The role of chemical and hydraulic signalling in the recovery of photosynthesis following a rootzone water deficit stress in *Rubus idaeus* (red raspberry)

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Abstract

With the changing UK climate, abiotic stresses are becoming more prevalent in the UK, causing major effects on crop yield and quality. Abiotic stresses have been researched extensively on crops, including tomato and grapevine, but are limited on the response and recovery of raspberry plants from a water deficit stress.

The aim of this PhD research was to improve knowledge of the impact of transient rootzone water deficit stress on red raspberries and to understand signalling mechanisms controlling the response. The effects of different rooting volume pots under water deficit stress conditions were also analysed. Moreover, the investigation studied the impacts of transient rootzone water deficit stress on marketable yield and quality of raspberries.

The recovery of leaf gas exchange depended on the duration and intensity of the rootzone water deficit stress. However, even for a deficit of four days, legacy effects on photosynthesis persisted for days after rewetting of the coir. More than a 50-fold increase in xylem-borne ABA was recorded after four days of coir drying, indicating a role of signalling mechanisms in the response to transient rootzone water deficit stress. Effects on Class 1 yield were substantial after a transient rootzone deficit stress reducing yield and impacting berry fresh weight for several weeks. Finally, results highlighted that the use of larger rooting volume pots would likely minimise the adverse effects of water deficit stress.

Even short durations of water deficit stress can significantly impact plant physiology, substantially affecting both yield and quality, highlighting the necessity of comprehending not only plant abiotic stress perception but also the recovery response. An integrated approach to understanding combined stress events is imperative for elucidating real-life scenarios. Ultimately, this study contributes to a deeper understanding of raspberry plant responses to water deficit stress, progressing toward preventing legacy effects on leaf gas exchange.

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ABA	Abscisic acid
°C	Degrees centigrade
µl	Microlitres
µmol	Micromoles
AcOH	Acetic acid
A_{max}	Maximum assimilation rate
ANOVA	One-way analysis of variance
ATP	Adenosine triphosphate
ca.	*Circa*
cm	Centimetres
cm^2	Square centimetres
CO_2	Carbon Dioxide
CSPS	Crop Science and Production Systems
CVMC	Coir volumetric moisture content
D6-ABA	Deuterium-labelled isotope of abscisic acid
DD	Dried down
DD10	Dried down for 10 days
DD4	Dried down for 4 days

DD7	Dried down for 7 days
e.g.	*Exempli gratia*
EC	Electrical conductivity
g	Grams
GC-MS	Gas chromatography coupled to mass spectroscopy
g_s	Stomatal conductance
h	Hour
h^{-1}	Per hour
HSD	Honestly significant difference
i.e.	*Id est*
kg/pot	Kilograms per pot
kPa	Kilopascal
L	Litres
LER	Leaf elongation rates
LSD	Least significant differences
m/z	Mass-to-charge ratio
m^{-2}	Per square metre
m^3	Cubic metre
m^{-3}	Per cubic metre

MeOH	Methanol
mins	Minutes
mL	Millilitres
mm	Millimeters
mol^{-1}	Per mol
MPa	Megapascal
MTBSTFA	N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide
N_2	Nitrogen
NADPH	Nicotinamide adenine dinucleotide phosphate
ng/µL	Nanograms per microlitre
nM	Nanomoles
NPQ	Non-photochemical quenching
PAR	Photosynthetically active radiation
pH	Potential of Hydrogen
PI	Precision irrigation
Pn	Photosynthetic rate
PP2C	Type 2C protein phosphatases
PPFD	Photosynthetically active photon flux density
PRD	Partial root-zone drying

PSI	Photosystem I
PSII	Photosystem II
qE	Energy-dependent quenching
RDI	Regulated deficit irrigation
RH	Relative humidity
ROS	Reactive oxygen species
RuBP	Ribulose bisphosphate
s^{-1}	Per second
SER	Stem elongation rates
SnRK2	Snf1-related protein kinases 2
SSC	Soluble solids content
SWP	Stem water potential
VPD	Vapour pressure deficit
w/w	Weight by weight
WW	Well-watered

1 Introduction

1.1 General introduction

The global population is estimated to reach 9.7 billion by 2050 (FAO, 2018), therefore meeting increased food demands to sustain an additional 2.1 billion people will require innovative approaches to avoid clearing non-arable land for further crop production. At the same time, agricultural production is facing unparalleled challenges from changing climate (Mbow *et al*., 2019). As a result, new approaches to increase crop yield and nutritional quality of the crops on the currently available land will be required (Godfray *et al*., 2010; Simkin, 2019; Simkin *et al*., 2019). Crop resilience needs to be improved to adapt to a changing climate, while also increasing crop yields in a sustainable way to guarantee future food security (Benitez-Alfonso *et al*., 2023).

Whilst many strategies are aimed at increasing yield and climate resilience of staple crops such as wheat, rice, and maize, other foods such as fruits and vegetables contain essential nutrients, and so consumption of such healthy foods is likely to increase (FAO, 2018) as consumers in some parts of the world switch to more plant-based diets. Increased demand for berries has driven a 25% increase in strawberry production, relative to the previous decade in the UK, which produced 132,000 tonnes of strawberries in 2018 (Evans, 2020; FAO, 2018) and raspberry production has grown by 8.19% from 2018 to 2019 (Berry Gardens, 2019). Horticultural statistics from DEFRA for the year of 2023, stated that 106 thousand tonnes of strawberries were produced, down 11% from 2022

(DEFRA, 2024). Despite the need for various crops for good nutrition, many global endeavours to increase plant productivity are focused only on increasing the yields of staple grain crops (Godfray *et al*., 2010; Simkin, 2019; Simkin *et al*., 2019). Hence, strategies to enhance crop yield and climate resilience are increasingly important for all crops.

Most vegetables and fruits are grown on a seasonal basis (Klein, 2020); consequently, achieving maximum yield and quality during the growing season is essential for growers, retailers, and consumers. The impact of climate change on crops has been detrimental, and like many other crops, fruits and vegetables, which are key components of healthy diets, are very vulnerable to climate change at their reproductive stages, where yield declines under higher temperatures (Mbow *et al*., 2019). Climate change has a direct impact on food systems and responding to these changes is important to maintain marketable yields, and to meet national targets around food and nutrition security.

Plants produce vital compounds, known as phytonutrients, which act as a protective mechanism against cell damage and are also used to combat the effects of biotic and abiotic stresses. For example, plants can produce phytonutrients to overcome the toxicity of ammonia and ensure no negative effects on nitrogen metabolism and photosynthetic processes (Sharpe *et al*., 2020). Not only do phytonutrients have benefits for the plants themselves, but they are also beneficial for consumers. It was found that phytonutrients appeared to maintain cardiovascular health (Nahar *et al*., 2020) and reduce the risk of cancer (Bhattacharya *et al*., 2021). Hence, understanding a plant's response to the changing climate is crucial for meeting consumer demands.

1.2 Photosynthesis

To sustain and thrive, all plants carry out the fundamental process of photosynthesis, whereby energy from sunlight is harnessed to convert atmospheric carbon dioxide (CO_2) into organic matter, which serves as the foundational component for all plant functions (Equation 1).

$$6CO_2 + 6H_2O + hv = C_6H_{12}O_6 + 6O_2 \qquad (\textit{Equation 1})$$

Photosynthesis is highly regulated (Tanaka & Makino, 2009), and occurs in two sequential phases, the light-dependent and light-independent reactions.

In the light-dependent reaction, which takes place in the chloroplast thylakoid membranes, chlorophyll absorbs photons (light energy - hv) and converts them to adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) *via.* the electron transport chain (Bassham *et al*., 1950; Berg *et al*., 2015). These products are subsequently used in the stroma-based light-independent reactions to fix inorganic carbon dioxide into organic compounds via the Calvin-Benson cycle (CB cycle; Raines, 2003; Figure 1-1). This results in the biosynthesis of carbohydrates that are essential for plant growth.

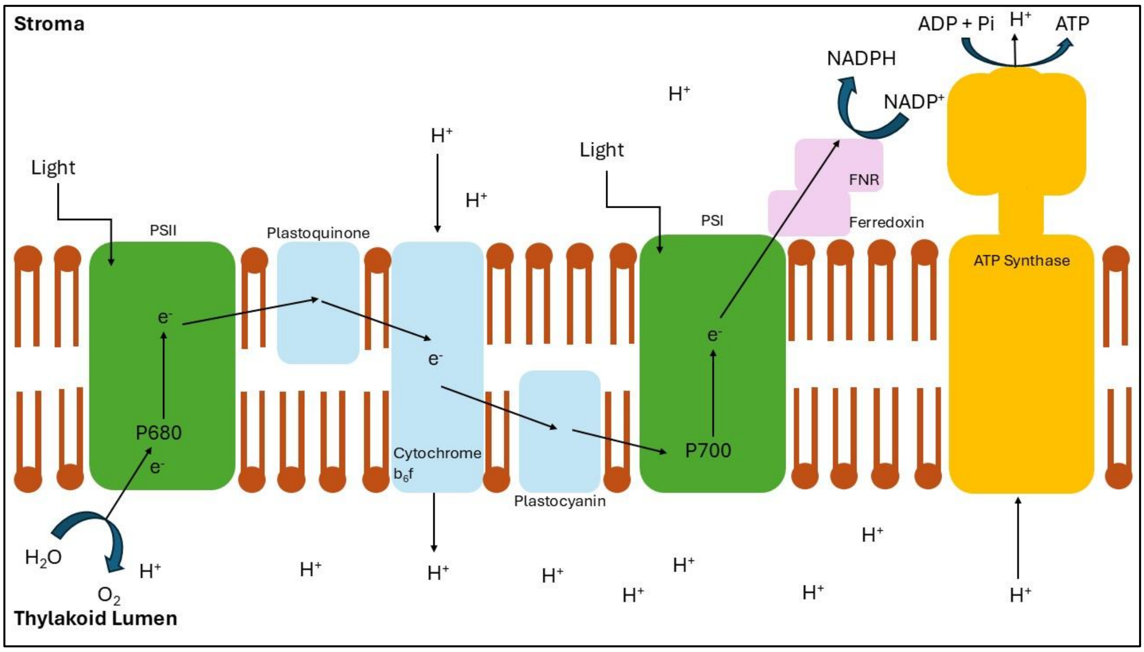

Figure 1-1. *The light-dependent reaction and the complexes that are part of it. Abbreviations in the image: H^+, proton; e-, electron; PSI, photosystem I; PSII, photosystem II; FNR, ferredoxin-NADP-reductase. Figure made using Inkscape and PowerPoint.*

There are five major protein complexes in the photosynthetic electron transport chain which produce ATP and NADPH, known as photosystem II (PSII), cytochrome b6f, photosystem I (PSI), ferredoxin and ATP synthase (Figure 1-1; Flugge *et al*., 2016). The light energy captured by PSII and PSI are at wavelengths 680 nm and 700 nm, respectively, and are channelled to the reaction centre. Electrons are transferred from PSII to cytochrome b_6f and then to PSI through two mobile carriers, plastoquinone and plastocyanin. Cytochrome b6f serves as an electrical conduit (Johnson *et al*., 2014) whereby plastoquinone is oxidised and plastocyanin is reduced (Cramer *et al*., 2011). The enzyme

Ferredoxin-NADP$^+$-reductase facilitates the conversion of NADP$^+$ to NADPH with the assistance of ferredoxin. Electrons entering the electron transport chain when light is captured by PSI can also contribute to NADPH production, contingent upon the electron count in the electron transport chain. The electron transport chain establishes a protein gradient, driving ATP synthase to synthesise ATP (Allen, 2002).

Photosynthetic CO_2 assimilation occurs *via*. the CB cycle and has three main stages (Figure 1-2). Stage 1 is known as carboxylation, whereby CO_2 is incorporated with ribulose 1,5-bisphosphate *via*. the enzyme Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) to form two molecules of 3-phosphogylcerate. Stage 2 is when 3-phosphogylcerate is reduced using NADPH to form hexose sugars. Stage 3 is the regeneration phase, in which ribulose 1,5-bisphosphate is regenerated to continue the cycle (Berg *et al*., 2015; Raines, 2003). The CB cycle consists of 11 different enzymes, catalysing 13 reactions in total (Raines, 2003). The CB cycle also supplies intermediates into different end products such as amino acids, isoprene, fatty acids, as well as isoprenoid biosynthesis (Lichtenthaler, 1999; Raines, 2003).

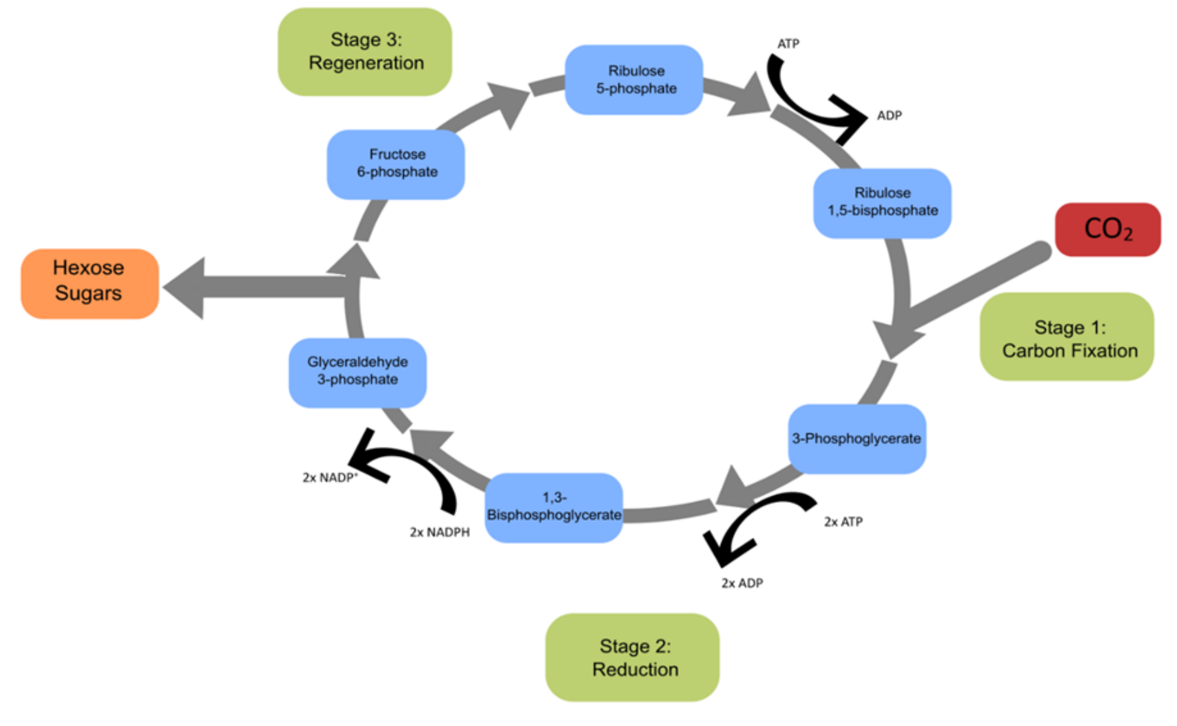

Figure 1-2. *The Calvin-Benson cycle which is also known as the light independent reaction of photosynthesis that occurs in the stroma of the chloroplasts. Figure made using Inkscape.*

Increasing photosynthetic capacity and efficiency can increase potential cropping yields (Parry *et al*., 2011), however, there are some limitations to the rate of photosynthesis that can be divided into three main groups: (1) the supply and utilisation of CO_2, (2) the supply and utilisation of light, and (3) the supply and utilisation of phosphate (Sharkey, 1985). In other words, carbon fixation, electron transport and triose phosphate utilisation determine the overall rate of photosynthesis. Importantly, the electron transport limitation can be divided into two steps; the limitation by light harvesting and the limitation by the maximum electron transport capacity (Kull & Kruijt, 1998). Photosynthesis is a series of reactions which do not all proceed at the same pace. Hence, the overall rate of photosynthesis is determined by the slowest step, which is believed to be, in most cases, the photosynthetic electron transport reaction (Sharkey, 1985).

1.3 Effects of stress events on photosynthetic rate

Photosynthetic efficiency is greatest at optimal conditions, and fluctuations in the environment can lead to plant stress and a lowered photosynthetic efficiency. Many changes in the plant's growing environment can impact on growth, productivity and produce quality, including: high/low temperature stresses, high/low light intensities, rootzone water-deficits or surfeits (flooding, waterlogging), nutrient deficiency, and high salinity. Alone and in combination, these conditions can decrease photosynthetic efficiency, which can affect plant health, growth, and cropping potential. An increase in the severity and/or frequency of these unfavourable environmental conditions can also lead to increased production of reactive oxygen species (ROS), including hydroxyl radicals, singlet oxygens, hydrogen peroxides and superoxide radicals (Sairam & Tyagi, 2004). Radicals damage many important macromolecules including chlorophyll, protein, DNA, and lipids (Imlay & Linn, 1988; Sairam & Tyagi, 2004), which will in turn limit the plant's functionality.

When a plant experiences stress, its adaptive responses can help ensure plant survival; however, the subsequent recovery phase to pre-stress state can cause legacy effects on photosynthesis, which can affect crop yield and berry quality.

For example, yields from UK raspberry crops were lower than usual in 2020 due to the variable weather (wetter and duller than average) in August (DEFRA, 2021), with a 15% decrease in average yield, a 23% reduction in production, and a 14% decrease in value, whereby yield refers to the amount of crop harvested per unit area of land and production refers to the total quantity harvested across a specific area (DEFRA, 2021). The impacts of these sub-optimal weather events on marketable yield, and therefore grower profit margins, are significant, and a better understanding of how crops plants respond to and recover from these variable weather conditions is needed to support new productions strategies, the identification and use of more resilient varieties, and to inform grower decision-making to help to safeguard commercial berry production.

1.3.1 Light stress

The process of photosynthesis begins with the absorption of light quanta, which highlights how crucial light is for photosynthesis (Belgio *et al*., 2012). Both low and high light intensity can impact plant survival and growth as well as productivity.

Like all stresses, low light stress can differ in the type and level of damage that occurs to the plant cells, including the destruction of the membrane integrity, leading to an increase in the cell permeability and intracellular conductivity (Zhu *et al*., 2017). In an experiment conducted by Zhu *et al*. (2017), malondialdehyde is produced during lipid peroxidation increased under low light stress, and can be used to evaluate how much damage has occurred and provide an understanding of plant tolerance to stress (Zhang *et al*., 2021a).

Excessive absorption of light energy at light intensities greater than saturation can cause photodamage (Haniewicz *et al*., 2015; Kok, 1956). Even slight fluctuations in light intensity can cause damage *via*. a reduction in the electron transport rate, which will, in turn, lead to a reduction in photosynthetic efficiency, known as photoinhibition (Powles, 1984). Photoinhibition is defined as the slow retardation of photosynthesis under high light intensity (Long *et al*., 1994).

Plants have an effective feedback mechanism to cope with the natural diurnal variation in light intensities known as non-photochemical quenching (NPQ) (Johnson & Ruban, 2010). Non-photochemical quenching is the dissipation of excess absorbed light as heat. There are many mechanisms which explain what occurs when there is excess light absorbed. Horton *et al*. (1991) published a model of the conformational changes (quenching) that the plant undergoes to overcome the excess light energy absorbed (Horton *et al*., 1991; Horton *et al*., 2005; Ruban *et a*l., 2007).

Many components contribute to NPQ; however, the major component is energy-dependent quenching (qE). Energy-dependent quenching is activated by the acidification of the thylakoid lumen (Teardo *et al*., 2007). Furthermore, it was found that there is a correlation between qE activation and the presence of zeaxanthin (Demmig *et al*., 1987) and the Photosystem II subunit S protein (Sacharz *et al*., 2017; Wilk *et al*., 2013) when plants were under photooxidative stress. Non-photochemical quenching is a competing process for energy, so under dynamic light conditions high NPQ can build up from sun flecks that then decrease photosynthesis when plants return to low light. Kromdijk *et al*. (2016)

found that when the relaxation of qE was accelerated, qE dissipated much quicker when plants were subjected to lower light intensity, and this rapid switching provided more energy for photosynthesis which results in increased yield. This approach could be used as a bioengineering technique to enhance crop productivity (Kromdijk *et al*., 2016), as the crops will be able to overcome the stress of high light much quicker to ensure maximum photosynthesis.

1.3.2 Salinity stress

In arid and semi-arid regions where the rainfall is low, and irrigation is practised without significant drainage, there is a greater likelihood of salt-induced land degradation (Qadir *et al*., 2014). Poor irrigation practices, without a natural or artificial drainage system, could lead to the accumulation of natural ionic salts, which could affect many soil properties, as well as decrease crop productivity (Qadir *et al*., 2014). In case studies analysed by Ghassemi *et al*. (1995), soil salinity affected approximately 20% of irrigated land and is considered to be a global problem (Ghassemi *et al*., 1995; Parihar *et al*., 2015; Qadir *et al.*, 2014).

Salt stress can inhibit plant growth in two ways: first, the osmotic effect of salinity can cause a reduction in the capability of water uptake by the plant, which then leads to a slower growth rate, and second, the ion-excess effect of salinity, whereby excessive amounts of salt enter the plant’s transpiration stream causing cell damage that, in turn, reduces growth rate (Greenway & Munns, 1980). Salinity affects many physiological traits including plant water relations, photosynthesis, ion homeostasis, and yield components (Negrao *et al*., 2017).

The initial response to excess salt build-up is similar to when plants experience rootzone water deficits; first, there is a decrease in the osmotic potential of the soil solution due to the accumulation of salts, which then creates water stress in plants (Sairam & Tyagi, 2004). The water stress can be caused by a negative osmotic potential in the plant cells (Betzen *et al*., 2019). The process of osmotic adjustment requires time as it is an adaptive process, whereby fast dehydration does not allow sufficient time for osmotic adjustment (Blum, 2017). This loss of water from the cells can cause changes in the cell size and membrane proteins affecting turgor pressure (Sairam & Tyagi, 2004), which activates the signalling cascade.

1.3.3 Water deficit stress

Water-deficit stress can occur when the water availability in the rooting zone of the plant becomes limited. 'Water-deficit stress' is also referred to as soil or substrate drying and often (though erroneously) as drought, which in fact is a meteorological term. The conceptual definition of drought is stated in relative terms and the operational definition is the attempt to classify the onset, severity, as well as termination, of drought episodes (Mishra & Singh, 2010; Wilhite & Glantz, 1985). Drought can also be based on standardised precipitation, whereby it is the "difference of the precipitation from the mean for a specified time period divided by the standard deviation where the mean and standard deviation are determined from past records" (McKee *et al.,* 1993). Plants do become more vulnerable to attack by disease and pests under drought conditions (Klein, 2020), as plants may reallocate resources away from defensive mechanisms to prioritise survival, resulting in increased susceptibility to diseases (Klutsch *et al*., 2017). Therefore, it is crucial to avoid water deficit stress events whenever possible, especially in commercial crop production, to reduce loss in revenue.

Drying of the soil or substrate can affect plant growth, development and functioning (Davies *et al*., 2002). Plant water balance can also be distorted when there are long periods without water and when soils capture less water in more intense storms (Ryan, 2011), leading to reduced soil water availability. Rootzone water deficits can have adverse effects as they can limit plant physiological processes (Tombesi *et al*., 2015). As water is a key component in photosynthesis, reduced water availability reduces photosynthesis through the decrease of stomatal conductance and the subsequent limitation of CO_2 uptake for photosynthesis, which will slow the photosynthetic rate (Medrano *et al*., 1997). In many cases, to reduce further water loss during a rootzone water deficit, stomatal conductance and leaf growth will be restricted (Zhang & Davies, 1989).

