hetherington, A. M. 1990. Abscisic acid-induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. *Nature,* 343**,** 186-188.

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.

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D. G. & Yepez, E. A. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist,* 178**,** 719-739.

Mencuccini, M., Mambelli, S. & Comstock, J. 2000. Stomatal responsiveness to leaf water status in common bean (*Phaseolus vulgaris* L.) is a function of time of day. *Plant, Cell & Environment,* 23**,** 1109-1118.

Merilo, E., Jõessar, I., Brosché, M. & Kollist, H. 2014. To open or to close: species-specific stomatal responses to simultaneously applied opposing environmental factors. *New Phytologist,* 202**,** 499-508.

Michel, J. C., Rivière, L. M. & Bellon-Fontaine, M. N. 2001. Measurement of the wettability of organic materials in relation to water content by the capillary rise method. *European Journal of Soil Science,* 52**,** 459-467.

Mielke, M. S., de Almeida, A. A. F., Gomes, F. P., Aguilar, M. A. G. & Mangabeira, P. A. O. 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. *Environmental and Experimental Botany,* 50**,** 221-231.

Mieszkalska, K. & Łukaszewska, A. 2011. Effect of the silicon and phosphorus-containing fertilizer on geranium (*Pelargonium hortorum* LH Bailey) response to water stress. *Acta Scientiarum Polonorum-Hortorum Cultus,* 10**,** 113-121.

Miralles-Crespo, J., Sánchez-Blanco , M. J., Navarro G, A., Martínez-Sánchez, J. J., Franco L, J. A. & Bańñón A, S. 2010. Comparison of Stem Diameter Variations in Three Small Ornamental Shrubs under Water Stress. *Hortscience,* 45**,** 1681-1689.

Morel, P., Crespel, L., Galopin, G. & Moulia, B. 2012. Effect of mechanical stimulation on the growth and branching of garden rose. *Scientia Horticulturae,* 135**,** 59-64.

Morison, J. I. L., Baker, N. R., Mullineaux, P. M. & Davies, W. J. 2008. Improving water use in crop production. *Phil. Trans. R. Soc. B*, 363, 639-658.

Morvant, J. K., Dole, J. M. & Cole, J. C. 1998. Irrigation Frequency and System Affect Poinsettia Growth, Water Use, and Runoff. *Hortscience,* 33**,** 42-46.

Mulholland, B. J., Hussain, A., Black, C. R., Taylor, I. B., & Roberts, J. A. 1999. Does root-sourced ABA have a role in mediating growth and stomatal responses to soil compaction in tomato (*Lycopersicon esculentum*)? *Physiologia Plantarum*, 107, 267-276.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant, Cell and Environment*, 25, 239-250.

Munns, R. & Cramer, G. R. 1996. Is coordination of leaf and root growth mediated by abscisic acid? Opinion. *Plant and Soil,* 185**,** 33-49.

Munns, R. & King, R. W. 1988. Abscisic Acid Is Not the Only Stomatal Inhibitor in the Transpiration Stream of Wheat Plants. *Plant Physiology,* 88**,** 703-708.

Munns, R., Passioura, J. B., Guo, J., Chazen, O. & Cramer, G. R. 2000. Water relations and leaf expansion: importance of time scale. *Journal of Experimental Botany,* 51**,** 1495-1504.

Nagata, T., Iizumi, S., Satoh, K., Ooka, H., Kawai, J., Carninci, P., Hayashizaki, Y., Otomo, Y., Murakami, K., Matsubara, K. & Kikuchi, S. 2004. Comparative analysis of plant and animal calcium signal transduction element using plant full-length cDNA data. *Molecular Biology and Evolution,* 21**,** 1855-1870.

Nambara, E. & Marion-PollL, A. 2005. Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology.*

Neales, T. F. & McLeod, A. L. 1991. Do leaves contribute to the abscisic acid present in the xylem sap of ‘draughted’ sunflower plants? *Plant, Cell & Environment,* 14**,** 979-986.