A rootzone water deficit stress does not necessarily involve the drying of the whole soil or substrate throughout the rhizosphere. Adapted from laboratory split-root experiments, partial rootzone drying (PRD; Blackman & Davies, 1985; Gowing *et al*., 1990; Sobeih *et al*., 2004) is a technique designed to improve water-use efficiency, as when the root system senses drying, and produces chemical or hydraulic signals, they are then transmitted to the shoots that cause the stomata to close, hence decreasing water loss (Gowing *et al*., 1990). PRD irrigation may maximise the physiological effects of abscisic acid (ABA) signalling that may increase horticultural water-use efficiency, and also have other benefits

(Dodd *et al*., 2006). In a review by Chaves and Davies (2010), it was suggested that Dodd's analysis (2009) indicates PRD could be an effective way to increase yield, providing the method and the extent of drying and crop are considered carefully.

Short-term effects of water deficit stress include altered plant-water relations, for example, in the leaf, xylem cavitation and cell collapse can occur (Sack & Scoffoni, 2012). Brunetti *et al*. (2019) showed that unstressed *Populus nigra* plants had a higher and more constant net photosynthetic rate compared to moderate and severely water-stressed plants. In that experiment, when comparing moderate water-stressed plants to those that experienced severe water stress, there were only differences in net photosynthesis, leaf hydraulic conductance, stomatal conductance, and mesophyll conductance at 09:00 (Brunetti *et al*., 2019). This indicates that even slight changes in the magnitude of the stress can have significant impacts on the plant. Also, in many well-watered plants, stomatal conductance becomes lower as the day progresses, but a consistent photosynthetic rate is maintained, which indicates that unstressed plants function in a more water-efficient mode by increasing intrinsic water use efficiency without curbing CO_2 fixation (Brunetti *et al*., 2019). As water is a valuable resource, ensuring that plants use it as efficiently as possible can also help to limit abstraction and subsequent effects on the wider environment. However, legacy effects from severe drought episodes on tree physiology and reductions in radial growth occurred only in the same year, and did not affect leaf photosynthesis or vegetation greenness the following year (Kannenberg *et al*., 2019).

When comparing the physiological responses of trees and fruit crops to low soil water potential, it can be noted that there are some similarities and differences. Similarities between non-tree plants (including fruiting crops) and trees during a water-deficit stress event include a slowing of leaf expansion, and partial stomatal closure, which are adaptive responses to try to limit transpirational water losses (Galvez *et al*., 2011; Ryan, 2011). In some cases, trees do this by shedding their leaves (McDowell *et al*., 2008). It was also noted that trees were more susceptible to attacks by pathogens and insects during a drought stress (McDowell *et al*., 2008). Whilst raspberry canes do not shed their leaves as a result of water-deficit stress, similar to other plants and trees, raspberries do exhibit lower transpirational water loss, stomatal conductance, and photosynthesis rate.

A closer comparison can be drawn in terms of height and fruit production between raspberries and grapevines. In a study using commercial vineyards to detect differences between well-watered and drought-stressed plants, drought-stressed vines had a 70% lower photosynthetic rate compared to well-watered vines, when predawn water potential was at -0.97 MPa for the drought-stressed plants (Maroco *et al.,* 2002). The drought-stressed plants had lower photosynthetic rates and lower stomatal conductance compared to well-watered plants (Maroco *et al*., 2002).

It is also important to remember that it is highly likely that two or more stressors occur concurrently. For example, heat stress and water deficit stress often occur simultaneously (Moroni *et al*., 2020), triggering a signalling cascade involving the generation of second messengers like calcium ions and ROS leading to the production of plant hormones like salicylic acid and ABA which lead to resistance and stress adaptation. An improved understanding of the possible signalling pathways invoked during a water deficit stress, and during the subsequent recovery phase will help to improve our scientific understanding of how photosynthesis in cane crops could be maintained or improved to minimise the legacy effects of crop yield and berry quality.

1.4 Signalling pathways triggered during stress events

In plants, abiotic stresses often trigger both hydraulic and chemical signalling cascades to coordinate adaptive responses. Signalling systems include ionic stress signalling, osmotic stress signalling, ABA signalling, cold and heat stress signalling, and systemic signalling. Many signalling pathways include chemicals such as calcium, nitric oxide, and ROS, where these signalling molecules are involved in both intracellular and intercellular communication, which mediate signalling pathways aiding the plants ability to perceive, respond and adapt to environmental stressors (Zhu, 2016). The aim of this PhD project will be to identify and understand how these signalling systems regulate stress responses and the recovery of leaf gas exchange from water deficit stress. This new information may inform strategies to mitigate stress legacy effects on photosynthesis and could provide targets for manipulating plants to improve stress resilience.

This PhD programme of research concentrates on the effect of water deficit stress in substrate-grown raspberry plants; thus, the focus of this research programme will be understanding the responses to rootzone water deficits and the signalling mechanisms that regulate the response and recovery. For many years, it was accepted that when the soil dries, a reduction in water uptake caused the leaf water status to decline (Davies & Zhang, 1991). These changes are likely triggered by hydraulic and chemical signalling in response to the stress event. However, it is widely accepted that hydraulic and chemical signalling are coupled (Brunetti *et al*., 2019) and should not be considered as two separate pathways operating sequentially.

1.4.1 Hydraulic signalling

Often when plants perceive water deficit stress it is generally (but not always) combined with higher air temperature and a rise in pore electrical conductivity. Elevated air temperatures can lead to increased water consumption in the substrate, and if irrigation is not performed frequently enough, it may cause water deficit stress. This substrate drying can result in higher concentration of ions, which in turn can increase the pore electrical conductivity of the substrate. The rate of soil/substrate drying will depend largely on the prevailing evaporative demand in the canopy area and leaf-to-air vapour pressure deficit (VPD) and to meet this increased evaporative demand under high air temperatures and VPD, plant hydraulic conductance must also increase (Yang *et al.*, 2020). In the absence of this mechanism, plants would experience dehydration as a result of substantial transpiration loss at elevated temperatures.

Hydraulic signals, including leaf turgor changes and cavitation events, play a role in stomatal response to drought (Salleo *et al*., 2001; Tombesi *et al*., 2015). Hydraulic signals that are triggered when cavitation occurs, are caused by high tension during water transport induced by “air seeding”, the change from liquid to water vapour (Salleo *et al.*, 2000; Torres-Ruiz *et al.*, 2015). In the xylem conduits, cavitation breaks the water columns, which reduces plant water transport capacity. The negative pressure of xylem sap increases the risk of conduit collapse, which would also affect the hydraulic efficiency of the plant (Hacke *et al.*, 2001). In rice, drought-induced stomatal closure could be largely explained by hydraulic signals (Wang *et al.*, 2018), highlighting the importance of hydraulic signals in mediating stomatal changes in response to water deficit stress. Leaf turgor changes caused by changes in soil water potential, osmotic pressure or hydraulic conductance can result in stomata responses (Rodriguez-Dominguez *et al*., 2016). However, another study in isohydric grapevine genotypes showed that the drought-induced decline in leaf hydraulic conductance was regulated by ABA accumulation by lowering aquaporin activity (Coupel-Ledru *et al.*, 2017), meaning that ABA could directly or indirectly regulate stomatal apertures by a decrease in leaf hydraulic conductance, a combination of hydraulic and chemical signalling acting together (Brunetti *et al.*, 2019).

1.4.2 Chemical signalling

Chemical signalling is a long-distance response to changes in the environment, including regulating plant stomatal behaviour in drying soil by providing the shoot with some measure of water availability (Davies *et al.*, 2002). Although many chemicals are involved in a network of signalling response pathways, in plants exposed to soil/substrate drying, much emphasis has been placed on the plant hormone, ABA (Davies *et al.*, 2002).

Abscisic acid is thought to originate from the roots when the soil is drying and can be found in the xylem sap (Davies & Zhang, 1991). However, ABA can also be synthesised in the leaves and can be loaded to the phloem and transported to the roots (Hartung *et al*., 2002). ABA is a weak acid and accumulates preferentially in more alkaline compartments of the leaf (Wilkinson & Davies, 1997). A major role of ABA is to promote stomatal closure when plants are experiencing rootzone water deficits, which helps to slow transpirational water loss (Comstock, 2002; Ng *et al.*, 2014). The closing of stomata and the resultant loss of evaporative cooling can cause leaf temperature to increase (Schroeder *et al.*, 2001a). Socias *et al.* (1997) found a inverse correlation between xylem ABA content and stomatal conductance, indicating that soil drying is communicated *via*. xylem-borne, root-to-leaf signalling, which ultimately induces stomatal closure (Reddy *et al.*, 2004). A study by Borel *et al.* (2001) reinforced the central role of ABA in controlling the stomatal response to drought in tobacco. In two grapevine varieties, at the beginning of the water deficit stress, the leaf and root ABA concentrations increased more rapidly in var. Mavrodafni compared to var. Sabatiano (Beis & Patakas, 2010), indicating that response rates can vary within species. There was a higher stomatal sensitivity to ABA in var. Mavrodafni and increased xylem pH values were also noted compared to var. Sabatiano (Beis & Patakas, 2010). Changes in xylem sap pH induced by drought stress can also act as a signal from the roots to the leaf, which can cause stomatal closure *via*. a redistribution of ABA from surrounding tissues (Else *et al.*, 2006) or a reduction in the rate of removal, both of which can lead to higher ABA in the apoplast surrounding the guard cells (Wilkinson & Davies, 2002). In droughted *Commelina communis* plants, the pH of the sap increased by 0.6 units compared to well-watered plants, even though at that point, stem water potential was unaffected or slightly reduced by the soil drying treatment (Wilkinson & Davies, 1997). The rate of sap flow can also affect xylem solute concentrations, including that of ABA (Else *et al.*, 1994) and so stress-induced changes in sap flow on xylem ABA should be taken into consideration when trying to assign causal status to an apparent increase in root-sourced ABA output.

The ABA-signalling pathway was reviewed by Ng *et al.* (2014) and Zhu (2016). The illustration of the ABA-signalling pathway provided by Ng *et al.* (2014) is shown in Figure 1-3. Stress signals induce ABA accumulation, activating PYL-ABA receptors which, in turn, inhibits group AtType 2C protein phosphatases (PP2Cs; Park *et al.*, 2009). The inhibition of PP2C allows the activation of Snf1-related protein kinases 2 (SnRK2) through the process of autophosphorylation, and then active SnRK2 can phosphorylate many downstream effectors (Fujii *et al.*, 2009; Ng *et al.*, 2014). Stomatal closure occurs when SnRK2s phosphorylate the SLAC1 and KAT1 ion channels in guard cells, which then lowers transpiration and slows water loss (Ng *et al.*, 2014).

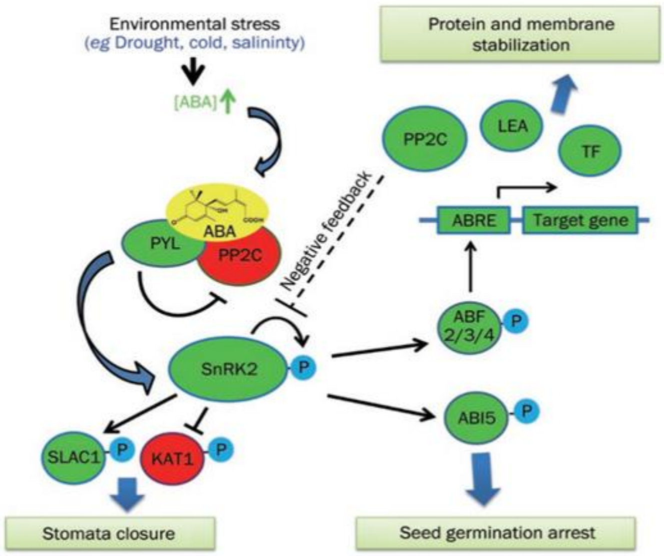

Figure 1-3. *The overview of the molecular events in the ABA-mediated abiotic stress response. Environmental stress causes an increase in the ABA concentration which results in a cascade of events. This can result in a range of responses, including stomatal closure, protein and membrane stabilisation, as well as seed germination arrest. In green and red are the positive and negative regulators, respectively, in the ABA-signalling pathway. Image taken from Ng et al. (2014).*

In well-watered tomato plants, the relatively low concentrations of endogenous ABA are vital to maintain shoot development, especially for leaf expansion (Sharp *et al.*, 2000). Brunetti *et al.* (2019) measured ABA accumulation in roots, xylem and leaves, and reported that in the leaves, ABA regulates the physiological parameters (stomatal conductance and leaf hydraulic conductance) and in the stem, ABA had a role in regulating water transportation and carbohydrate metabolism, by mobilising non-structural carbohydrates to preserve xylem hydraulic conductivity. Additionally, it was suggested that ABA accumulation in the roots under “water stress conditions may enhance root hydraulic conductivity” (Brunetti *et al.*, 2019). A study has shown that a decrease in root hydraulic conductance correlated with a strong decline in the abundance of aquaporin (Aroca *et al*., 2006). These results support the view that both hydraulic and hormonal (chemical) signals have a role in the regulation of stomatal conductance under water deficit stress (Brunetti *et al.*, 2019; Christmann *et al.*, 2007; Wilkinson & Davies, 2002).

Much research has proven that ABA is a root-to-shoot signal that regulates plant physiological changes as a stress response, where increases in root ABA concentrations, then increases in xylem ABA concentrations followed by foliar ABA concentrations increases. There have also been several mutant and transgenic studies as well as grafting experiments that have provided strong evidence that ABA acts as a root-to-shoot signal in response to drought stress. Liu *et al*., (2024) demonstrated that regardless of scion genotype, drought resistance can be conferred by the rootstock, indicating that long-distance signals that originate from the roots can cause the increase of ABA levels in leaves. Studies using transgenic plants like Arabidopsis PYL8, an ABA receptor which functions in drought conditions, provided evidence that hypersensitivity to ABA of this transgenic plant resulted in higher degrees of stomatal closure (Lim *et al*., 2013). Research on Arabidopsis mutants have also provided evidence that *roa* mutants (Root growth Overly sensitive to ABA) display defects in ABA-mediated drought tolerance (Dong *et al*., 2020). Finally, the application of exogenous ABA on ABA-deficit tomato mutants was sufficient to close stomata and reduce transpiration (Herde *et al*., 1997), highlighting the important role of ABA in regulate stomatal changes. All these studies have provided evidence that ABA is a root-to-shoot signal and the important role that ABA plays in regulating stress-induced responses.

However, grafting experiments have also provided evidence that ABA in fact is not necessarily a root-sourced signal that regulated stomatal closure. In tomato, stomata closed in all grafted plants when soil was dried down and did not require ABA production by roots, however, other chemical signals (like pH changes in xylem sap) from the roots causing changes to apoplastic ABA levels in leaves could be responsible for stomatal closure (Holbrook *et al*., 2002). McAdam *et al*. (2016) provided evidence that foliar ABA has a significant effect on root growth, and suggested that leaf hydration is the main signal for regulating plant responses to moisture.

Many experiments have investigated whether ABA acts as a root-to-shoot signal, and although some experiments suggest that ABA is not a root-sourced signal and other chemicals may be involved, the theory that chemical and/or hydraulic signals, regulated by ABA or not, regulate plant physiological responses to abiotic stresses like water deficit stress, should be investigated and responses may not be the same for all plant species.

1.5 Limitations to photosynthesis

Plants respond to biotic and abiotic stress events differently according to the stress event. Reddy *et al.* (2004) summarised three categories of responses to a water deficit stress in higher plants: physiological, biochemical, and molecular. Some of the main physiological responses include loss of turgor and osmotic adjustment, a decrease in stomatal conductance, reduced internal CO_2 concentration, a decline in net photosynthesis, and slowed growth rates. Biochemical responses stated by Reddy *et al.* (2004), included a lowered efficiency of Rubisco, reduced ROS accumulation, accumulation of stress metabolites, and an increase in antioxidative enzymes. Finally, the molecular responses included an increased expression of ABA biosynthetic genes, stress responsive genes, and drought stress tolerance genes (Reddy *et al.*, 2004).

However, some of these stress-induced responses can persist after the stress has subsided (Romero *et al*., 2017) and a key aim of this PhD programme was to identify the signalling processes that regulate these post-stress recovery responses.

1.5.1 Stomatal limitations to photosynthesis

Partial stomal closure lowers the rate of CO_2 entering and water vapour exiting and can result in a diffusional limitation. Under a moderate water deficit, the short-term restrictions in net photosynthesis are often caused by stomatal limitation (Flexas & Medrano, 2002; Pena-Rojas *et al.*, 2004). However, under changing environmental conditions, stomatal responses are fractionally slower than photosynthetic responses (Lawson *et al.*, 2012), and this difference in response time can lead to a disconnect between stomatal conductance and photosynthetic carbon assimilation (Lawson *et al.*, 2010). During short periods of shading (~3 mins) stomatal conductance was unaffected, whereas when longer periods of shading (~25 mins) were imposed, stomatal conductance declined further for a few more minutes (~5 mins) even after the period of shading (Lawson *et al.*, 2012). This quicker response in decline in stomatal conductance than photosynthetic rate was supported by Socias *et al.* (1997), where drought stress was first imposed a decline in photosynthesis rate was less noticeable than in stomatal conductance.

1.5.2 Metabolic limitations to photosynthesis

Metabolic impairments, also known as metabolic limitations, can reduce the rate of net photosynthesis in stressed plants (Lawlor, 2002). Under mild water stress, metabolic limitations correlated with the loss of ATP content (Flexas & Medrano, 2002; Tezara *et al.*, 1999). Further to ATP, Flexas and Medrano (2002) suggested that RuBP (ribulose-1,5-bisphosphate) content also decreases, suggesting that “RuBP regeneration and ATP synthesis were impaired” by water stress. Non-stomatal limitation is also known to inhibit key enzymes involved in CO_2 metabolism, including Rubisco (Maroco *et al.*, 2002; Medrano *et al.*, 1997).

Overall, it can be concluded that stomata close partially and then fully under developing water deficits and a reduced net photosynthetic rate often follows. However, when water was reapplied after a 3-week water deficit stress, it was noted that gas exchange recovered in each of four plants species (*Callitris gracilis, C. preissii, C. columellaris* and *C. rhomboidea*); however, the rates recovery varied, with the recovery of *C. rhomboidea* described as extremely slow (Brodribb *et al.*, 2010). Therefore, the variation in the recovery of leaf gas exchange depends not only on the species but also on the type, magnitude, and duration of the stress. Therefore, it is important to understand the magnitude and duration of the stress that a given species can withstand before plant growth and development is impacted, and in the case of horticultural crop plants, when marketable yield and fresh produce quality is compromised.

1.6 Types of water deficit stress

Two deficit irrigation techniques commonly used to better understand the effects of rootzone water deficit stress on plants are regulated deficit irrigation (RDI) and partial rootzone drying (PRD). Regulated deficit irrigation involves irrigating crops with a lower volume of water than is needed to match demand with supply; the resulting stress can be relatively mild and have minimal effects on yield (English & Raja, 1996). A more severe stress can develop that reduces apple fruit size at harvest but also raises soluble solids content (Ebel *et al*., 1993). On the other hand, RDI applied to almond trees during pre- and post-harvest periods was shown to have no significant effects on bud development, bloom, fruit growth, or fruit abscission, although differences in physiological parameters, such as leaf expansion rate and earlier leaf abscission were observed (Romero *et al*., 2004). However, effects on yield and crop quality may vary depending on the crop, the stage of development, the phytoclimate and many other factors. Published work indicates that RDI can effectively conserve a substantial volume of irrigation water while concurrently enhancing water use efficiency, although in both of these experiments, yields had significantly reduced (Romero *et al*., 2004; Topcu *et al*., 2007).

In the second deficit irrigation technique, PRD, half of the rootzone is irrigated and the other half is left to dry for a varying but critical length of time, and then the irrigation is switched so that the previously well-watered side of the rootzone begins to dry while the previously dry side is rewetted (Dodd *et al*., 2006; Dodd *et al*., 2015; Loveys *et al*., 2000; Sobeih *et al*., 2004; Topcu *et al*., 2007). The technique of PRD was developed based on knowledge of root-to-shoot chemical signalling in drying soil (Dodd *et al*., 1996). The alternations of the wet and dry sides can improve crop yield compared to other deficit irrigation techniques by sustaining root-sourced phytohormonal signalling (Dodd *et al*., 2015) and can also improve water use efficiency (Davies *et al*., 2002). A PRD treatment applied to strawberries resulted in water savings of up to 30% water without compromising strawberry Class 1 yield or berry quality attributes (Dodds *et al*., 2007).

1.7 Cane crops – Raspberries

Raspberries (*Rubus idaeus*) are grown mainly in Northern Europe and are cropped during the summer and autumn. Raspberries that fruit in the summer bear fruit on canes produced from the previous year (floricanes) and raspberries that fruit in the autumn form fruit on the current season’s growth (primocanes; Klein, 2020). Raspberries are defined as aggregate fruits as their flowers have multiple carpels and are not joined together. The fruits develop from the mature ovary that contains the seeds. The seeds of berries are embedded in a fleshy mass (Taiz *et al.*, 2018). According to horticulture statistics published for the year of 2020 by DEFRA, many raspberry crops are now grown in substrate, like coir, as the crop continues to move towards the pot-grown sector (DEFRA, 2021).

Raspberries are rich in vitamins including vitamins C, A, B, B_1, B_2, E, folic acid, phytochemicals and many mineral elements such as iron and potassium (Bobinaitė *et al.*, 2016). In a study by de Souza *et al.* (2014), five fruits: blackberry, red raspberry, strawberry, blueberry, and cherry, were picked to evaluate the chemical composition of each. Out of the five fruits, red raspberries had the second highest moisture content and when comparing antioxidant capacity, red raspberry were determined to be good sources of antioxidants (de Souza *et al.*, 2014). Natural antioxidants in foods are important in counteracting radicals that lead to diseases (Weber & Hai Liu, 2002).

Many chemical compounds contribute to the typical smell of raspberries, however a compound known as the ‘raspberry ketone’ is known to have a major impact on the aroma. ‘Raspberry ketone’ or *p*-Hydroxyphenylbutan-2-one is often referred to as RK (Rao *et al*., 2021). The pleasant scent has seen RK be used in cosmetics as well as food additives. Raspberry ketone has a similar chemical structure to capsaicin and synephrine. It was reported that capsaicin, found in hot red pepper, enhances energy metabolism, decreasing adipose tissue weight (Kawada *et al.*, 1986a; Kawada *et al.*, 1986b). In contrast, synephrine, found in Citrus plants, was found to exert a lipolytic activity (Carpéné *et al.*, 1999). When the effects of RK on obesity were tested, it prevented obesity and fatty liver in mice while significantly increasing norepinephrine (Morimoto *et al.*, 2005). Raspberry ketone is just one of the chemical compounds found in raspberries that could confer major health benefits to consumers.

Raspberry breeders aim to produce varieties with a good appearance and flavour as well as high productivity and ease of harvesting. Experiments in this PhD were carried out on a variety known as Malling™ Bella, which was released in 2017. Malling^{TM} Bella is a *Rubus idaeus* variety with a patent number emr 20171 belonging to NIAB East Malling. This variety has not yet been used in the published literature and so the results from this PhD programme will constitute the first evidence of changes in leaf gas exchange in Malling^{TM} Bella exposed to a root water deficit stress, and also the subsequent recovery following rewetting. Malling^{TM} Bella is a primocane raspberry (Graham & Brennan, 2018) that currently is known for its production of heavy yields, with large berries, and is suitable for double cropping. Double cropping is a pruning technique that allows two harvests per year from the same plant. In the winter months, the strongest canes are selected and the upper fruited part of the cane is pruned off and the other canes are cut to ground level. These then fruit in the summer, and once these have finished fruiting they can be cut to ground level, leaving only the current year’s stems to fruit beginning late summer and continuing to fruit into autumn. It is a popular commercial variety and is grown in Spain, Mexico, and other countries where high temperature and associated VPDs mean that transient and more prolonged drought stresses are more likely than in the UK.

1.8 Measuring the effects of stress

The effects of water-deficit stress and other stressors on raspberry plants can be measured using a variety of methods. These include examining physiological parameters such as plant-water relations, stomatal conductance, leaf water potential, net photosynthesis rate, and crop yield and quality.

As each category could help to identify and elucidate the signalling pathways that occur when a raspberry plant experiences a water deficit stress, it is important that each physiological parameter is evaluated individually. For example, although there is a close relationship between photosynthesis and stomatal conductance (Wong *et al.*, 1979), it is known that stomatal movements are a magnitude slower than photosynthetic responses (Lawson & Blatt, 2014; Lawson & Vialet-Chabrand, 2019). On the other hand, stomatal regulation cannot fully explain the maintenance of leaf water potential since other variables, like soil water availability, hydraulic conductance and leaf conductance are involved in the regulation of leaf water potential (Martinez-Vilalta & Garcia-Forner, 2017). Direct stress effects on the rate of photosynthesis in fruit could also be investigated, as stress could have effects on berry weight and total Class 1 yield. It is known that changes in SWP can be an early and reliable indication that a plant is perceiving stress (McCutchan & Shackel, 1992) and midday SWP measurements have been used as a reference for irrigation scheduling (Moriana *et al*., 2012; Shackel *et al*., 1997). Therefore, measuring midday SWP can provide valuable insights into the plant's stress levels and prevent further stress on the plant by acting immediately. Finally, examining xylem-borne and foliar ABA and leaf hydraulic conductance can help understand the signalling pathway that regulates a raspberry plant's response to a water deficit stress and why the legacy effects on photosynthesis still persist for days after rewetting of the coir commences. All these techniques are destructive measurements and take time to obtain results. Therefore, these techniques may not be practical measurements for growers to use as an irrigation scheduling technique. However, the results obtained from these measurements will suggest possible signalling mechanisms that regulate stress response in raspberry plants.

In this thesis, stress legacy effects on leaf gas exchange were measured using portable infra-red gas-exchange (IRGA) Li-Cor systems. Putative hydraulic signals were identified by measuring changes in pre-dawn and midday xylem hydrostatic potential with a Scholander pressure chamber and changes in xylem sap delivery of putative chemical signals were quantified using gas chromatography-mass spectrometry (GC-MS). The impacts of these legacy effects on Class 1 yields, berry quality and percentage of waste fruit were determined. The results from this work will help to inform strategies to optimise productivity and consistency of berry quality in commercial raspberry production.

1.9 Research objectives

It is clear that the rate of photosynthesis is important in plant growth and development, and that stress events that result in a water deficit can lower photosynthetic rates. Plant responses to stress can differ between species, and understanding their responses is vital in ensuring high crop yield production. This knowledge is especially important if berry crop resilience to environmental stress events is to be improved under the UK’s changing climate. The objectives of the research were (i) to improve our understanding of a raspberry plant's response to water deficit stress, (ii) to investigate the specific response times to a water deficit stress, (iii) to investigate the causal signalling that regulates stress perception, response and recovery, (iv) to understand the persistence of legacy effects on photosynthesis following rewatering, (v) to understand the effects of using different rooting volume on stress perception and recovery, and (vi) to investigate the effects of a transient rootzone water deficit stress on marketable yield and quality in commercial production systems.

2 Leaf physiological responses to a rootzone water deficit stress in Malling™ Bella (red raspberry)

2.1 Introduction

The global population is estimated to grow by 2.1 billion people to 9.7 billion by 2050 (FAO, 2018), and ensuring enough food to feed the growing population is a major political and scientific concern. It has been suggested that new approaches to increase yield and nutritional quality of crops currently grown on available land and marginal lands will be required to ensure food security (Godfray *et al*., 2010; Simkin *et al*., 2019). Many strategies are also aiming at increasing climate resilience of crops (FAO, 2018). Whilst this is important, understanding plant responses to changing climate conditions is important for ensuring that we can maximise yield without compromising quality.

The changing UK climate means that growers are experiencing different growing conditions, not just between seasons but within a growing season. Abiotic stress events like heatwaves and droughts are becoming more frequent, so understanding how plants respond during and after such stresses is important. Additionally, stress events often occur in combination. In the experiments described here, the effects of a rootzone water deficit stress on red raspberry were investigated.

Plant growth, development, and functioning are affected in many ways when water availability is limited (Davies *et al*., 2002). Water is a key component in photosynthesis, and makes up the bulk of the plant, reducing water availability often triggers stomatal closure which, in turn, can limit CO_2 uptake, thereby lowering photosynthesis (Medrano *et al*., 1997). Therefore, even mild and temporary rootzone water deficits can have adverse effects on marketable yield and berry flavour. As photosynthesis is a fundamental process whereby light energy is converted to chemical energy, improving photosynthesis directly affects agricultural productivity (Croce *et al*., 2024).

In 2020, reported yields of commercial raspberry crops in the UK were lower than usual due to variable weather in August (DEFRA, 2021), with an overall 15% decrease in average yield per hectare, a 13% reduction in production at 15 thousand tonnes, and a 14% decrease in value (DEFRA, 2021). This highlights the importance of understanding plant responses to stress events.

The work reported here aimed to establish the timing of leaf physiological responses to different durations of water deficit stress in raspberry plants grown in a polytunnel. It was proposed that water deficit stress would decrease both stomatal conductance and photosynthetic rate. The polytunnel growing system was similar to that on a commercial soft fruit farm, allowing us to study how leaf physiology responds to a water deficit stress event in a typical growing environment.

2.2 Materials and methods

2.2.1 Plant material

Twenty-four three-year-old plants of the raspberry variety Malling™ Bella were grown in 7.5 L pots containing Cocogreen™ coir on the Eastern row in a polytunnel at NIAB East Malling, Kent. The first six plants on the southern end of the row were allocated as guard plants; the next 24 plants were used for the experiment (Figure 2-1). There were four cropping canes per pot, with two pots per linear metre, and so *ca*. eight canes per linear metre.

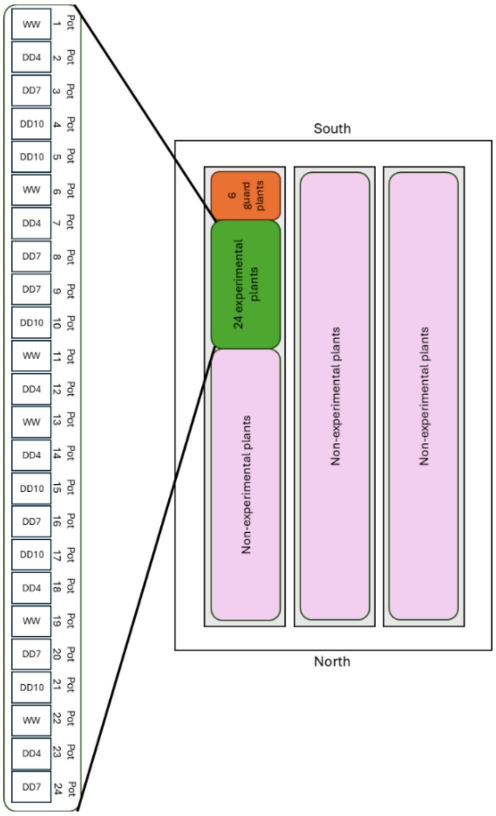

Figure 2-1. *Schematic diagram of planting arrangement for the Malling™ Bella plants used in the experiment.*

All pots were well-watered during establishment with a target daily run-off volume of *ca*. 15% of input volume – this has been found to be the most productive and resource use efficient strategy for cane crops in previous research at East Malling. A coir drying treatment was first imposed on eighteen plants on 02 August 2021 to coincide with a full crop load; the aim was to identify, measure, and quantify the leaf physiological responses to a transient rootzone water deficit in Malling™ Bella.

Throughout the experiment, advice on crop husbandry, fertigation programmes, and pest and disease control were provided by a Berry Gardens Growers agronomist, and all recommendations were implemented promptly by the crop science and production systems (CSPS) technical team or NIAB East Malling's farm team.

2.2.2 Experimental design

A complete randomised experimental design was used: there were four treatments and six plants per treatment, arranged in four replicate blocks. Each block contained one plot for each treatment, with four plants per plot. In this experiment, all pots were fertigated using the same irrigation line, but the volume of irrigation water delivered to each pot was adjusted by altering the number of emitters/drippers per pot; well-watered (WW) pots had two drippers, with each dripper in diagonal corners, and pots to be dried down (DD) had only a single dripper which was repositioned to the centre of the pot. This arrangement meant that DD pots received half of the daily volume of irrigation water that WW plants received, and so coir volumetric moisture content (CVMC) fell gradually as transpirational losses were not fully replaced. Four irrigation treatments were imposed: (i) WW, (ii) dried down for 4 days – DD4, (iii) dried down for 7 days – DD7, and (iv) dried down for 10 days – DD10.

2.2.3 Irrigation application and scheduling

Irrigation water and fertigation to Malling™ Bella were delivered to each pot via two dripper stakes, each connected to a 1.2 L h^{-1} Netafim non-return dripper. The timing and duration of irrigation events to the experimental plants were not controlled separately from the remainder of the plants in the tunnel that were being used for other industry-funded research at East Malling. Irrigation was scheduled using precision irrigation (PI) control, whereby changes in CVMC were monitored using a sensor-based closed-loop irrigation system, and when CVMC reached pre-determined values, irrigation was automatically supplied. A total of four Delta-T SM150T sensors (Delta-T Devices Ltd., Cambridge, UK) were used and with two of the four positioned in the eastern row of the tunnel. The sensors were connected to a Delta-T GP2 Advanced Datalogger and Controller unit. The mean value from the SM150T sensors was calculated automatically by the GP2 unit using a preloaded script, and if the mean CVMC value was equal to or less than the pre-determined irrigation set point, the GP2 opened the solenoid valve. The duration of irrigation events was adjusted to deliver the target mean daily run-off volume of 15% of the input volume.

2.2.4 Coir volumetric moisture content and pore electrical conductivity

Weekly “spot” measurements of CVMC and coir pore electrical conductivity (EC) were made using a hand-held WET-2 sensor connected to an HH2 meter (Delta-T Devices Ltd., Cambridge, UK) calibrated for coir. Measurements were carried out in all pots (dimensions: 21.2 cm x 25.6 cm), with sampling holes drilled at 60 and 160 mm from the top of each pot to allow insertion of the WET sensor prongs, and the mean value was calculated per pot. The weekly “spot” measurements were taken at least twice a day during the drying down phase.

2.2.5 Measurement of physiological parameters

Physiological measurements consisting of stomatal conductance (g_{s}), photosynthetic rate (Pn) and midday stem water potential (SWP) were carried out between 02 August and 27 August 2021. During the drying down and subsequent recovery phases, measurements were carried out at the same time of day.

Midday SWP was measured following the method described by (McCutchan & Shackel, 1992) using a pressure chamber (Skye Instruments, UK). A terminal leaf from the eastern side on one cane in every pot for all plots was covered in foil for 90 mins before excision. A single sharp cut was then made while the leaf was still covered in foil, and then it was sealed quickly around the petiole and placed into the pressure chamber in which a damp tissue had been placed. After sealing, the chamber was gradually pressurised and the xylem elements at the cut surface of the protruding petiole were observed using a hand lens. The endpoint was recorded once the xylem sap darkened the xylem vessels at the cut surface; any minor bubbles emitted before this endpoint were dabbed with a tissue. All measurements were carried out between 11:00 and 12:30.

Leaf gas exchange parameters were also collected using an LCi T Compact Photosynthesis System (ADC BioScientific Ltd., UK). An automatic leaf chamber was used with the same conditions (6 cm^2 leaf area, flow rate of 500 µmol s^{-1}, CO_2 at 400 µmol mol^{-1} and a 1,500 µmol saturation point m^{-2} s^{-1} photosynthetically active radiation). Photosynthetic rate and g_s were measured on all measurement dates between 13:00 and 14:00 using a fully expanded leaf that was exposed to sunlight on the eastern side of the canopy.

2.2.6 Statistical analyses

Graphs were plotted using RStudio (version 2023.06.0). Statistical analyses were carried out using GenStat 20th Edition (VSN International Ltd.). To determine whether differences between the treatments were statistically significant, one-way analysis of variance (ANOVA) tests were carried out and least significant differences (LSD) values for $p < 0.05$ were calculated.

2.3 Results

2.3.1 Coir volumetric moisture content

A coir moisture deficit was imposed gradually by the removal of one dripper from each DD pot, and the leaf physiological responses that were triggered in Malling™ Bella were measured. Since specific irrigation control for each treatment was not possible in this experiment, CVMC values in DD pots could not be controlled independently of WW values. Prior to coir drying, CVMC was maintained within a narrow range in all four treatments, with a mean CVMC value of 0.675 $m^3\ m^{-3}$ (Figure 2-2A).

The mean CVMC value for WW plants was maintained above 0.6 $m^3\ m^{-3}$ for most of the experiment, except on 24 August 2021 when mean CVMC values were temporarily lowered in each of the four treatments (Figure 2-2B). Following the imposition of the different treatments on 02 August 2021, CVMC values fell in the DD pots and the lowest CVMC value measured in each treatment was a function of the duration of the drying down treatment imposed. Values of CVMC first differed significantly between WW and the three DD treatments on 03 August 2021 (Figure 2-2B). The lowest mean CVMC value for each of the DD treatments was 0.363 $m^3\ m^{-3}$ (36% ≡ 0.36 $m^3\ m^{-3}$) for DD4, 0.347 $m^3\ m^{-3}$ (35% ≡ 0.35 $m^3\ m^{-3}$) for DD7 and 0.301 $m^3\ m^{-3}$ (30% ≡ 0.30 $m^3\ m^{-3}$) for DD10, respectively.

Re-wetting of the coir commenced at 07:00 the day after the drying down phase ended by returning the second dripper back to the pot. In the DD treatments, the recovery of CVMC to WW values took seven days in pots previously under the DD4 treatment, four days in the previous DD7 treatments, and 13 days in the previous DD10 treatment. Following re-wetting, mean CVMC values for all DD pots remained above 0.51 $m^3\ m^{-3}$.

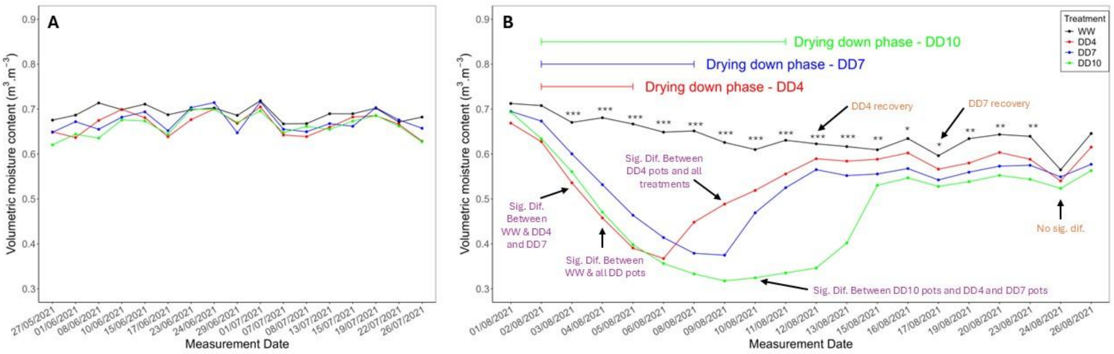

Figure 2-2. *"Spot" measurements of coir volumetric moisture content made using a hand-held WET sensor (A) before the imposition of the drying-down treatment and (B) during the drying down recovery phases. Horizontal lines indicate the drying down phase for each of the treatments. Measurements were carried out throughout the day, and mean values are plotted. Asterisks indicate statistical significance and annotations describe significance differences between treatments.*

2.3.2 Leaf physiological responses to the drying down

Measurements of leaf physiological parameters were carried out to understand how Malling™ Bella responded to different durations of water deficit stress. Since separate sensor-based irrigation scheduling could not be used for each treatment, CVMC values could not be maintained within a certain threshold and so it was not possible to identify the CVMC values at which leaf physiological responses were first triggered. Instead, the presence or absence of a leaf physiological response to the drying down treatment and the persistence of the response following rewetting were recorded.

Although the coir drying treatments were imposed at the same time for all plants, differences in responses were noted. A significant difference in midday SWP was measured by Day 3 in DD4 and DD10 plants ($p < 0.05$), however differences between WW and DD7 plants were only detected on Day 7 ($p < 0.05$). In this preliminary experiment, measurements were not carried out on consecutive days, so the date on which recovery to WW values occurred in each treatment could not be determined. Once rewetting of the coir commenced for DD4 on Day 5, SWP values had recovered by Day 7. The same trend was evident for DD7 and DD10 plants, whereby the next time the measurements were carried out (three to four days after rewetting), midday SWP values had recovered. On Day 14 and thereafter, there were no differences ($p = 0.335$) between WW plants and those in the three DD treatments (Figure 2-3).

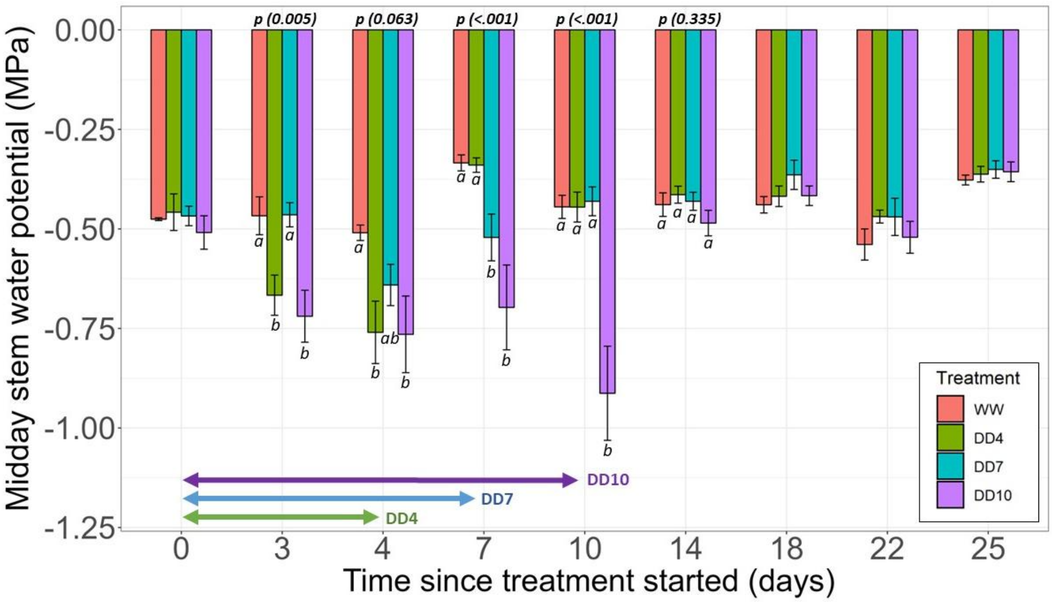

Figure 2-3. *Effects of different durations of coir drying treatments on stem water potential of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Measurements of Pn and g_s were not, unfortunately, carried out on Day 0 of this experiment due to the limited availability of equipment caused by damage to one of East Malling's infrared gas analysers by another student. However, measurements of g_s carried out on Day 4 showed significant differences between WW plants and DD7 and DD10 plants ($p < 0.05$), but not between WW and DD4 plants, although g_s values were lowered in the latter. The g_s values remained depressed until rewetting commenced for DD4 and DD7 plants, with g_s recovering three days later. However, g_s values for DD10 plants had not recovered by the end of the experiment; differences between WW and DD10 plants persisted even on Day 25, 15 days after coir rewetting commenced ($p < 0.05$; Figure 2-4).

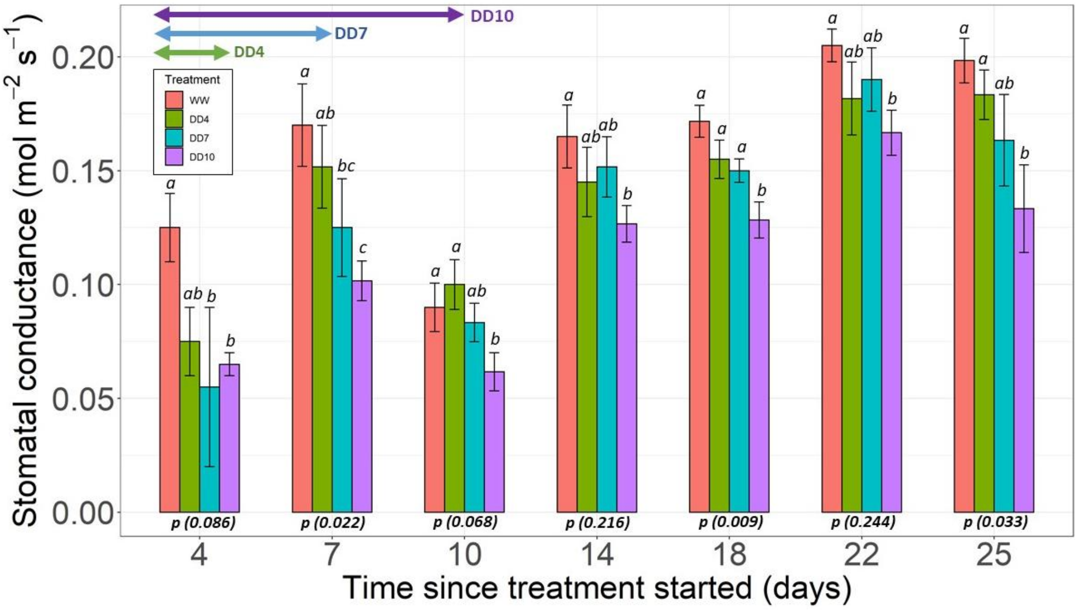

Figure 2-4. *Treatment effects on stomatal conductance of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

The coir drying treatment also affected Pn, with lower values measured in DD plants than in WW plants. In the DD4 treatment, although Pn values were lower when measured on Day 4, the difference from WW values was not significant ($p = 0.315$). The Pn values between WW plants and DD7 plants were also similar throughout the experiment, but varied more from day-to-day in DD10 plants. On Day 25, 15 days after coir rewetting, Pn values of DD10 plants remained significantly lower than in WW plants ($p < 0.05$; Figure 2-5).