Netting, A. G., Theobald, J. C. & Dodd, I. C. 2012. Xylem sap collection and extraction methodologies to determine in vivo concentrations of ABA and its bound forms by gas chromatography-mass spectrometry (GC-MS). *Plant Methods,* 8.

Nora, L., Dalmazo, G. O., Nora, F. R. & Rombaldi, C. V. 2012. Controlled Water Stress to Improve Fruit and Vegetable Postharvest Quality. *In:* Rahman, I. M. M. (ed.) *Water Stress.* http://www.intechopen.com/books/water-stress/controlledwater-stress-to-improve-fruit-and-vegetable-postharvest-quality: InTech.

Oktem, A., Simsek, M. & Oktem, A. G. 2003. Deficit irrigation effects on sweet corn (*Zea mays* saccharata Sturt) with drip irrigation system in a semi-arid region: I. Water-yield relationship. *Agricultural Water Management,* 61**,** 63-74.

Ouma, G. 2007. Effect of different container sizes and irrigation frequency on the morphological and physiological characteristics of mango (*Mangifera indica*) rootstock seedlings. *International Journal of Botany,* 3**,** 260-268.

Owen, J. & Stoven, H. 2010. Irrigation Strategies to Conserve Water in Container Nurseries. Best Management Practices Guide for Climate Friendly Nurseries.

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.

Pantin, F., Simonneau, T. & Muller, B. 2012. Coming of leaf age: control of growth by hydraulics and metabolics during leaf ontogeny. *New Phytologist*, 196**,** 349-366.

Pardey, P. G., Beddow, J. M., Hurley, T. M., Beatty, T. K. M. & Eidman, V. R. 2014. A Bounds Analysis of World Food Futures: Global Agriculture Through to 2050. *Australian Journal of Agricultural and Resource Economics*, 58**,** 571-589.

Passioura, J. B. 1988. Root Signals Control Leaf Expansion in Wheat Seedlings Growing in Drying Soil. *Australian Journal of Plant Physiology*, 15, 687-693.

Patonnier, M. P., Peltier, J. P. & Marigo, G. 1999. Drought-induced increase in xylem malate and mannitol concentrations and closure of *Fraxinus excelsior* L. stomata. *Journal of Experimental Botany*, 50**,** 1223-1229.

Peacock, C. H. & Dudeck, A. E. 1984. Physiological-response of St-Augustinegrass to irrigation scheduling. *Agronomy Journal*, 76**,** 275-279.

Peak, D. & Mott, K. A. 2011. A new, vapour-phase mechanism for stomatal responses to humidity and temperature. *Plant Cell and Environment*, 34**,** 162-178.

Pérez-Alfocea, F., Ghanem, M. E., Gómez-Cadebas, A., & Dodd, I. C. 2011. Omics of root-to-shoot signaling under salt and water deficit. *OMICS: A Journal of Integrative Biology*, 15, 893-901.

Pérez-Pérez, J. G. & Dodd, I. C. 2015. Sap fluxes from different parts of the rootzone modulate xylem ABA concentration during partial rootzone drying and re-wetting. *Journal of Experimental Botany*.

Pierce, M. & Raschke, K. 1980. Correlation between loss o turgor and accumulation of abscisic-acid in detached leaves. *Planta,* 148**,** 174-182.

Pour, A. T., Sepaskhah, A. R. & Maftoun, M. 2005. Plant water relations and seedling growth of three pistachio cultivars as influenced by irrigation frequency and applied potassium. *Journal of Plant Nutrition,* 28**,** 1413-1425.

Puértolas, J., Alcobendas, R., Alarcón, J. J. & Dodd, I. C. 2013. Long-distance abscisic acid signalling under different vertical soil moisture gradients depends on bulk root water potential and average soil water content in the root zone. *Plant Cell and Environment,* 36**,** 1465-1475.