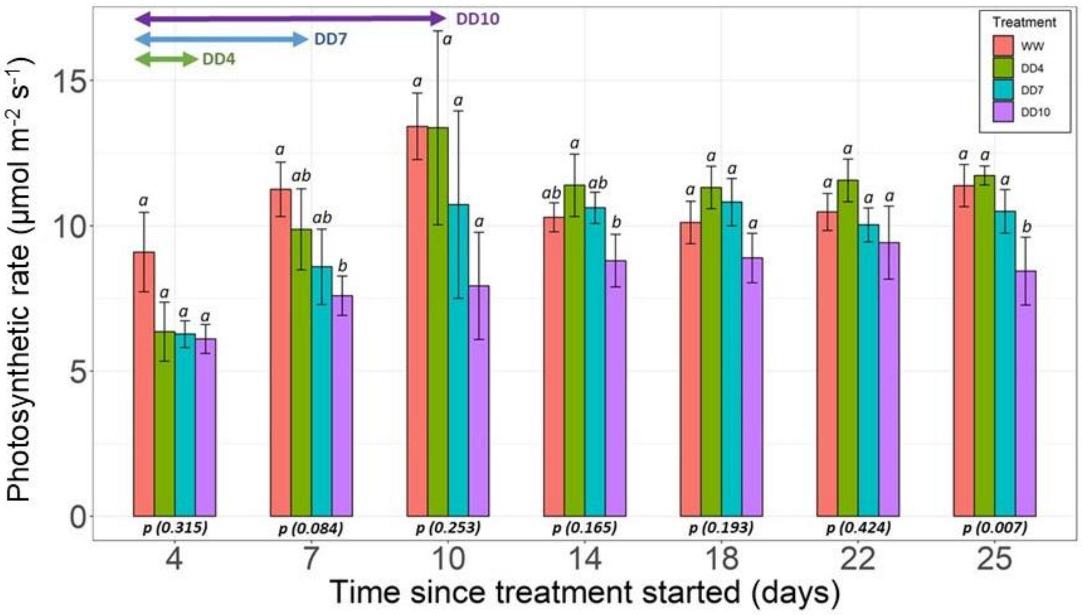

Figure 2-5.*Treatment effects on photosynthesis (assimilation rate) of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

In order to make comparisons between the differences in the responses during the drying down and recovery phase, further analysis of the photosynthetic rate and stomatal conductance values were carried out. While the values of stomatal conductance and photosynthesis fluctuated for the WW plants, no trend was observed. However, with the plants that were dried down (DD4, DD7 and DD10) plants the decrease of stomatal conductance values during the drying down led to a decrease in photosynthetic rate, and when values of stomatal conductance increased during the recovery phase, so did photosynthetic rate.

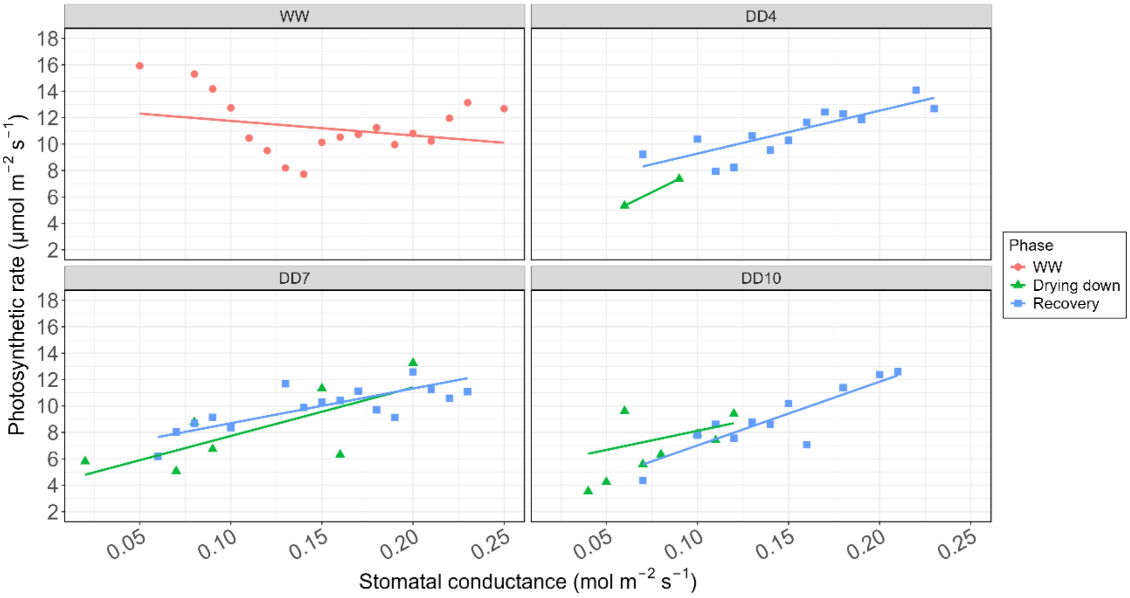

Figure 2-6. *Relationship between the g_s and Pn of Malling™ Bella during the drying down and recovery phase. Each panel is separated according to the treatment and therefore the number of replication is different dependent on the length of drying down.*

In an effort to understand if a hysteresis of stomatal conductance occurred during the recovery phase, values of stomatal conductance were plotted against CVMC values. No trends have been identified.

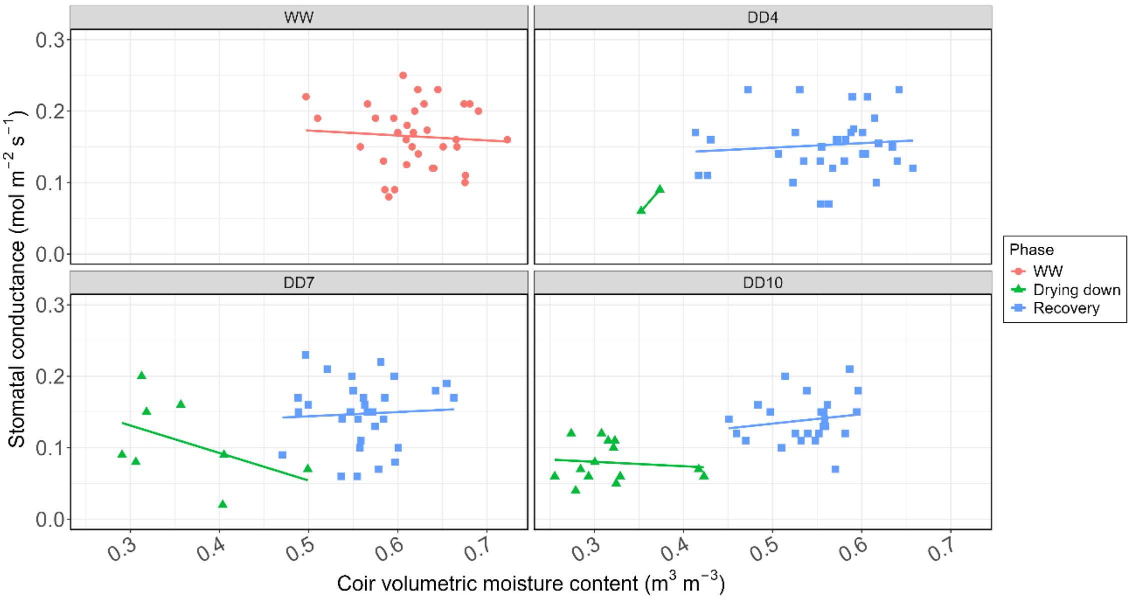

Figure 2-7. *Relationship between the g_s and CVMC of Malling™ Bella during the drying down and recovery phase. Each panel is separated according to the treatment and therefore the number of replication is different dependent on the length of drying down.*

Reducing the volume of water available for plants can affect various plant physiological processes (Bradford & Hsiao, 1982), as water is an important component for plant growth and survival. Higher temperatures and drought episodes will become more prevalent and current crop-growing regions are likely to be less productive, putting food security at risk (Godfray *et al*., 2010). Since plants are likely to experience more stress events than ever before, it is important to understand how plant physiological responses to single and combined stress events impact on marketable yield and quality. Here, the extent and timing of leaf physiological responses induced by a temporary rootzone water deficit stress, and during recovery were measured and quantified in primocane Malling™ Bella.

2.4.1 Irrigation application and the drying of the coir

During plant establishment, the PI automated control system enabled consistent control of coir moisture with minimal fluctuations. Precision irrigation is needed to increase productivity and ensure food security, and so it is important for farmers and researchers to utilise appropriate technologies, but the relatively high cost and level of expertise needed to use them optimally are significant challenges (Abioye *et al*., 2020). For example, the timing of manual measurements of CVMC relative to an irrigation event is important and should be standardised to avoid large fluctuations which could hamper decision making. The timing of manual measurements of CVMC should also be relative to physiological measurements, ensuring to be an accurate value of the moisture content at the time of when physiological measurements are recorded. This information will also then be helpful in understanding whether dropping below a certain CVMC threshold triggers responses in leaf physiological responses, which growers would then be able to use the minimum CVMC value an irrigation scheduling target to prevent legacy effects on photosynthesis. Future measurements of CMVC carried out in our experiments should ensure consistency in the time of day as well as the time elapsed after an irrigation event and consistent and relative to physiological measurements.

Other than monitoring volumetric moisture content, there are also sensors that can measure soil/substrate matric potential, which measures how tightly water is bound to the soil/substrate particles, which ultimately provides information on how much energy is required to be exerted from the plant to extract water from the soil/substrate (Montesano *et al*., 2015).

Research carried out on ornamental crop grown in containers has shown that at higher water potential (-0.6 MPa) water became inaccessible to plants that were grown in peat amended bark compared to other experimental substrates (Fields *et al*., 2018). The addition of coir to peat improved aeration and retained sufficient water in the optimal growing water potential range, which suggests that in a water deficit event higher proportions of water can be used at lower water potentials (Fields *et al*., 2018), hence raspberry grown in soil may exert different physiological responses to water deficit stress to those grown in coir in pots. Many different combinations of substrates have been used and tested to reduce the phenomena of hysteresis (Fields *et al*., 2018), where the relationship between substrate matric potential versus soil water content is different depending on whether the soil/substrate is drying or wetting. Despite an increase in soil water content during the rewetting phase, the soil matric potential may remain lower due to hysteresis, which would reduce water availability to the plant and therefore leading to sustained stomatal closure or partial reopening (Carminati & Javaux, 2020). As a delayed stomatal recovery once rewetting commenced may be due to a hysteresis of substrate matric potential versus substrate water content, the analysis of stomatal conductance and CVMC values highlighted different relationships during the drying-down and recovery phases (Figure 2-7). However, more frequent measurements may give a better understanding of this and therefore should be taken into account in future experiments (see Chapter 3).

For many years, deficit irrigation strategies have been used as a water-saving approach, whereby the volume of water applied to plants is lower than the demand. The subsequent plant physiological responses *e.g*. partial stomatal closure can help to increase the efficiency of plant water use (Khapte *et al*., 2019). Two common deficit irrigation techniques used in research include: (i) regulated deficit irrigation (RDI), whereby irrigation volumes are reduced by a relative amount but are always less than the evaporative demand (Proebsting *et al*., 1989) and (ii) PRD, a technique deliberately used to impose a heterogeneity in soil moisture by watering different parts of the rootzone independently (Loveys *et al*., 2000). In both techniques, irrigation volumes applied to the plants are carefully controlled to ensure that physiological responses can be quantified and the effects on crop yield can be investigated. In the experiment described here, removing a dripper meant that the water received by DD plants would be halved, which is similar to some RDI treatments imposed by supplying less than the evapotranspirational water need (Romero *et al*., 2010). The moving of the remaining dripper to a more central position prevented the scenario where only one side of the pot would receive irrigation, somewhat similar to a PRD treatment. Although there was limited control of the volume of water applied in the DD treatments, removing a dripper did result in a water deficit stress, and the rate of coir drying in all DD treatments was similar. However, the rate of recovery during the subsequent rewetting phases differed for each treatment, and this made it difficult to quantify the timing of the recovery of the physiological responses. More independent and precise control of coir rewetting in the different treatments is needed in future experiments (see Chapter 3). Having separate irrigation lines per treatment would also ensure better control of coir moisture content which would help in quantifying the timing and extent of physiological responses to different irrigation treatments. Furthermore, retaining two drippers per pot during the drying down phase would also improve the distribution of the applied irrigation and maintain a better consistency with the WW pots.

Experiments in vineyards have also previously used drip irrigation as a watering technique (Douthe *et al*., 2018) allowing close control of irrigation input. However, other experiments have used a pre-determined specific volume of water and nutrient supply (Medyouni *et al*., 2021). However, this does not consider the plant's varying water requirements through the development of the plant as well as due to changes in environmental conditions. The PI control used at East Malling and in this experiment considers these, ensuring that the plants get sufficient water without the risk of flooding or drought.

2.4.2 Leaf physiological responses to a water deficit stress

The changes in leaf physiological responses were measured in response to the imposed water deficit stress. In many cases, the first indication that a plant is perceiving a water deficit stress can be detected by a lower midday SWP, a sensitive and reliable measure for plant-based water stress (McCutchan & Shackel, 1992), and a common approach to measure shoot water status (Davies & Zhang, 1991). When differences between WW and DD7 plants were evident on Day 3, the differences in mean CVMC value could explain this slower response from the DD7 plants; this suggests that CVMC must be below a certain threshold for midday SWP differences to be measured. Since measurements were not carried out on consecutive days, the exact recovery dates for midday SWP values for each treatment could not be determined; however, recovery by the next measurement date suggests that the causal signal that led to the fall in midday SWP is quickly lost upon rewetting of the coir. Furthermore, as measurements were not carried out on consecutive days, whether midday SWP is more sensitive than changes in leaf gas exchange would only be able to be determined when consecutive measurements of all measurements are carried out.

Wilkinson and Davies (1997) reported that even though an increase in the pH of the sap was identified in droughted plants compared to well-watered ones, SWP was unaffected or slightly reduced, suggesting that a change in sap pH does not affect SWP values. A review of hydraulic and chemical signalling suggested that soil drying triggers stomatal closure even without a noticeable decline in shoot water potential (Comstock, 2002), suggesting that separate signalling mechanisms can affect midday SWP and stomatal closure.

Due to a constraint on available equipment, changes in Pn and g_s caused by the different treatments were only measured from Day 4 onwards. Daily fluctuations in g_s values were likely caused by differences in environmental conditions such as VPD (Figure-A1) and photosynthetically active radiation (PAR; Figure-A2), and these environmental variables can also exacerbate the degree of stress perceived. In natural environments, plants experience sun/shade flecks resulting from cloud cover, sun angle, shading from other plants (Way & Pearcy, 2012) and wind-driven movements (Lawson *et al*., 2010). Therefore, stomata and photosynthesis respond continually to changing environmental cues (Lawson & Blatt, 2014). When light absorption exceeds the rate at which the captured energy can be used for carbon fixation, cell damage can occur (Lawson *et al*., 2012). Also, fluctuations in light intensity can lower electron transport rate, leading to a reduction in photosynthetic efficiency (Powles, 1984). Photosynthetically active radiation values during the experiment (Figure-A2), measured some fluctuations on most days. When evaluating the effect of VPD, research carried out on lettuce showed that drastic VPD fluctuations (1.63 kPa for 6 mins and 0.63 kPa for 3 mins) throughout the day caused a decrease in stomatal conductance, while moderate VPD fluctuations (1.32 kPa for 7 mins, 0.86 kPa for 3 mins) had no effect (Inoue *et al*., 2021). The environmental sensors did not measure moderate fluctuations, indicating that the drop in stomatal conductance in this experiment would not have been affected by the VPD conditions. Reviewing the plant responses to VPD, Grossiord *et al*. (2020) reported that under high VPD, stomatal conductance decreased until a given VPD threshold when photosynthesis rate was then impacted (Grossiord *et al*., 2020).

Lower values of g_s on Day 4 for DD-treated plants suggest that the applied water deficit stress triggered partial stomatal closure. The signalling response involves many chemicals, including cytokinin and ethylene, however much of the emphasis is placed on the plant hormone ABA (Davies *et al*., 2002). Research carried out on different crops proves that ABA plays a role in regulating stomatal behaviour when experiencing a drought episode (Davies *et al*., 2002; Comstock, 2002; Ng *et al*., 2014). Abscisic acid accumulates under stressful environmental conditions, causing the activation of SnRK2 by the inhibition of PP2C and in guard cells SnRK2s can phosphorylate ion channels resulting in guard cell closure to reduce water loss (Ng *et al*., 2014).

The different recovery rate of g_s in the DD10 treatment, compared to that in DD4 and DD7, following the rewetting of the coir suggests that the duration and extent of water deficit stress can result in different recovery of leaf physiological parameters. Previous research on drought-resistant trees found that the rate of recovery in gas exchange was highly variable following drought stress depending on the species and the magnitude of the stress (Brodribb *et al*., 2010). However, the same report also observed that all plants eventually recovered, suggesting that if measurements had continued here for longer, g_s values in DD10 plants might have recovered to those of WW plants. Brodribb *et al*. (2010) mentioned that hydraulics influenced the dynamics of recovery from extreme drought, as when significant embolism was induced, the gas exchange recovery matched the rate of hydraulic repair. In that report, the duration of the extreme drought was one month, which was significantly longer compared to the duration of the stress in our experiment.

As the soil dries, ABA accumulates in the roots due to enhanced biosynthesis but lowered water uptake during drought stress reduces the transport of this additional ABA via the xylem to the shoots. Following rewetting, a ‘pulse’ of xylem ABA can sometimes be measured (Dodd *et al*., 2006; Dodd *et al*., 2015). This extra supply of ABA could contribute to continued stomatal closure following rewetting, and this could help to explain the prolonged stomatal closure in DD10 plants which had experienced a longer duration of water deficit stress. Stomatal behaviour can also be regulated by hydraulic signals (Else *et al*., 2001), and reports have shown that hydraulic signals can affect leaf ABA concentrations, which in turn regulate stomatal behaviour (Christmann *et al*., 2007). The literature suggests that both chemical and hydraulic signals are involved in the leaf physiological changes when drought stress is imposed; however, understanding the responses and signalling for each crop is vital in order to optimise crop yields when drought stress is present.

Research carried out on other crops, including wheat (Vassileva *et al*., 2011), kidney bean (Miyashita *et al*., 2005) and field-grown grapevines (Romero *et al*., 2017) showed similar results, with g_s and Pn values taking longer to recover following longer durations of drought stress. In olive trees, 18 hours after rewetting, g_s values were still low. However, six days after rewetting, g_s values had significantly increased (Torres-Ruiz *et al*., 2015), indicating legacy effects on stomatal conductance.

Despite partial stomatal closure, measured differences in Pn values between WW plants and DD-treated plants on Day 4 were not significant. These results are similar to those of Socias *et al.* (1997) and Miyashita *et al*. (2005), who reported a greater decline in stomatal conductance compared to the rate of photosynthesis in the first instance of drought stress. Lower rates of Pn can be caused by a limitation of CO_2 supply by reduced g_s (Lawlor, 2002), differences between WW plants and DD4 and DD7 plants in this experiment were not significant, suggesting that CO_2 supply was not limited at that specific value of g_s. However, the intermittent recovery of Pn values for DD10 plants suggests the legacy effects on stomatal conductance may be the cause, as short-term restrictions in net photosynthesis can be caused by a stomatal limitation (Flexas & Medrano, 2002; Pena-Rojas *et al*., 2004). Plants can achieve lower rates of transpiration without affecting CO_2 assimilation (Pirasteh-Anosheh *et al*., 2016). However, as stomatal conductance reduced, so did photosynthetic rate (Figure 2-6) so when stomatal opening is further reduced, CO_2 assimilation and net photosynthesis are then decreased (Pirasteh-Anosheh *et al*., 2016).

Compared to other crops, measured values of maximum assimilation rate (A_{max}) were lower in raspberry (var. Malling™ Bella), with mean values of 10 μmol m^{-2} s^{-1} in the WW plants and a high value of 14 μmol m^{-2} s^{-1} in this experiment. While this range is usual for raspberry (Qiu *et al*., 2016; Qiu *et al*., 2017), in other crops under non-stressed conditions, including wheat (Fischer *et al*., 1998), apple (Lebese *et al*., 2011), and strawberry (Le *et al*., 2021), A_{max} values of *ca*. 20 μmol m^{-2} s^{-1} are commonplace. On the other hand, grapevines show more similar A_{max} values to raspberries, with light-saturated values around 10 μmol m^{-2} s^{-1} (Cortázar *et al*., 2005).

As stomata respond to variations in photosynthetically active photon flux density (PPFD), slow stomatal opening can also limit Pn (Lawson & Blatt, 2014). In this experiment, differences in Pn values between WW and DD10 plants were measured on Days 14 and 25 when VPD was relatively low (< 1 kPa) and consistent. Maximum PAR values on these days were 650 and 500 μmol m^{-2} s^{-1} (Figure-A2), respectively, which is below the light saturation point of 700 μmol m^{-}

^{2} s^{-1} for Malling™ Bella (Figure-A3). With light limiting on those days, treatment differences in photosynthesis may have been easier to detect due to a decrease in absorption of light quanta and less stress on the plant during recovery, highlighting the importance of taking into account changes in light intensity during such measurements (Belgio *et al*., 2012).

Drought stress can lower yields as it affects physiological and reproductive processes in plants, with reductions usually due to impaired photosynthesis, reductions in nutrient uptake and compromised reproductive development (Qiao *et al*., 2024). The effects of different irrigation regimes on yield and fruit quality in red raspberry were investigated by Ortega-Farias *et al.* (2022); yield and fruit number were significantly impacted when irrigated with 50% evapotranspiration of the crop, but there were no significant effect in the berry soluble solids content. Stomatal limitation of Pn when integrated over a growing season could have significant detrimental effects on crop yield (Lawson & Blatt, 2014). It follows that water deficit stress events could have detrimental consequences for commercial raspberry production, with yield penalties resulting in lost revenue for growers. Hence, a better understanding of legacy effects on stomatal conductance and photosynthesis following a water deficit stress is vital to ensure profitable raspberry production in a changing climate.

Environmental conditions in typical raspberry polytunnel production are highly variable, and a more controlled environment is needed in which to carry out experiments to improve our understanding of the signalling mechanisms that regulate stress legacy effects. Consequently, subsequent experiments were carried out in a more controlled environment, with an automatic sensor-based irrigation system to enable independent control of coir moisture content in the different treatments.

As outlined in this chapter, several potential signalling mechanisms could play a role in the response and recovery of stomatal conductance and photosynthesis following a rootzone water deficit stress. However, in order to schedule sampling times to identify putative causal signals, more detailed time-courses of physiological responses to transient rootzone water deficits are needed – this work is described in Chapter 3.

2.5 Conclusions

This preliminary experiment demonstrated that legacy effects on stomatal conductance and photosynthesis are likely in raspberry following a water deficit stress. The drying-down treatments caused a reduction in midday SWP, g_s and Pn values. The duration of the water deficit stress imposed also affects the recovery response, with longer drying episodes taking longer to recover.

3 Recovery of leaf gas exchange in Malling™ Bella from rootzone water deficit stresses in a controlled environment

3.1 Introduction

Plants frequently experience different abiotic stresses, and there is a need for a better understanding of how plants respond to and recover from single or combined stress factors. Periods of drought are becoming a frequent occurrence which causes stress to the plants and can affect plant development (Davies *et al*., 2002; Medyouni *et al*., 2021; Romero *et al*., 2004). Understanding a plant's response and recovery from stress is essential for enhancing and advising mitigation strategies for commercial growers.

Stomatal apertures adjust in response to various environmental cues, including changes in soil moisture (Belko *et al*., 2012). Stomatal responses to water deficit stress have been studied extensively in many model species and also in crop plants (Beis & Patakas, 2010; Chaves *et al*., 2010; Khonghintaisong *et al*., 2017; Miyashita *et al*., 2005); however, there is limited understanding of the response and the recovery to a water deficit stress in soft fruit such as raspberry and the impacts of water deficits on berry yield and quality.

Red raspberries (*Rubus idaeus*) are cultivated mainly in Europe as well as North and South America (Sargent *et al*., 2007), and are used both as a dessert fruit and in processing, making them highly valued berries of economic importance (Sargent *et al*., 2007; Ward *et al*., 2013). Variable weather conditions in August 2020, including heatwaves followed torrential rainfall resulted in a 15% decrease in average yield that year (DEFRA, 2021) . Climate change is likely to bring about more unpredictable and extreme weather conditions, including increasing drought episodes, and so a better understanding of how crop plants respond to, and recover from these episodes will play an important role in helping to maintain or even improve crop yield and quality.