Puértolas, J., Conesa, M. R., Ballester, C. & Dodd, I. C. 2014. Local root abscisic acid (ABA) accumulation depends on the spatial distribution of soil moisture in potato: Implications for ABA signalling under heterogeneous soil drying. *Journal of Experimental Botany*.

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 radioummunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. *Planta,* 173**,** 330-339.

Rabino, I. & Mancinelli, A. L. 1986. Light, temperature, and anthocyanin production. *Plant Physiology,* 81**,** 922-924.

Radin, J. W., Mauney, J. R. & Kerridge, P. C. 1989. Water-uptake by cotton roots during fruit filling in relation to irrigation frequency. *Crop Science,* 29**,** 1000-1005.

Regan, R. 1999. Nursery irrigation scheduling. Proc. 2nd Annu. Nursery Growers Short Course. Wilsonville, Ore.

Rhizopoulou, S. & Davies, W. J. 1991. Influence of soil dryin on root development, water relations and leaf growth of *Ceratonia siliqua* L. *Oecologia,* 88**,** 41-47.

Ritchie, J. T. 1981. Water dynamics in the soil-plant-atmosphere system. *Plant and Soil,* 58**,** 81-96.

Rodriues, M. L., Santos, T. P., Rodrigues, A. P., de Souza, C. R., Lopes, C. M., Maroco, J. P., Pereira, J. S. & Chaves, M. M. 2008. Hydraulic and chemical signalling in the regulation of stomatal conductance and plant water use in field grapevines growing under deficit irrigation. *Functional Plant Biology,* 35**,** 565-579.

Romero, P., Gil-Muñoz, R., Del Amor, F. M., Valdés, E., Fernández, J. I. & Martinez-Cutillas, A. 2013. Regulated Deficit Irrigation based upon optimum water status improves phenolic composition in Monastrell grapes and wines. *Agricultural Water Management,* 121**,** 85-101.

Rothwell, S. & Dodd, I. 2014. Xylem sap calcium concentrations do not explain liming-induced inhibition of legume gas exchange. *Plant and Soil,* 382**,** 17-30.

Ruiz, L. P., Atkinson, C. J. & Mansfield, T. A. 1993. Calcium in the xylem and its influence on the behaviour of stomata. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 341**,** 67-74.

Saab, I. M. & Sharp, R. E. 1989. Non-hydraulic signals from maize roots in drying soil: inhibition of leaf elongation but not stomatal conductance. *Planta*, 179, 466-474.

Sack, L. & Holbrook, N. M. 2006. Leaf hydraulics. *Annual Review of Plant Biology,* 57**,** 361-381.

Sade, N., Gebremedhin, A. & Moshelion, M. 2012. Risk-taking plants: Anisohydric behavior as a stress-resistance trait. *Plant signaling & behavior,* 7**,** 767-770.

Saeed, I. A. M. & El-Nadi, A. H. 1998. Forage sorghum yield and water use efficiency under variable irrigation. *Irrigation Science,* 18**,** 67-71.

Saliendra, N., Sperry, J. & Comstock J. 1995. Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta,* 196**,** 357-366.

Sánchez-Blanco , M. J., Álvarez, S., Navarro, A. & Bañón, 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.

Sauter, A., Davies, W. J. & Hartung, W. 2001. The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. *Journal of Experimental Botany,* 52**,** 1991-1997.

Sauter, A., Dietz, K. J. & Hartung, W. 2002. A possible stress physiological role of abscisic acid conjugates in root-to-shoot signalling. *Plant Cell and Environment,* 25**,** 223-228.

Savé, R., Olivella, C., Biel, C., Adillón, J. & Rabella, R. 1994. Seasonal patterns of water relationships, photosynthetic pigments and morphology of *Actinidia deliciosa* plants of the Hayward and Tomuri cultivars. *Agronomie,* 14**,** 121-126.