Here, the legacy effects on leaf gas exchange in Malling™ Bella following a rootzone water deficit stress imposed as described in Chapter 2 were investigated further. The focus of this study was on measuring diurnal changes in leaf gas exchange that were triggered by different durations of rootzone water deficits, and also the subsequent rates of recovery following rewetting of the coir. This information was needed to help inform sampling strategies so that the role of signalling processes in the response to, and recovery from, a rootzone water deficit stress could be investigated (see Chapter 4).

3.2 Materials and methods

3.2.1 Plant material and growing conditions

Two-year-old Malling™ Bella plants, each grown in 7.5 L rectangular pots (dimensions: 21.2 cm x 25.6 cm) of Cocogreen™ coir substrate, were used in each experiment. Malling™ Bella plants in experiment one were arranged in two rows of 10 plants, and in experiment two, the plants were arranged in two rows of 12 plants. There were three cropping canes per pot in experiment one and four cropping canes per pot in experiment two. Pots were spaced at approximately two pots per linear metre. A 14-hour photoperiod between the hours of 06:00 and 20:00 was achieved using LED lights. The average natural daylight during both experiments was approximately 13 hours. The air temperature in the glasshouse compartment was set to 22°C during the light period and 18°C at night.

3.2.2 Experimental design

A complete randomised experimental design was used in both experiments: there were two treatments. The two irrigation treatments applied were: (i) WW with a target daily run-off volume of water (and fertilisers) of 15%, and (ii) a DD treatment, where irrigation was withheld so that the CVMC fell gradually to facilitate accurate measurements of the onset of plant physiological responses to limited coir water availability. In experiment 1 there were 10 plants per treatment, arranged in 10 replicate blocks; in experiment 2 there were 12 plants per treatment, arranged in 12 replicate blocks. Each block contained one plot for each treatment, and separate irrigation lines were used for each treatment allowing separate irrigation control for each treatment.

3.2.3 Fertigation system

Plants were watered and fed using a drip fertigation system where each pot had two dripper stakes connected to Netafim CNL emitters (1.2 L h^{-1}). Irrigation was scheduled using PI control, whereby changes in CVMC were monitored using a sensor-based closed-loop irrigation system (Delta-T Devices Ltd., Cambridge, UK), and when CVMC reached pre-determined values, irrigation was automatically supplied. Five Delta-T SM150T sensors (Delta-T Devices Ltd., Cambridge, UK) were used per treatment, with three sensors positioned in row one and two sensors in row two.

Fertiliser was added at each irrigation event (fertigation) using three Dosatrons attached to a 3-wire Galcon timer with two solenoid valves. Fertiliser recipes were provided with three tanks, where tank A contained macronutrients, tank B contained micronutrients and tank C contained nitric acid. The fertiliser recipes were adjusted for vegetative and fruiting stages following advice from the Berry Gardens Growers agronomist. The EC and pH of the fertigation input were measured weekly and adjusted accordingly to maintain recommended inputs.

3.2.4 Environmental conditions

The glasshouse compartment was fitted with a GP2 data logger and controller (Delta-T Devices Ltd., Cambridge, UK) with environmental sensors taking measurements of environmental conditions every 2 mins. Data from the environmental sensors and the Delta-T SM150T soil sensors was uploaded to a cloud report that was updated every 15 mins. With remote access to the cloud report, environmental and CVMC conditions could be checked frequently.

The environmental sensor was positioned 180 cm from the floor, and the solar radiation sensor was positioned 220 cm from the floor to prevent overshading from raspberry leaves. These measurements included relative humidity (RH), coir moisture temperature, air temperature, solar radiation and a PAR sensor. Vapour pressure deficit was calculated from temperature and RH data. Photosynthetically active radiation was measured using a PAR Sensor (Apogee Instruments, Utah, USA) attached to a Decagon Em50G Data Logger (Decagon Devices Inc., Pullman, WA). The information from these sensors was used to inform the interpretation of the plant physiological data sets.

3.2.5 Coir water deficit treatment

In the first experiment, a 10-day coir drying treatment was imposed from 15 to 25 August 2022; this is referred to as the 10-day DD experiment. The second experiment was a 7-day drying-down treatment, imposed from 18 to 25 May 2023, which from now on is referred to as the 7-day DD experiment.

In both the 10-day and 7-day DD experiments, two irrigation treatments were imposed: a WW control and a coir DD deficit irrigation treatment. Gradual coir drying was imposed by lowering the irrigation trigger point for DD plants by 5% each day. For the 10-day DD treatment, the trigger point was dropped until the lowest trigger point of 15% was reached; from then onwards, the 15% trigger point was maintained until the rewetting of the coir. The trigger point for the 7-day DD treatment was also imposed by dropping the irrigation point by 5% until 25% and then was rewetted.

At the end of each DD treatment, DD pots were re-wetted until leaf physiological parameters had fully recovered. To re-wet the coir, the irrigation trigger point was increased to 50% and then raised more gradually to achieve the target of *ca*. 10% run-off, as in the WW controls.

3.2.6 Coir volumetric moisture content

Measurements of CVMC values were made using a hand-held WET-2 sensor connected to an HH2 meter (Delta-T Devices Ltd., Cambridge, UK) calibrated for coir. Measurements were made once a day around 09:00, where two readings were taken, at the top and bottom of the pots, with sampling holes drilled at 60 and 160 mm from the top to allow insertion of the WET sensor prongs. An average value each pot was calculated and reported as the ratio of water volume in the coir to the total volume of coir (m^3 m^{-3}). The WET-2 sensor also measured coir pore EC and coir temperature.

3.2.7 Leaf physiological measurements

Measurements of leaf physiological parameters for the 10-day DD treatment consisted of g_s and midday SWP. For the 7-day DD treatment, physiological measurements consisted of g_s, Pn, SWP and leaf temperature.

Midday SWP measurements for both experiments were carried out between 11:00 and 12:00, as described in Section 2.2.5.

In the 10-day DD treatment, g_s was measured using a Delta-T AP4 porometer (Delta-T Devices Ltd., Cambridge, UK) every three hours between 07:00 and 19:00. Measurements were carried out on a fully expanded leaf that was exposed to sunlight on the eastern side of the canopy. With one of the Licor photosynthesis systems owned by East Malling back with the manufacturer to rectify issues, only one LICOR photosynthesis system was available to be shared with many ongoing experiments. For that reason, during the 10-day DD treatments, leaf gas exchange parameters were measured only on three occasions (on days 9, 18 and 25) using the LI-6400XT Portable Photosynthesis system (LICOR Biosciences, Inc., Lincoln, Nebraska, USA). An automatic leaf chamber was used with the same conditions in each of the systems (6 cm^2 leaf area, flow rate of 500 $\mu mol\ s^{-1}$, CO_2 at 400 $\mu mol\ mol^{-1}$ and a 1,500 µmol saturation point $m^{-2}\ s^{-1}$ PAR), and measurements were carried out between 12:00-13:00.

During the 7-day DD treatment, g_s and Pn measurements were carried out using the LI-6400XT Portable Photosynthesis system (LICOR Biosciences, Inc., Lincoln, Nebraska, USA). An automatic leaf chamber was used with the same conditions as described above. Leaf surface temperatures of fully expanded leaves were measured using a thermal camera (Teledyne FLIR TG167 Thermal Imaging IR Thermometer).

3.2.8 Berry yield and quality

During the 10-day DD experiment, ripe fruit was harvested twice a week, starting from 05 August to 09 September 2022. All berries were graded into Class 1 and waste, and the number and fresh weight of each category were recorded using a ± 0.1 g Ohaus digital scale (Ohaus Corp., USA). The total number of berries per plant was calculated, and the average fresh weight of individual fruit was determined.

Berry soluble solids content (SSC or %BRIX) was measured with a digital refractometer (Palett 100, Atago & Co. Ltd., Tokyo, Japan) using a pooled sample of juice expressed from five individual berries collected from each experimental plot. These measurements were taken every time fruit was collected.

3.2.9 Statistical analyses

Graphs were produced and statistical analyses were carried out using RStudio (version 2023.06.0). To determine whether differences between irrigation treatments were statistically significant, one-way analysis of variance (ANOVA) tests were varied out and Tukey HSD (honestly significant difference) values for $p < 0.05$ were calculated.

3.3 Results

3.3.1 Coir volumetric moisture content during the drying down treatments

Coir volumetric moisture content was measured frequently during the DD treatments to quantify the rate and severity of coir drying. For information, weekly measurements made before the onset of the coir drying are referred to as negative (-) days before the treatment was imposed, and Day 1 is the day that the drying down treatment commenced; thereafter, daily measurements are referred to as positive (+) days since the beginning of the DD treatment.

In each experiment, CVMC in WW plants was maintained between approximately 0.5 and 0.6 $m^3 m^{-3}$ throughout (Figure 3-1 and 3-2). The imposition of coir drying resulted in lower CVMC values in DD plants in both experiments, and differences between these and WW control values became statistically significant ($p < 0.001$). In the 10-day DD treatment, CVMC values were significantly lower ($p < 0.001$) after Day 1 (Figure 3-1), whereas in the 7-day DD treatment, values were significantly lower ($p < 0.05$) after Day 2 (Figure 3-2). During the 10-day DD treatment, the lowest mean CVMC value for DD plants was 0.24 m^3 m^{-3}, a decrease of more than 50% (Figure 3-1), whilst the lowest mean CVMC value measured during the 7-day DD treatment was 0.33 m^3 m^{-3} (Figure 3-2). The rate of coir drying in these experiments differed, with the coir drying more quickly in the 7-day DD treatment (rate = -0.038 m^3 m^{-3} per day) compared to the 10-day DD treatment (rate = -0.029 m^3 m^{-3} per day).

Following rewetting of the coir in both DD treatments, CVMC values recovered to those in WW plants within 3 days, recovering on Day 13 for the 10-day DD experiment and Day 10 for the 7-day DD experiment. Coir volumetric moisture content values then remained similar in WW and DD treatments.

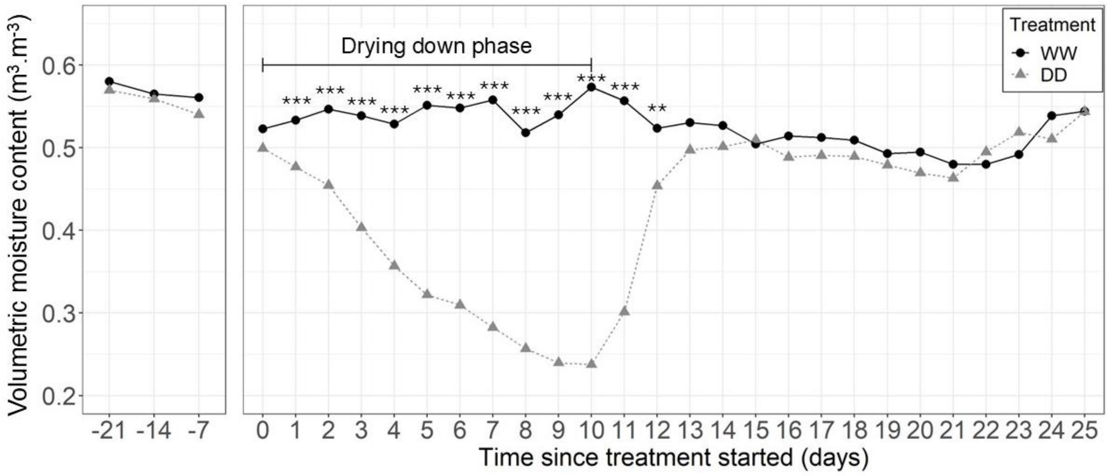

Figure 3-1. *The effects of the 10-day DD treatment on coir volumetric moisture content (CVMC) of Malling™ Bella. Each point represents a mean CVMC value during the drying down (n = 10) and recovery (n = 5) phases. The duration of the drying down treatment is shown for reference. X-axis values refer to measurements made since the onset of water deficit stress in days. Asterisks indicate statistical significance.*

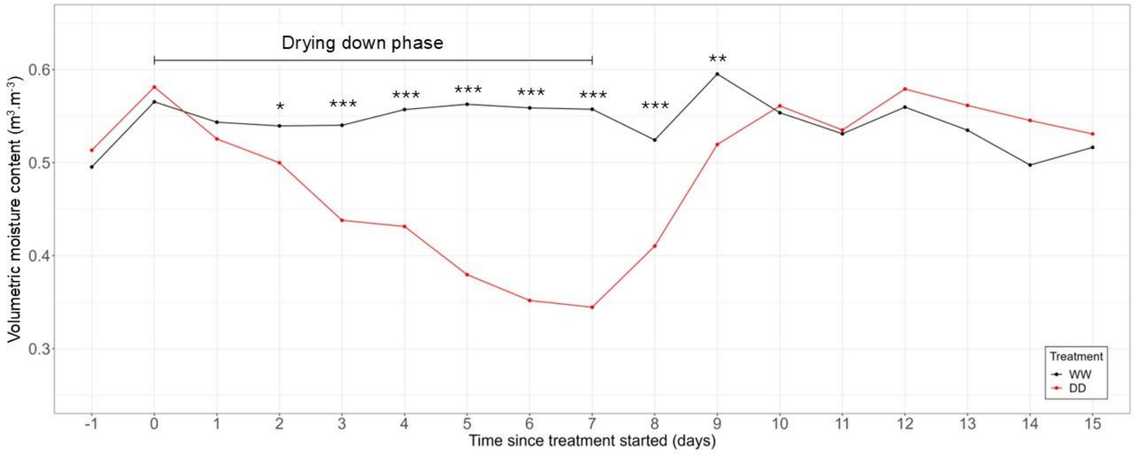

Figure 3-2. *The effects of the 7-day DD treatment on coir volumetric moisture content (CVMC) of Malling™ Bella. Each point represents an average CVMC value during the drying down (n = 12) and recovery (n = 6) phases. The duration of the drying down treatment is shown for reference. X-axis values refer to measurements made since the onset of water deficit stress in days. Asterisks indicate statistical significance.*

3.3.2 Midday stem water potential changes

Measurements of midday SWP were made at the same time each day in both experiments. On Day 0, mean midday SWP values in both experiments were below -0.5 MPa for WW and DD plants (Figures 3-3 and 3-4). The first detectable physiological response to drying coir was a statistically significant decrease in midday SWP in both experiments. In both experiments, midday SWP became more negative as the coir dried. A significant decrease in midday SWP was detected on Day 2 during the 10-day DD treatment ($p < 0.001$), while the decrease was significant on Day 3 during the 7-day DD treatment ($p < 0.001$). Under the prevailing conditions in these experiments, a CVMC value of less than 0.45 $m^3 m^{-3}$ measured at 09:00 was required to trigger a fall in midday SWP.

Once rewetting commenced after the 7-day and 10-day DD treatments, midday SWP recovered quickly. Recovery to values similar to WW values occurred within two days after the 10-day DD treatment ($p = 0.113$; Figure 3-3) and within 5 h after the 7-day DD treatment ($p = 0.604$; Figure 3-4). Measurements were made for five consecutive days after the rewetting of the coir following the 10-day DD treatment, to ensure that no further treatment differences were detected. Midday SWP measurements were then periodically conducted until the experiment was terminated.

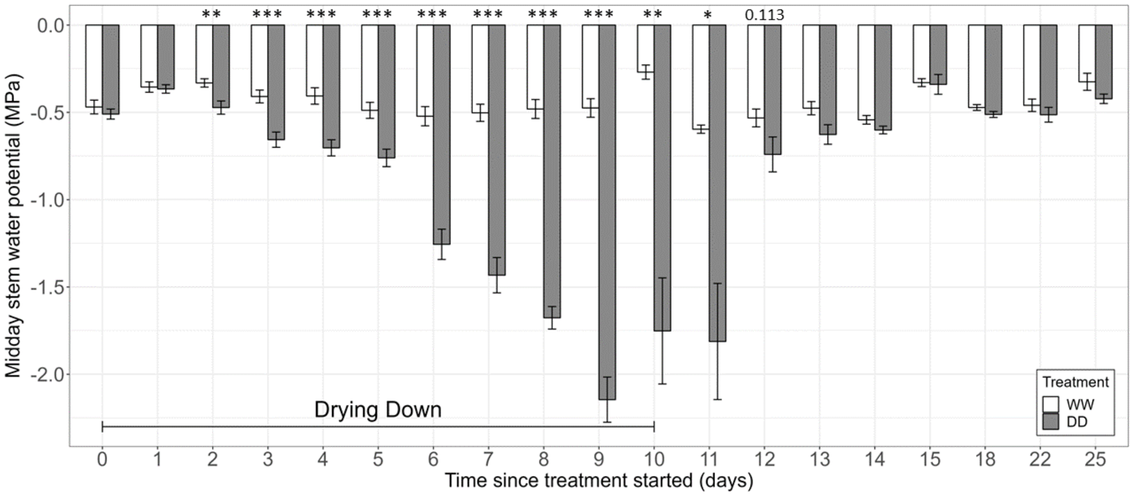

Figure 3-3. *The effects of the 10-day DD treatment on midday stem water potential. The results are an average of the values from the blocks. Error bars are ±SE, where n = 20 during the DD treatment and n = 10 during the recovery phase. The duration of the drying-down treatment is shown for reference. The asterisks indicate the significance of the difference, and the p-value indicates no difference thereafter.*

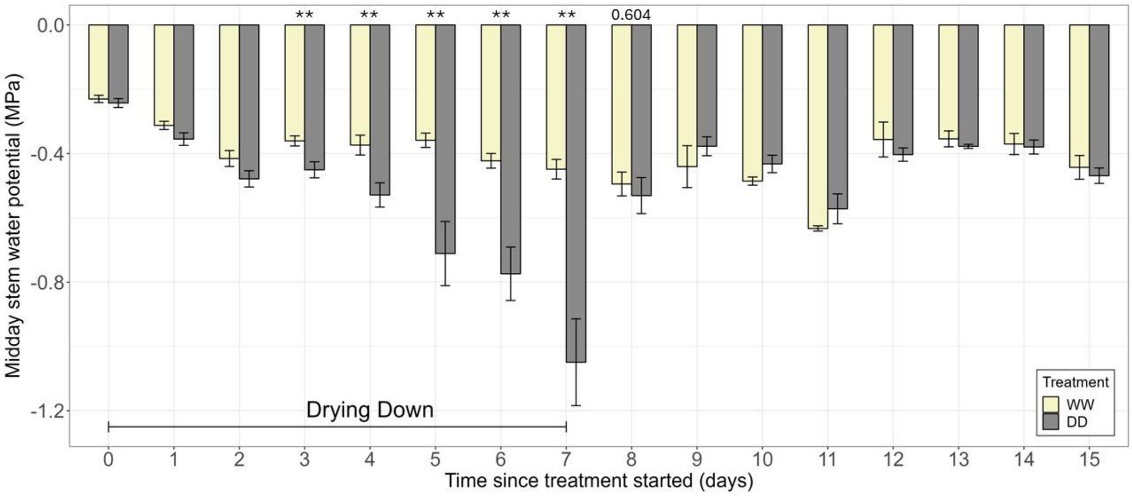

Figure 3-4. *The effects of the 7-day DD treatment on midday stem water potential. The results are an average of the values from the blocks. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on day 8, and n = 6 during the recovery phase. The duration of the drying-down treatment is shown for reference. The asterisks indicate the significance of the difference, and the p-value indicates no difference thereafter.*

3.3.3 Midday stomatal conductance and photosynthesis response to the drying down treatments

Following changes in midday SWP, further adaptive responses to coir drying were evident in midday g_s and Pn values, which decreased over time (Figures 3-5, 3-6 and 3-7). Although these experiments were carried out in a glasshouse compartment where environmental conditions were more stable, external changeable weather conditions did still influence the phytoclimate within the glasshouse compartment, and hence values of midday g_s and Pn fluctuated from day-to-day.

In both experiments, gradual drying of the coir caused g_s values to decrease over time: significant decreases ($p < 0.05$) were measured on Day 5 in the 10-day DD treatment (Figure 3-5), and Day 4 of the 7-day DD treatment (Figure 3-6). Values of g_s continued to fall until the last day of each DD-treatment. Similar changes in Pn were measured during the 7-day DD treatment, with a significant decrease ($p < 0.05$) also measured on Day 4 (Figure 3-7), and Pn values continued to fall until the end of the 7-day drying down phase.

Following coir rewetting in each experiment, g_s and Pn values recovered at different rates. Intermittent recovery of g_s values was evident following the 10-day and 7-day DD treatments. Fifteen days after rewetting following the 10-day DD treatment (Day 25), midday g_s values had recovered, even though a day before, values were significantly lower (Figure 3-5). After rewetting commenced (following the 7-day DD treatment), while g_s values measured were not significantly different between WW and DD plants apart from on Day 11 (Figure

3-6), a significant decrease in Pn values between WW and DD plants were noted until Day 12 (Figure 3-7).