Schachtman, D. P. & Goodger, J. Q. D. 2008. Chemical root to shoot signaling under drought. *Trends in Plant Science,* 13**,** 281-287.

Scheiber, S. M., Beeson, R. C., JR., Chen, J., Wang, Q. & Pearson, B. 2008. Evaluation of irrigation frequency and quantity on leaf gas exchange, growth, and nitrate leaching of coleus in a simulated landscape. *Hortscience,* 43**,** 881-884.

Scholander, P. F., Hammel, H. T., Bradstreet, E. D. & Hemmings, H. E. A. 1965. Sap pressure in vascular plants – Negative hydrostatic pressure can be measured in plants. *Science,* 148**,** 339-&.

Schultz, H. R. 2003. Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant Cell and Environment,* 26**,** 1393-1405.

Schurr, U., Gollan, T. & Schulze, E. D. 1992. Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. II. Stomatal sensitivity to abscisic acid imported from the xylem sap. *Plant, Cell & Environment,* 15**,** 561-567.

Sezen, S. M., Yazar, A. & Eker, S. 2006. Effect of drip irrigation regimes on yield and quality of field grown bell pepper. *Agricultural Water Management,* 81**,** 115-131.

Sharp, R. E. & Davies, W. J. 1985. Root Growth and Water Uptake by Maize Plants in Drying Soil. *Journal of Experimental Botany,* 36**,** 1441-1456.

Sharp, R. G. & Davies, W. J. 2009. Variability among species in the apoplastic pH signalling response to drying soils. *Journal of Experimental Botany,* 60**,** 4361-4370.

Sharp, R. G., Else, M. A., Cameron, R. W. & Davies, W. J. 2009. Water deficits promote flowering in Rhododendron via regulation of pre and post initiation development. *Scientia Horticulturae,* 120**,** 511-517.

Shashidhar, V. R., Prasad, T. G. & Sudharshan, L. 1996. Hormone Signals from Roots to Shoots of Sunflower (*Helianthus annuus* L.). Moderate Soil Drying

Increases Delivery of Abscisic Acid and Depresses Delivery of Cytokinins in Xylem Sap. *Annals of Botany,* 78**,** 151-155.

Shen, Y.-Y., Wang, X.-F., Wu, F.-Q., Du, S.-Y., Cao, Z., Shang, Y., Wan, X.-L., Peng, C.-C., Yu, X.-C., Zhu, S.-Y., Fan, R.-C., Xu, Y.-H. & Zhang, D.-P. 2006. The Mg-chelatase H subunit is an abscisic acid receptor. *Nature,* 443**,** 823-826.

Simmoneau, T., Barrieu, P. & Tardieu, F. 1998. Accumulation rate of ABA in detached maize roots correlates with root water potential regardless of age and branching order. *Plant, Cell & Environment,* 21**,** 1113-1122.

Sobeih, W. Y., Dodd, I. C., Bacon, M. A., Grierson, D. & Davies, W. J. 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjected to partial root-zone drying. *Journal of Experimental Botany,* 55**,** 2353-2363.

Souza, R. P., Machado, E. C., Silva, J. A. B., Lagôa, A. & Silveira, J. A. G. 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environmental and Experimental Botany,* 51**,** 45-56.

Sperry, J. S., Hacke, U. G., Oren, R. & Comstock, J. P. 2002. Water deficits and hydraulic limits to leaf water supply. *Plant Cell and Environment,* 25**,** 251-263.

Stagnari, F., Galieni, A., Speca, S. & Pisante, M. 2014. Water stress effects on growth, yield and quality traits of red beet. *Scientia Horticulturae,* 165**,** 13-22.

Steyn, W. J., Wand, S. J. E., Holcroft, D. M. & Jacobs, G. 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *New Phytologist,* 155**,** 349-361.

Stoll, M., Loveys, B. & Dry, P. 2000. Hormonal changes induced by partial rootzone drying of irrigated grapevine. *Journal of Experimental Botany,* 51**,** 1627-1634.