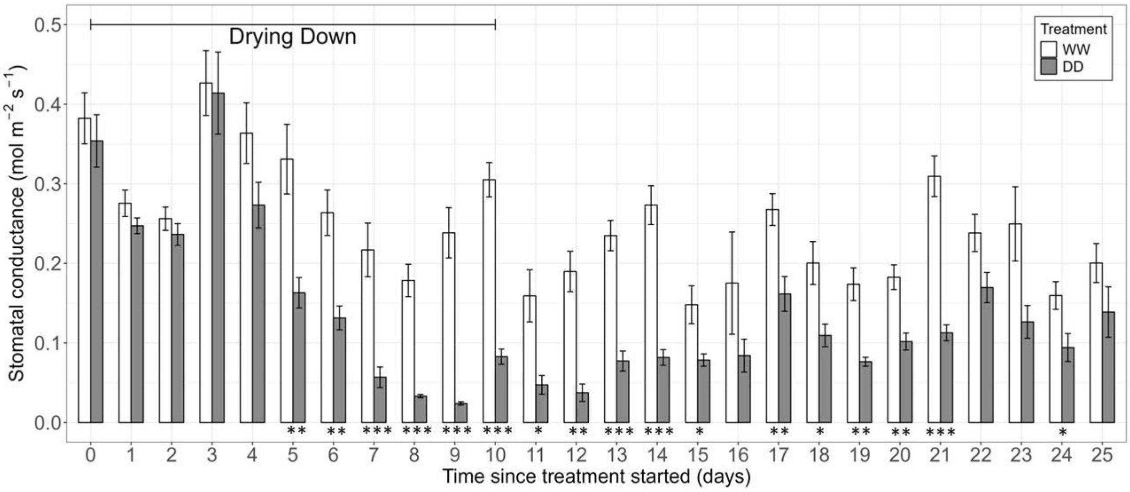

Figure 3-5. *The effects of the 10-day DD treatment on stomatal conductance measured at midday. Results are an average value from all the blocks. Error bars are ±SE, where n = 20 during DD treatment and n = 10 during the recovery phase. The horizontal line represents the duration of the DD treatment. The asterisks indicate the significance of the difference.*

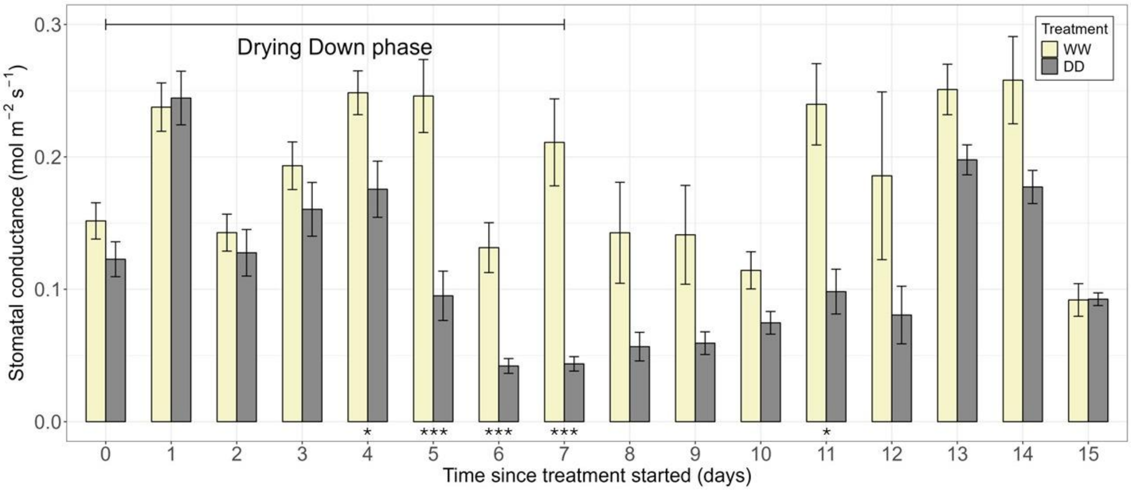

Figure 3-6. *The effects of the 7-day DD treatment on stomatal conductance at midday. Results are an average value from all the blocks. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on day 8, and n = 6 during the recovery phase. The horizontal line represents the duration of the DD treatment. The asterisks indicate the significance of the difference.*

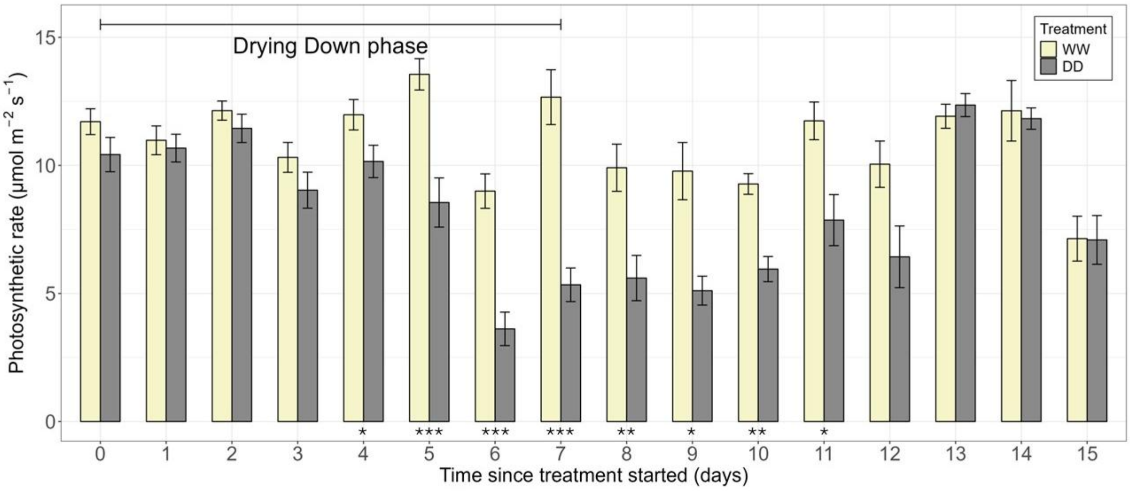

Figure 3-7. *The effects of the 7-day DD treatment on photosynthesis at midday. Results are an average value from all the blocks. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on day 8, and n = 6 during the recovery phase. The horizontal line represents the duration of the DD treatment. The asterisks indicate the significance of the difference.*

In order to analyse the relationship between g_s and CVMC and compare between experiments results were further analysed. The relationship differed between the two experiments. Where the recovery of stomatal conductance values with the increase of CVMC values for the 7-day DD treatment (Figure 3-8B) was parallel to the drying down phase. Whereas the recovery of stomatal conductance values during the 10-day DD treatment were interesting (Figure 3-8A).

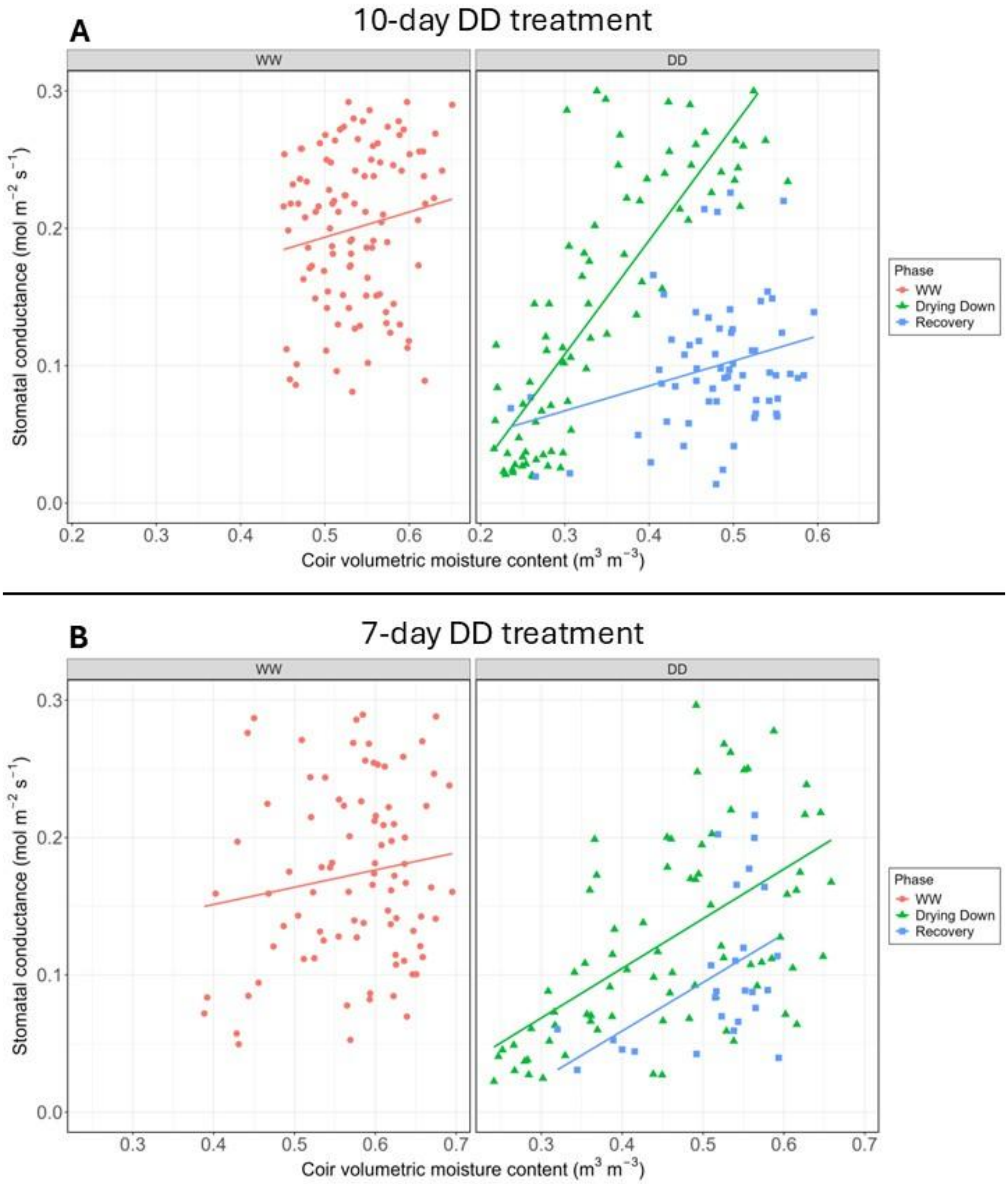

Figure 3-8. *Relationship between the g_s and CVMC of Malling™ Bella during the drying down and recovery phase. The panel are separated according to treatment and the experimental phase are highlighted in different colours.*

There was a linear relationship between stomatal conductance and photosynthetic rate (Figure 3-9). While this was also true for the DD plants during the drying down and recovery phase, the slope differed between them. During the recovery phase, as the coir rewetted, higher photosynthetic rates were achieved at the same CVMC value compared to during the drying down phase (Figure 3-9).

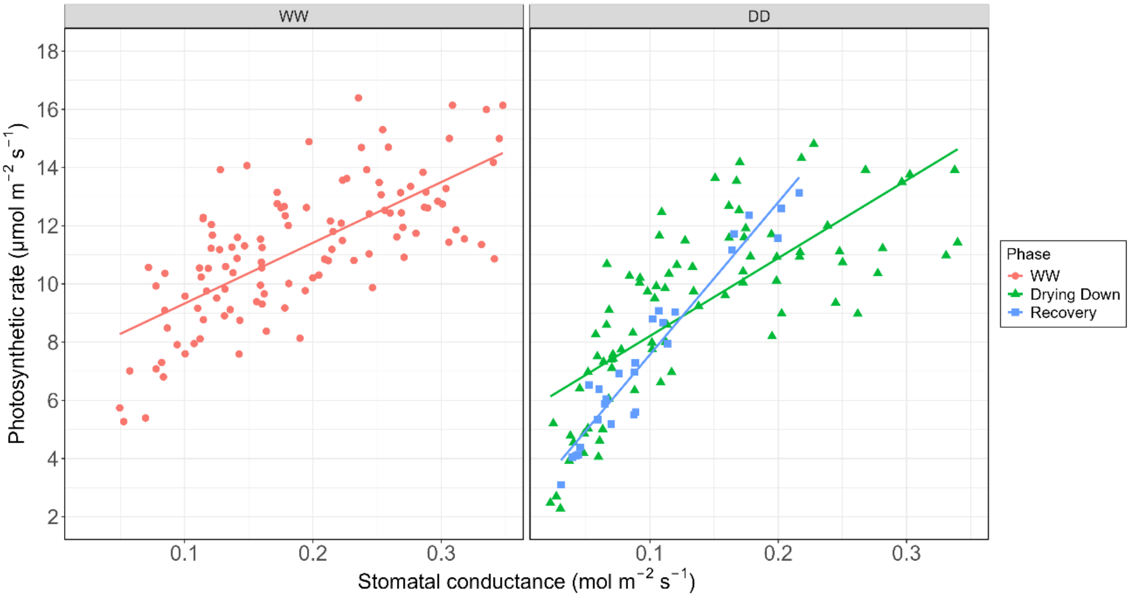

Figure 3-9. *Relationship between the g_s and Pn of Malling™ Bella during the drying down and recovery phase. The panel are separated according to treatment and the experimental phase are highlighted in different colours.*

3.3.4 Diurnal leaf gas exchanges responses to the drying down treatment

Diurnal g_s and Pn responses to the different irrigation treatments were measured to better understand the response and recovery of leaf gas exchange parameters in raspberry plants following different durations and severities of coir drying (Figures 3-10 to 3-18). On most measurement days, values of g_s and Pn peaked in the late morning before falling progressively during the afternoon to low values in the evening, creating a single peak curve. However, other diurnal patterns were evident, including a double peak with a midday depression (Figure 3-10 – Day 1) and a constant fall from a peak early morning value to lower values throughout the day (Figure 3-11 – Day 6). Several factors, including the irrigation treatments and the environmental conditions, such as light intensity, RH, VPD and air temperature in the glasshouse compartment at the time influenced the diurnal patterns of g_s and Pn. The environmental conditions in the glasshouse compartment would have also influenced irrigation events during the day, which could have also impacted the patterns of g_s and Pn.

The first measurable responses of g_s and Pn to coir drying were significant reductions in values later in the day, and as the drying-down treatments continued, significant differences in both parameters became evident earlier in the day. During the 10-day DD treatment, significant differences in g_s values between WW and DD plants at all 5-time points were first noted on Day 6 (Figure 3-11). During the 7-day DD treatment, significant differences between WW and DD plants at all 5-time points for g_s and Pn values first occurred on Day 5 (Figure 3-16). Following rewetting, the recovery of g_s in the DD treatments to values similar to WW values took longer in the 10-day DD treatment than in the 7-day DD treatment. During this recovery phase, significant differences were still measured at all five measurement times until Day 15 following the 10-day DD treatment (Figure 3-12). However, following the 7-day DD treatment, g_s values were no longer significantly different at all five measurement times on Day 8

(Figure 3-17). In the shorter 7-day DD treatment, the recovery of Pn values were different to that of g_s values. After coir rewetting, partial recovery of both parameters were noted at different times during the day (Figure 3-17), but by Day 13, Pn and g_s values at all five measurement times had recovered to pre-stress values with no significant differences between WW and DD plants (Figure 3-18).

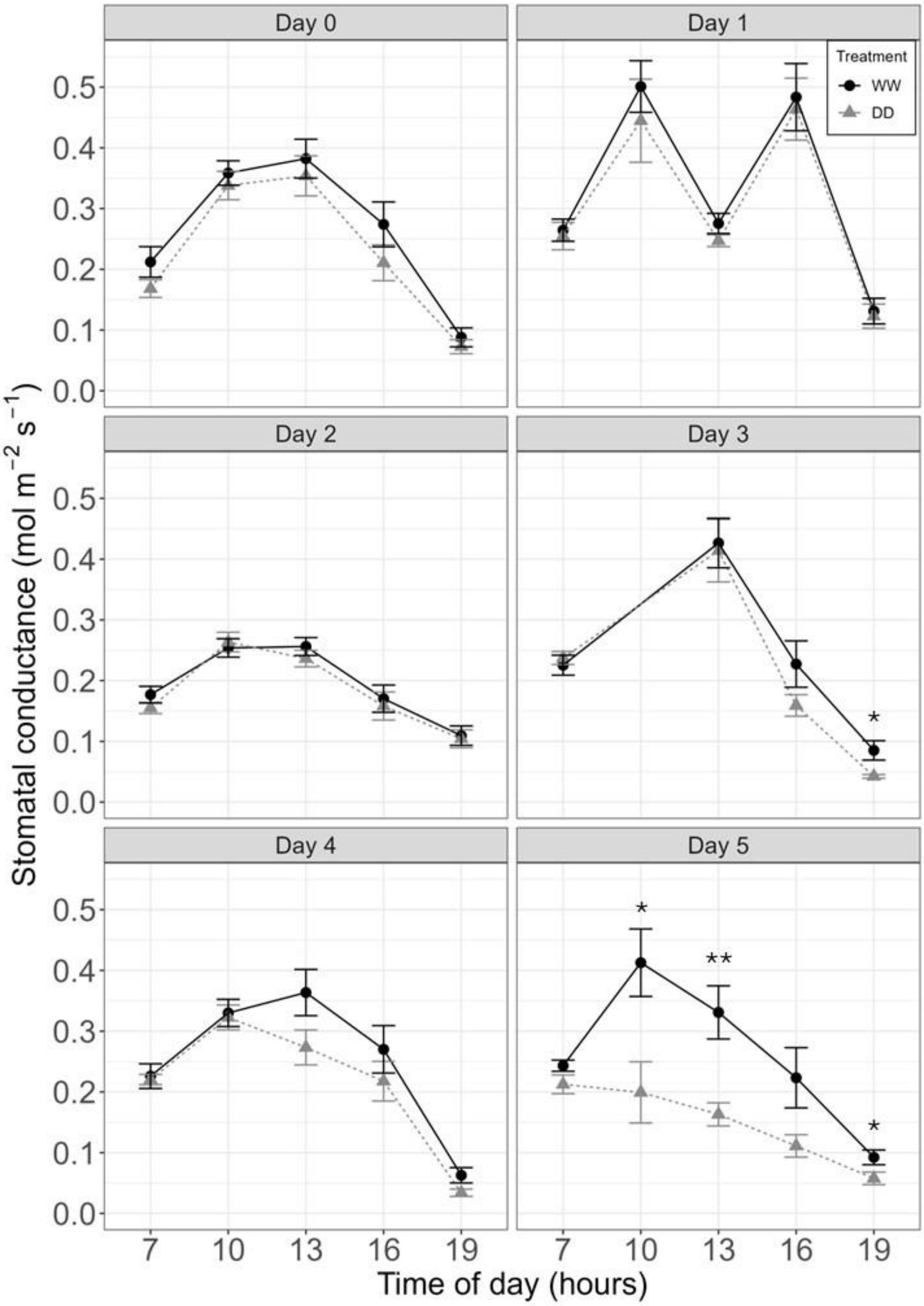

Figure 3-10. *The effects of the 10-day DD treatment on diurnal stomatal conductance between Days 0-5. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 20 during DD treatment and n = 10 during the recovery phase. Each diurnal graph represents a single day.*

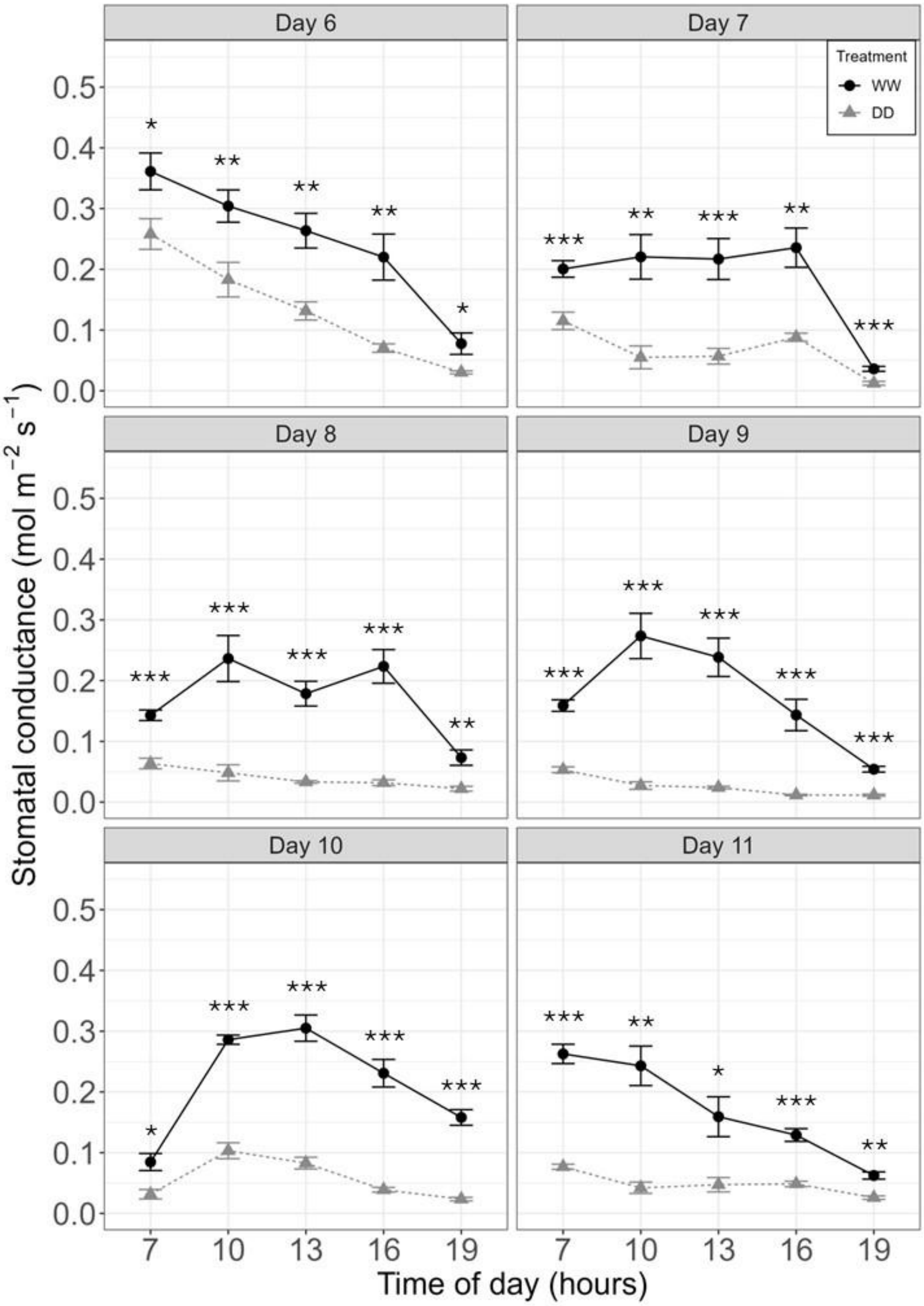

Figure 3-11. *The effects of the 10-day DD treatment on diurnal stomatal conductance between Days 6-11. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 20 during DD treatment and n = 10 during the recovery phase. Each diurnal graph represents a single day.*

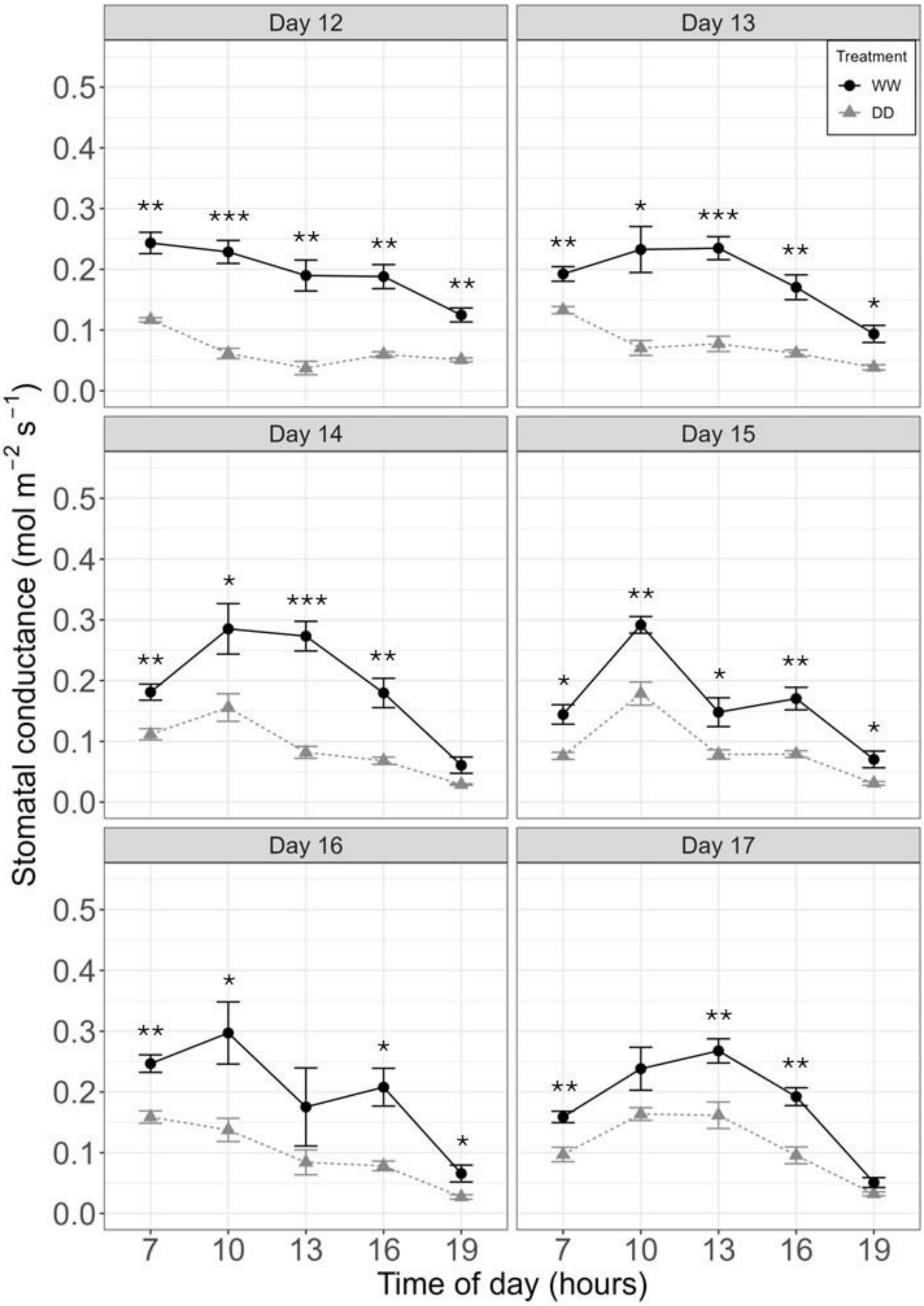

Figure 3-12. *The effects of the 10-day DD treatment on diurnal stomatal conductance between Days 12-17. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 20 during DD treatment and n = 10 during the recovery phase. Each diurnal graph represents a single day.*

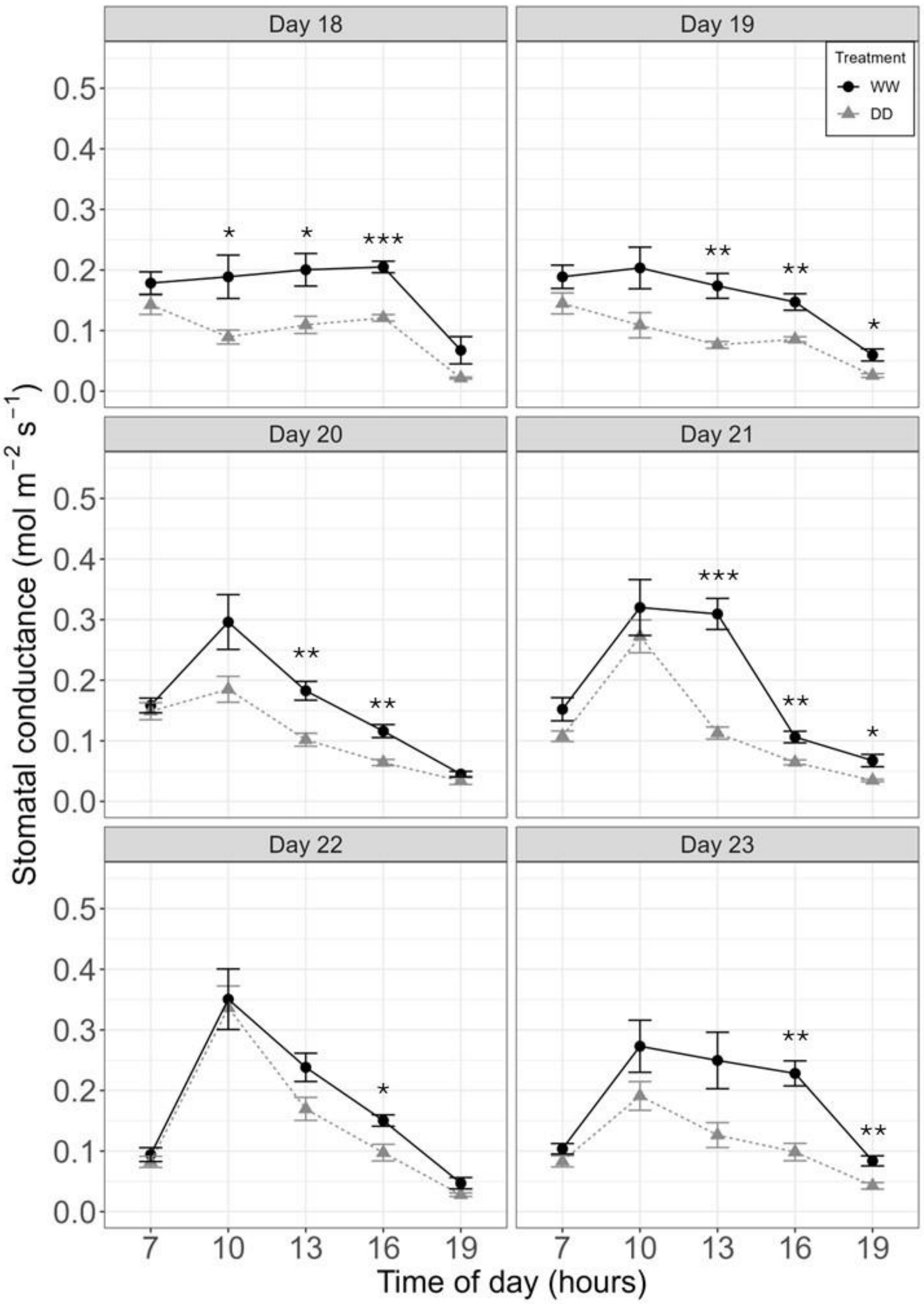

Figure 3-13. *The effects of the 10-day DD treatment on diurnal stomatal conductance between Days 18-23. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 20 during DD treatment and n = 10 during the recovery phase. Each diurnal graph represents a single day.*

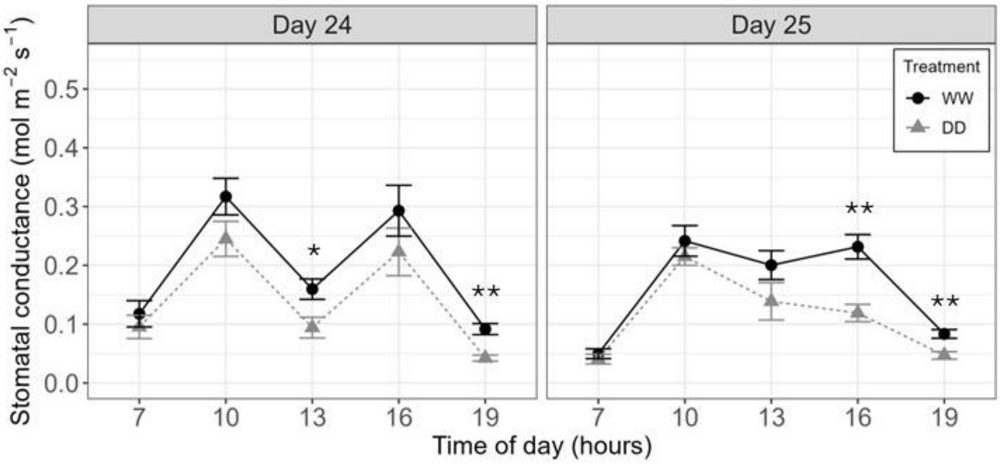

Figure 3-14. *The effects of the 10-day DD treatment on diurnal stomatal conductance between Days 24-25. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 20 during DD treatment and n = 10 during the recovery phase. Each diurnal graph represents a single day.*

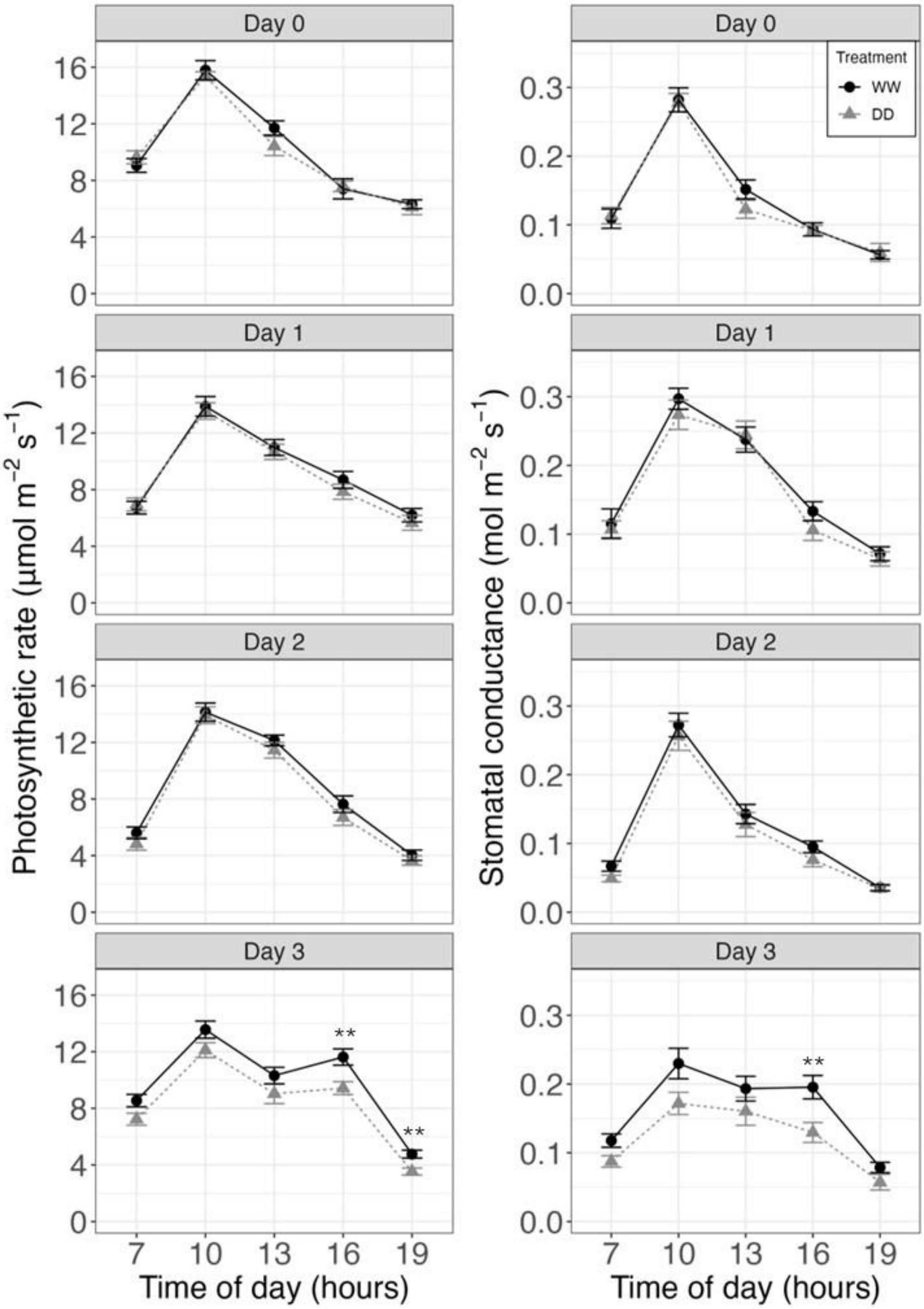

Figure 3-15. *The effects of the 7-day DD treatment on diurnal stomatal conductance and photosynthesis between Days 0-3. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on Day 8, and n = 6 during the recovery phase. Each diurnal graph represents a single day.*

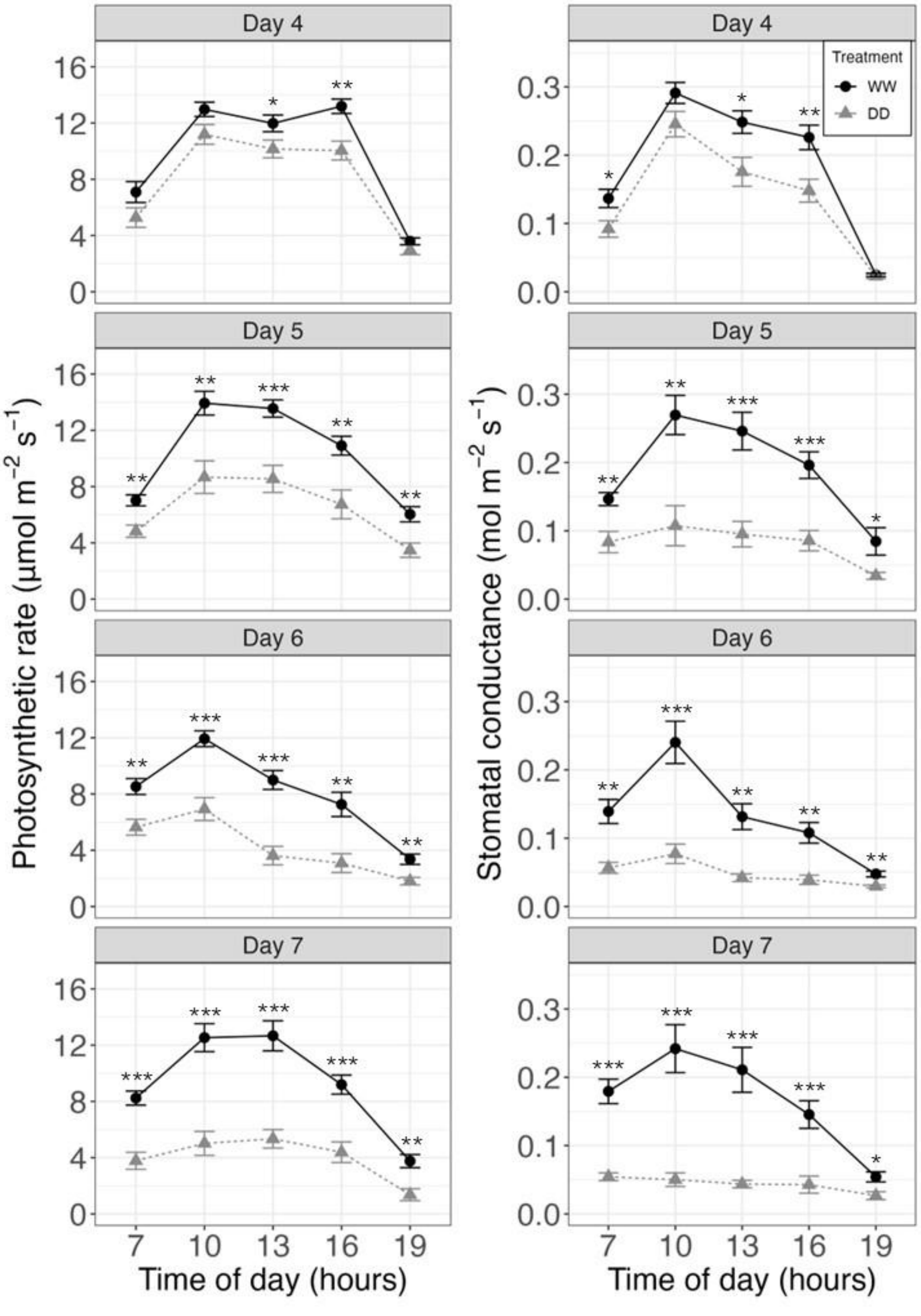

Figure 3-16. *The effects of the 7-day DD treatment on diurnal stomatal conductance and photosynthesis between Days 4-7. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on Day 8, and n = 6 during the recovery phase. Each diurnal graph represents a single day.*

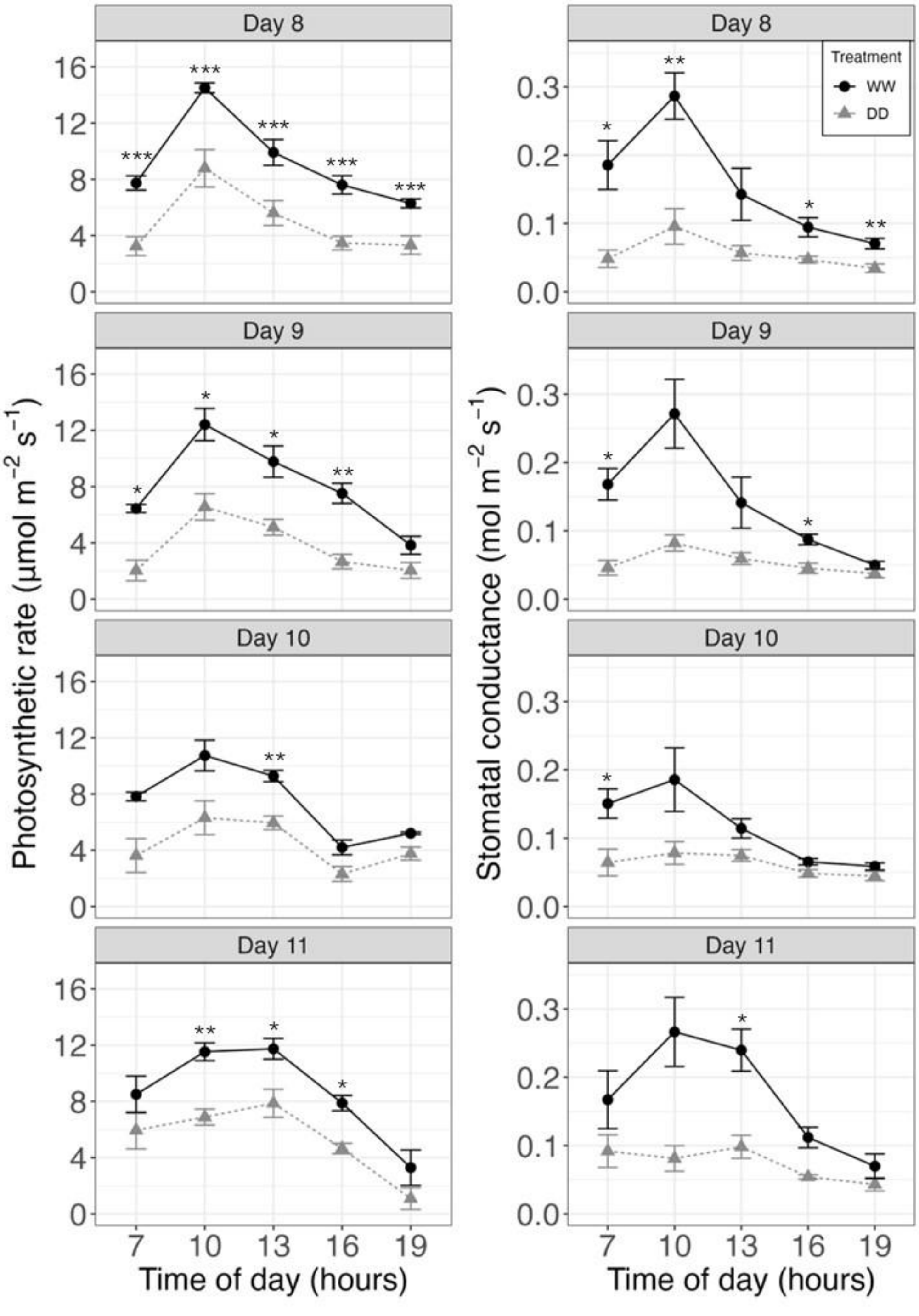

Figure 3-17. *The effects of the 7-day DD treatment on diurnal stomatal conductance and photosynthesis between Days 8-11. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on Day 8, and n = 6 during the recovery phase. Each diurnal graph represents a single day.*

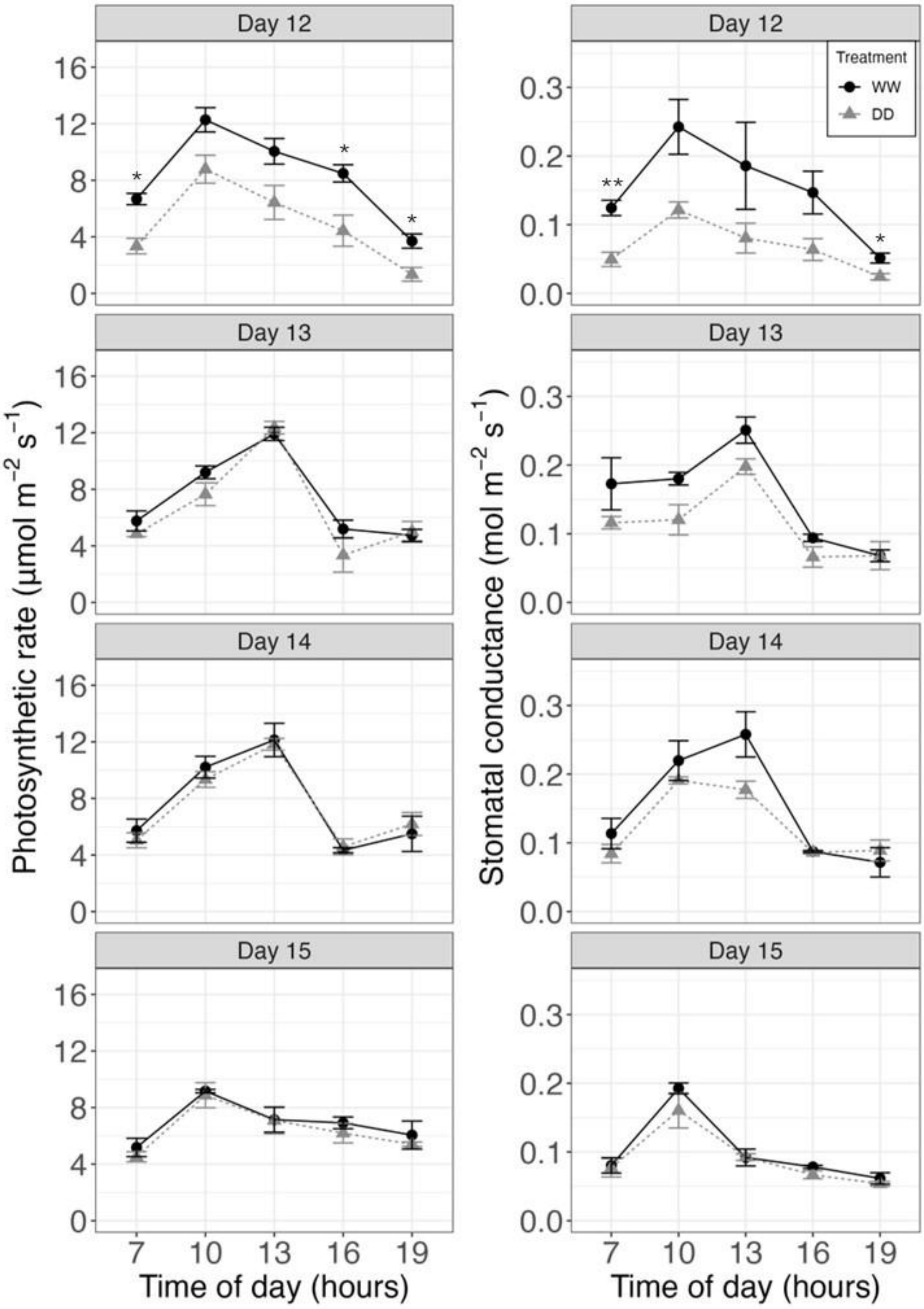

Figure 3-18. *The effects of the 7-day DD treatment on diurnal stomatal conductance and photosynthesis between Days 12-15. Results are mean values from all experimental blocks. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on Day 8, and n = 6 during the recovery phase. Each diurnal graph represents a single day.*

3.3.5 Leaf gas exchange measurements during the 10-day drying down experiment

As equipment availability was limited during the 10-day DD experiment, an infrared gas analyser was used only on some days to check for changes in Pn values. Measurements of Pn and g_s on Day 9, the penultimate day of the drying down phase, were significantly different between WW and DD plants (Figure 3-19). Measurements made at midday on Day 25 indicated that significant differences were no longer evident in g_s and Pn values (p = 0.161 and 0.350, respectively).