Tardieu, F. & Davies, W. J. 1992. Stomatal response to abscisic-acid is a function of current plant water status. *Plant Physiology,* 98**,** 540-545.

Tardieu, F., Parent, B., & Simonneau, T. 2010. Control of leaf growth by abscisic acid: hydraulic or non-hydraulic processes? *Plant, Cell and Environment*, 33, 636-647.

Tardieu, F., Reymond, M., Hamard, P., Granier, C. & Muller, B. 2000. Spatial distributions of expansion rate, cell division rate and cell size in maize leaves: a synthesis of the effects of soil water status, evaporative demand and temperature. *Journal of Experimental Botany,* 51**,** 1505-1514.

Tardieu, F., & Simonneau, T. 1998. Variability among species of stomatal control in fluctuating soil water status and evaporative demand. Modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany*, 49, 419-432.

Tardieu, F., Zhang, J. & Gowing, D. J. G. 1993. Stomatal control by both [ABA] in the xylem sap and leaf water status: a test of a model for draughted or ABA-fed field-grown maize. *Plant, Cell & Environment,* 16**,** 413-420.

Tomás, M., Medrano, H., Pou, A., Escalona, J. M., Martorell, S., Ribas-Carbó, M. & Flexas, J. 2012. Water-use efficiency in grapevine cultivars grown under controlled conditions: effects of water stress at the leaf and whole-plant level. *Australian Journal of Grape and Wine Research,* 18**,** 164-172.

Toscano, S., Scuderi, D., Giuffrida, F. & Romano, D. 2014. Responses of Mediterranean ornamental shrubs to drought stress and recovery. *Scientia Horticulturae,* 178**,** 145-153.

Touchette, B. W., Iannacone, L. R., Turner, G. E. & Frank, A. R. 2007. Drought tolerance versus drought avoidance: A comparison of plant-water relations in herbaceous wetland plants subjected to water withdrawal and repletion. *Wetlands,* 27**,** 656-667.

Trejo, C. L., Clephan, A. L., & Davies, W. J. 1995. How Do Stomata Read Abscisic Acid Signals? *Plant Physiology*, 109, 803-811.

Trejo, C. L. & Davies, W. J. 1991. Drought-induced Closure of *Phaseolus vulgaris* L. Stomata Precedes Leaf Water Deficit and any Increase in Xylem ABA Concentration. *Journal of Experimental Botany,* 42**,** 1507-1516.

Tyree, M. T. & Sperry, J. S. 1989. Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology,* 40**,** 19-38.

Wakrim, R., Wahbi, S., Tahi, H., Aganchich, B. & Serraj, R. 2005. Comparative effects of partial root drying (PRD) and regulated deficit irrigation (RDI) on water relations and water use efficiency in common bean (*Phaseolus vulgaris* L.). *Agriculture, Ecosystems & Environment,* 106**,** 275-287.

Wang, F.-X., Kang, Y. & Liu, S.-P. 2006. Effects of drip irrigation frequency on soil wetting pattern and potato growth in North China Plain. *Agricultural Water Management,* 79**,** 248-264.

Wang, H., Zhang, L., Dawes, W. R. & Liu, C. 2001. Improving water use efficiency of irrigated crops in the North China Plain — measurements and modelling. *Agricultural Water Management,* 48**,** 151-167.

Wang, H. F., Zhang, J. H., Liang, J. S. & Yin, W. L. 1999. Root and xylem ABA changes in response to soil drying in two woody plants. *Chinese Science Bulletin,* 44**,** 2236-2241.

Wang, Y., Liu, F. & Jensen, C. R. 2012. Comparative effects of deficit irrigation and alternate partial root-zone irrigation on xylem pH, ABA and ionic concentrations in tomatoes. *Journal of Experimental Botany,* 63**,** 1907-1917.

Wang, Z., Liu, Z., Zhang, Z. & Liu, X. 2009. Subsurface drip irrigation scheduling for cucumber (*Cucumis sativus* L.) grown in solar greenhouse based on 20 cm standard pan evaporation in Northeast China. *Scientia Horticulturae,* 123**,** 51-57.

Warsaw, A. L., Fernandez, R. T., Cregg, B. M. & Andresen, J. A. 2009. Container-grown Ornamental Plant Growth and Water Runoff Nutrient Content and Volume Under Four Irrigation Treatments. *Hortscience,* 44**,** 1573-1580.

Whitmore, A. P. & Whalley, W. R. 2009. Physical effects of soil drying on roots and crop growth. *Journal of Experimental Botany,* 60**,** 2845-2857.

Wilkinson, S., Bacon, M. A. & Davies, W. J. 2007. Nitrate signalling to stomata and growing leaves: interactions with soil drying, ABA, and xylem sap pH in maize. *Journal of Experimental Botany,* 58**,** 1705-1716.

Wilkinson, S., Clephan, A. L. & Davies, W. J. 2001. Rapid low temperature-induced stomatal closure occurs in cold-tolerant *Commelina communis* leaves but not in cold-sensitive tobacco leaves, via a mechanism that involves apoplastic calcium but not abscisic acid. *Plant Physiology,* 126**,** 1566-1578.

Wilkinson, S., Corlett, J. E., Oger, L., & Davies, W. J. 1998. Effects of Xylem pH on Transpiration from Wild-Type and *flacca* Tomato Leaves. *Plant Physiology,* 117, 703-709.

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.

Wilkinson, S. & Hartun, W. 2009. Food production: reducing water consumption by manipulating long-distance chemical signalling in plants. *Journal of Experimental Botany,* 60**,** 1885-1891.

Wilkinson, S., Kudoyarova, G. R., Veselov, D. S., Arkhipova, T. N. & Davies, W. J. 2012. Plant hormone interactions: innovative targets for crop breeding and management. *Journal of Experimental Botany,* 63**,** 3499-3509.

Xu, G., Lekovitch, I., Soriano, S., Wallach, R. & Silber, A. 2004. Integrated effect of irrigation frequency and phosphorus level on lettuce: P uptake, root growth and yield. *Plant and Soil,* 263**,** 297-309.

Zhang, J. & Davies, W. J. 1987. Increased Synthesis of ABA in Partially Dehydrated Root Tips and ABA Transport from Roots to Leaves. *Journal of Experimental Botany,* 38**,** 2015-2023.

Zhang, J. & Davies, W. J. 1989a. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. *Plant, Cell & Environment,* 12**,** 73-81.

Zhang, J. & Davies, W. J. 1990. Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. *Plant, Cell & Environment,* 13**,** 277-285.

Zhang, J., Schurr, U. & Davies, W. J. 1987. Control of Stomatal Behaviour by Abscisic Acid which Apparently Originates in the Roots. *Journal of Experimental Botany,* 38**,** 1174-1181.

Zhang, J. H. & Davies, W. J. 1989b. Sequential response of whole plant water relations to prolonged soil dryin and the involvement of xylem sap ABA in the regulation of stomatal behaviour in sunflower plants. *New Phytologist,* 113**,** 167-174.

Zhan, J. H. & Davies, W. J. 1991. Antitranspirant activity in xylem sap of maize plants. *Journal of Experimental Botany,* 42**,** 317-321.

Zhang, J. H., Jia, W. S., Yang, J. C. & Ismail, A. M. 2006. Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Research,* 97**,** 111-119.

Zhang, J. H., Sui, X. Z., Li, B., Su, B. L., Li, J. M. & Zhou, D. X. 1998. An improved water-use efficiency for winter wheat grown under reduced irrigation. *Field Crops Research,* 59**,** 91-98.

Zhang, J. H. & Tardieu, F. 1996. Relative contribution of apices and mature tissues to ABA synthesis in droughted maize root systems. *Plant and Cell Physiology,* 37**,** 598-605.