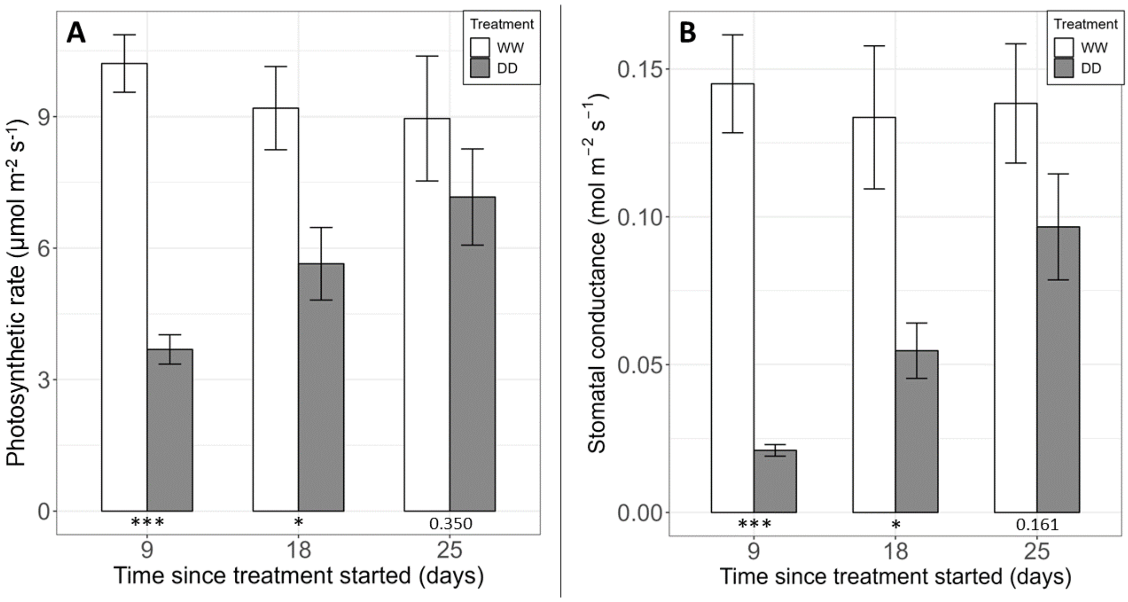

Figure 3-19. *A) Photosynthetic rate and B) stomatal conductance of Malling™ Bella on Day 9 during the drying down phase and Days 18 and 25 during the recovery phase. The results are an average of the values from all the blocks. Error bars are ±SE, where n = 20 during DD treatment and n = 10 during the recovery phase. The asterisks indicate the significance of the difference, and the p-values indicate no difference.*

3.3.6 Changes in leaf temperature during the 7-day drying down treatment

In the 7-day DD treatment, mean leaf temperature in DD plants was higher compared to WW values on Days 6, 8 and 9, but the differences were not statistically significant (*p* > 0.05; Figure 3-20).

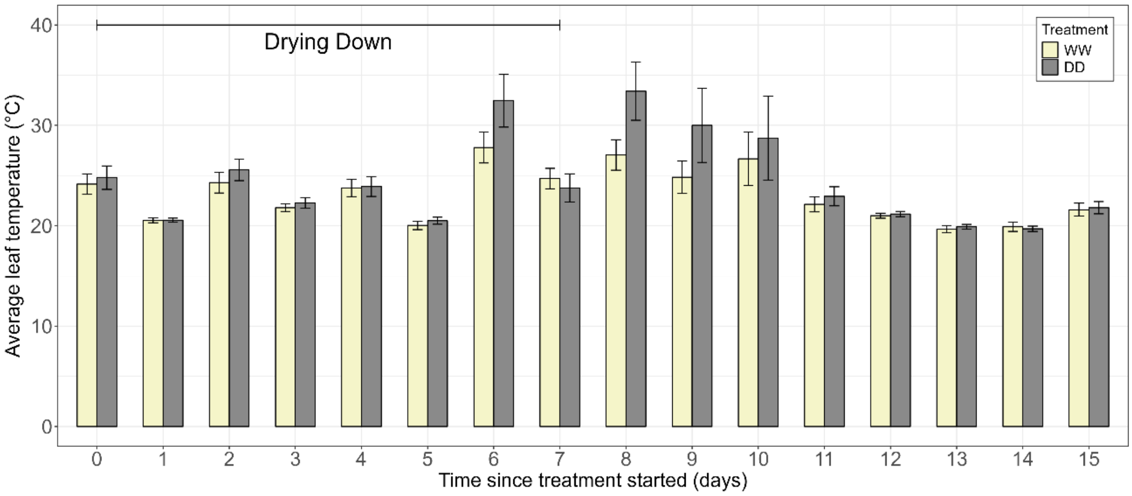

Figure 3-20. *The effects of the 7-day DD treatment on leaf temperature. Results are mean values from all experimental the blocks. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on Day 8, and n = 6 during the recovery phase. The horizontal line represents the duration of the DD treatment.*

3.3.7 Coir water deficit stress effects on berry yield and quality

In the 10-day DD experiment, ripe fruit from primocane Malling™ Bella was first harvested on 05 August 2022, and Class 1 yield and berry quality attributes were measured until 09 September 2022, although cropping continued for another two weeks. Over this time, the mean cumulative Class 1 yield for WW plants was 1.86 kg/pot compared to 1.22 kg/pot for the 10-day DD plants; this drying down effect on Class 1 yield was statistically significant (p < 0.05). Total Class 1 berry numbers per pot were significantly lower in DD than WW plants, with 291 and 385 berries per pot, respectively.

A significantly lower (p < 0.05) Class 1 yield was harvested from DD-treated plants compared to WW plants on 19 August 2022, in only the second pick after coir drying was imposed (Figure 3-21); the mean CVMC value in DD plants at this time was 0.36 $m^3 m^{-3}$ (Figure 3-1). Class 1 yield remained significantly lower from DD plants until 30 August 2022, but there was no significant difference at harvest on 02 September 2022.

A significant decrease in mean individual berry fresh weight was first measured in DD plants (Figure 3-22) on the final harvest day of the DD phase. On 26 August 2022, the day coir rewetting commenced; berries from DD plants (2.34 g) were less than half the mean fresh weight of those from WW plants (5.28 g), but mean berry fresh weight in DD plants had recovered by 02 September 2022, 8 days after rewetting (Figure 3-22).

The effects of the 10-day DD treatment on berry SSC (%BRIX) were also measured between 05 August and 09 September 2022. Soluble solids content values of berries harvested from DD plants were significantly higher (p < 0.01) on 23 August 2022 (Figure 3-23). Significant differences in SSC values between WW and DD plants were evident until 02 September 2022, apart from measurements carried out on 26 August 2022 when differences were just outside of significance ($p = 0.053$).

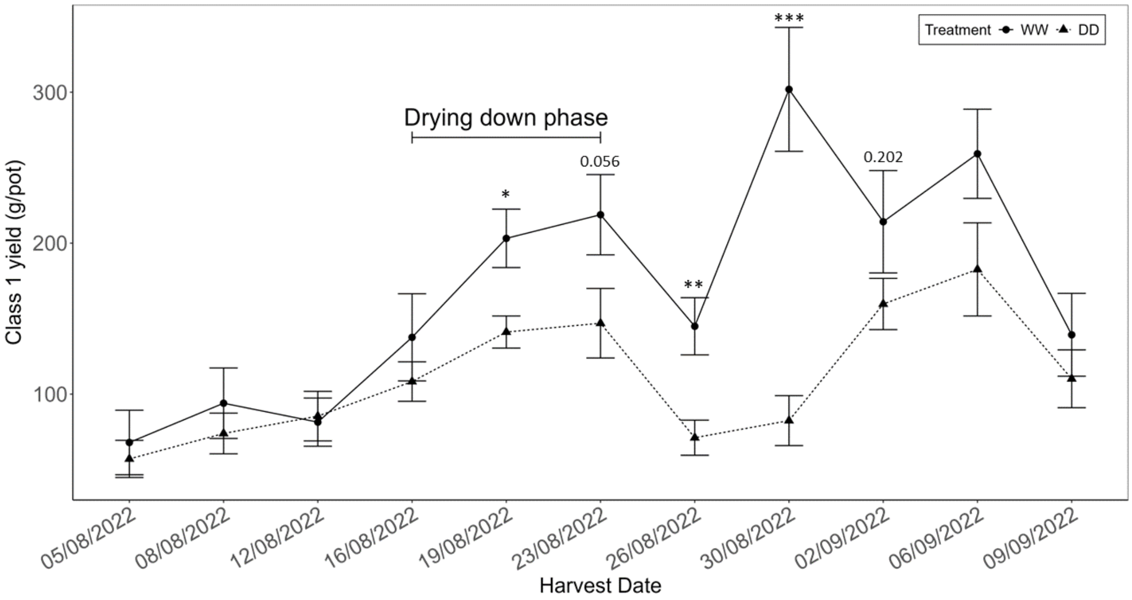

Figure 3-21. *The effects of the 10-day DD treatment on the average Class 1 yield harvested per pot from Malling™ Bella on each pick from 05 August to 09 September 2022. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on day 8, and n = 6 during the recovery phase. The horizontal line represents the duration of the DD treatment. The asterisks indicate the significance of the difference, and the p-values indicate no difference.*

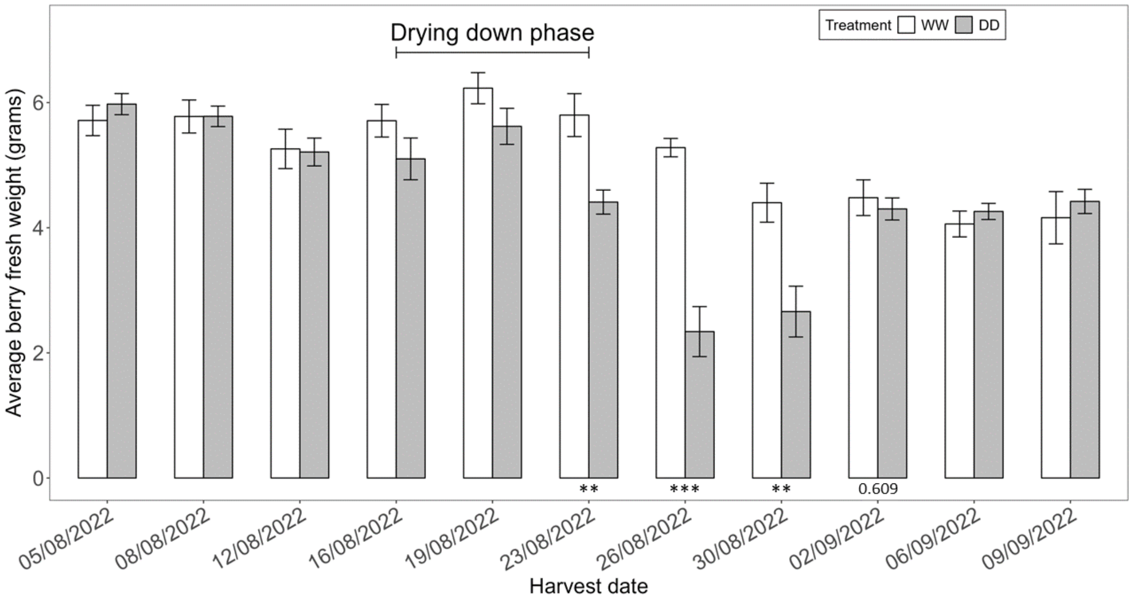

Figure 3-22. *The effects of the 10-day DD treatment on mean berry fresh weight of Malling™ Bella from 05 August to 09 September 2022. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on day 8, and n = 6 during the recovery phase. The horizontal line represents the duration of the DD treatment. The asterisks indicate the significance of the difference, and the p-values indicate no difference.*

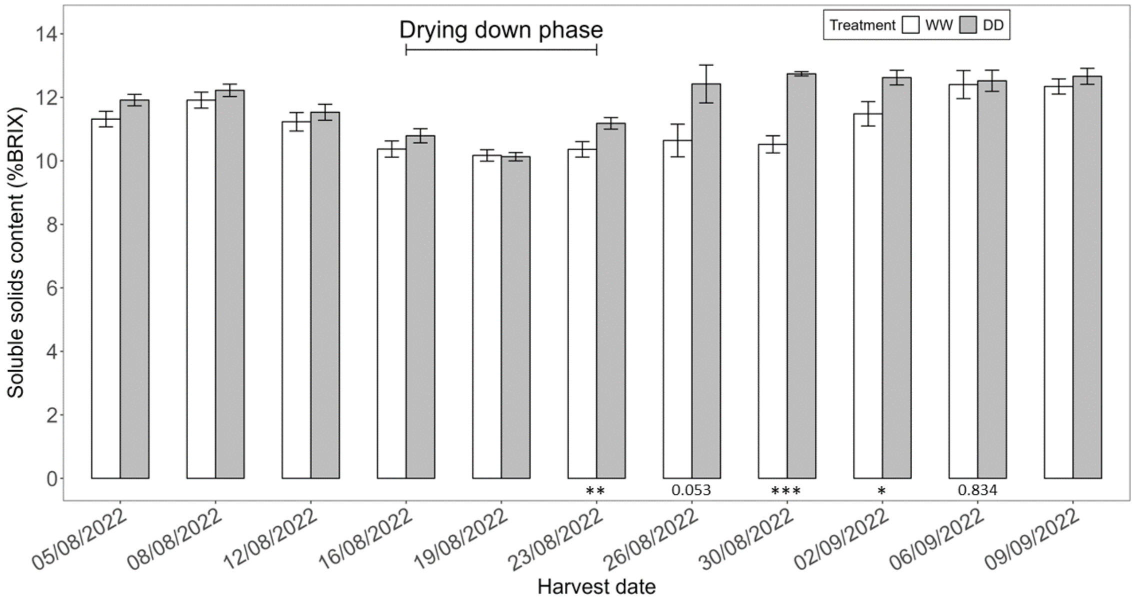

Figure 3-23. *The effects of a 10-day DD treatment on the berry soluble solids (%BRIX) of five randomly selected fruit of Malling™ Bella between 05 August to 09 September 2022. Error bars are ±SE, where n = 24 during DD treatment, n = 12 on Day 8, and n = 6 during the recovery phase. The horizontal line represents the duration of the DD treatment. The asterisks indicate the significance of the difference, and the p-value indicates no difference.*

3.4 Discussion

The experiments in this chapter were designed to better understand the legacy effects on photosynthesis, stomatal conductance and ultimately yield parameters of a temporary rootzone water deficit stress in Malling™ Bella. To help achieve this aim, experiments were carried out in a glasshouse compartment where environmental variables could be controlled more readily than in a polytunnel. A sensor-based automated precision irrigation system that enabled independent irrigation in each treatment was used in these experiments to ensure that CVMC values could be maintained with a narrow pre-determined range in WW plants and to better control the rate of change in CVMC of plants under the coir drying treatments. This flexibility was necessary to detect the first measurable physiological responses to the imposed coir water deficits.

3.4.1 Different durations and the rate of coir drying affect the response and recovery of leaf physiological parameters

Following legacy responses on g_s and Pn measured after a 10-day DD treatment described in Chapter 2, the experiment was repeated here in a more controlled environment and with more detailed measurements. However, when differences in g_s and Pn were measured before the end of the 10-day DD treatment, the experiment was then repeated on a new set of plants with a shorter drying-down duration. As measurements were carried out on consecutive days, it was easier to identify the response and recovery times. Along with a different drying duration, the other difference was that the 10-day DD treatment was carried out during the fruiting stage, whereas the 7-day DD treatment occurred during the vegetative stage.

The use of PI control ensured that the CVMC of WW plants was maintained within a relatively narrow range and mostly above 0.5 $m^3\ m^{-3}$ throughout the experiment. Avoiding large swings in CVMC in control plants is important when trying to detect the early effects of deficit irrigation treatments on plant morphology and physiology to ensure that comparisons between treatments can be made. For example, research carried out on *Cymbidium* found that plants grown at 0.25 $m^3\ m^{-3}$ compared to higher volumetric water contents had significantly smaller leaves, biomass and lower photosynthesis rates (An *et al*., 2020). An *et al*. (2020) used a sensor-based automatic irrigation system to achieve consistent volumetric water contents and reported that the system could be successfully adapted to support the growth of young *Cymbidium*. Here, the sensor-based automatic irrigation system enabled independent control of CVMC in WW and DD plants, which, in turn, enabled the legacy effects of transient coir drying on leaf gas exchange to be investigated with the required precision.

However, differences in the drying rate between the two experiments were evident, even though PI was used in both experiments. The rate of coir drying depends on the rate of evapotranspiration, which, in turn, can be influenced by many factors, including VPD, atmospheric aridity, high temperatures, leaf area, light intensity, and stomatal density (Qing *et al*., 2023). During the 10-day experiment, higher mean values of VPD (2.8 kPa), air temperature (22.5°C), PAR (324 μmol m^{-2} s^{-1}) and relative humidity (65%) were recorded over the drying down phase compared to the drying down phase of the 7-day DD treatment (2.6 kPa, 19.2°C and 44%, respectively – data not shown), apart from PAR (with a mean of 510 μmol m^{-2} s^{-1} during the DD phase). The differences in the drying rate and absolute mean CVMC values at the start of each day could explain the difference in the relative timing of the leaf physiological responses discussed below.

As irrigation was supplied in the same way following each drying down treatment, rewetting of the coir occurred at the same rate in each experiment. Coir has a high rewetting capacity (Wever *et al*., 1997), and so CVMC recovered to pre-stress values within three days of rewetting in both experiments despite the different durations of coir drying. This is likely due to limited hysteresis of as CVMC increased at similar rates to coir matric potential. Midday SWP values of WW plants remained consistent with minimal fluctuations from day to day; this was important in ensuring that comparisons could be made between the WW plants and the DD plants' response to the rootzone water deficit stress. Midday SWP is a sensitive and reliable indicator of water stress and changes as small as 0.05 MPa can be detected (McCutchan & Shackel, 1992). The mean midday SWP values for WW plants in each experiment were not different, although, during the 10-day DD treatment, values were slightly more negative when the plant was at the cropping stage. Measurements carried out on peach trees also found that there are seasonal patterns of midday SWP, with values of control plants becoming more negative as the year progresses (Marsal *et al*., 2015), likely due to plants use of water increases and the soil is much less wetter than at the beginning of the year. The minimal variability of the control WW plants in each experiment allowed for comparisons between WW and DD plants on the effect of drying.

As midday SWP measurements were carried out on consecutive days, the timings of the response to the drying-down treatment and during the recovery phase following the rewetting of the coir could be detected more accurately. With differences in midday SWP evident a day before in the 10-day DD treatment, the absolute CVMC may have been a factor for the earlier response time compared to the 7-day DD treatment. Therefore, causal signals that regulate water potential are likely to be triggered after a drop in a specific CVMC threshold. When water availability decreases, stomata close and hydraulic conductance is adjusted to reduce water loss and maintain cell turgor which can adjust water potential gradients within the plant (Scharwies & Dinneny, 2019). Stomatal closure can ensure that water loss is minimised, preserving a more favourable water balance during drought stress (Tombesi *et al*., 2015). Once the coir was rewetted, the quick recovery of midday SWP for both experiments suggests that the water potential was quickly restored. Research carried out on five common European trees showed that even after experiencing severe water stress, midday leaf water potential recovered quickly, and it was suggested that the quick refilling of the embolised conduits in the xylem was responsible for restoring water potential (Li *et al*., 2015).

The response and recovery of leaf gas exchange parameters to coir drying were also measured. The WW plants in the 10-day DD treatment measured higher g_s values compared to the WW plants in the 7-day DD treatment. Measurements of g_s values of the WW plants during the 7-day DD treatment correlate more closely with values in the literature (Morales *et al*., 2013). Environmental conditions varied from day-to-day, with higher PAR values during the 7-day DD treatment, despite lower readings. This could have resulted because of the use of two different systems, a porometer and an infrared gas analyser, for the 10-day and 7-day DD treatment, respectively. Along with different growth stages when measurements were carried out, the use of two systems from different manufacturers may have been the reason for the differences in g_s values between the experiments. Keel *et al*. (2007) reported that measurements made with a LI-6400 (steady-state photosynthesis system) measured lower leaf conductances compared to the AP4 porometer. It was further stated that as the data generated with the AP4 porometer is reliant on the calibration process with pore plates and does not ventilate leaves when taking measurements, data collected with the LI-6400 are more trustworthy as it is based on mass flow and gas concentrations (Keel *et al*., 2006).

The response time to the drying of the coir in g_s values occurred at similar times, with differences being measured on Day 4 (7-day DD treatment) and Day 5 (10-day DD treatment). It is well-known that chemical signals, such as ABA can control stomatal closure in response to a drought stress (Liu *et al*., 2022). When the coir begins to dry, ABA is synthesised in the roots and then transported to the leaves via the xylem sap (Davies & Zhang, 1991). Research has also shown that ABA can be synthesised in the leaves, which can be loaded to the phloem to be transported to the roots (Hartung *et al*., 2002). However, ultimately, the accumulation of foliar ABA can limit gas exchange (Tombesi *et al*., 2015), as the accumulation of ABA in the leaf around the guard cells promotes stomatal closure by regulating downstream signalling components (Liu *et al*., 2022). Water deficit stress can generate hydraulic signals that reduce turgor, subsequently causing an increase in solute concentration because water is withdrawn from cells (Christmann *et al*., 2013). Hydraulic signals can control stomatal behaviour, and can promote stomatal sensitivity to root-derived chemical signals (Jia & Zhang, 2008). Differences in stomatal response times between the two experiments may have been due to a hysteresis in CVMC and the matric potential during the 10-day treatment. As water content increased, the coir matric potential may have remained lower due to hysteresis, which would have affected the water availability to the plant (Carminati & Javaux, 2020), and therefore prolong stomatal opening in the 10-day DD treatment compared to the 7-day DD treatment.

The closing stomata prevents excessive water loss through transpiration and can aid in the control of leaf temperature (Reynolds-Henne *et al*., 2010). In this experiment, increases in leaf temperature measured in the DD plants occurred when g_s values were low. However, these values weren't significant, indicating that either partial stomatal closure was able to control leaf temperature increases, or the fact that the thermal camera was not as sensitive to pick up the slight differences that occurred. In future experiments more sensitive equipment that measures leaf temperature outdoors should be used to pick up subtle differences.

Following the rewetting of the coir, the response of g_s differed depending on the duration of the drying treatment. Longer durations of drought can result in embolism and a slower recovery (Brodribb *et al*., 2010), which could explain the slow and intermittent recovery of g_s values measured in the 10-day DD treatment. Also, the accumulation of foliar ABA during the drying-down phase could have subsequently reduced stomatal opening after rewetting as foliar ABA can prevent recovery of stomatal aperture upon rewetting, as embolism repair is favoured with long-term downregulation of transpiration (Tombesi *et al*., 2015). Stomatal conductance values following the 7-day DD treatment recovered quicker than the

10-day DD treatment, suggesting quicker stomatal opening after a shorter water deficit stress.

The similar response time of g_s and Pn during the 7-day DD treatment supports the notion that that decreasing relative water content causes g_s and Pn to decrease, approximately in parallel (Lawlor, 2002). When plants are subjected to a drought stress, this causes the production of ROS which can cause extensive cellular damage and impair photosystems by affecting photosystem II and the electron transport chain (Qiao *et al*., 2024). Metabolic limitations can reduce the rate of net photosynthesis (Lawlor, 2002), as RuBP and ATP decrease early on the onset of a drought, even though high g_s values were measured, suggesting RuBP regeneration and ATP synthesis are impaired (Flexas & Medrano, 2002). Stomatal limitations are also known to affect photosynthesis, short-term restrictions in net Pn can be caused by stomatal limitation, reducing CO_2 availability (Pena-Rojas *et al*., 2004). Following rewetting of the coir, reduced Pn values in the 7-day DD treatment (Figure 3-9) and the 10-day DD treatment (Figure 3-19) suggests that either metabolic or stomatal limitations or both were still the cause of reduced Pn values.

3.4.2 Diurnal stomatal conductance and photosynthetic rate

Since there are no published data on legacy effects on photosynthesis following a rootzone water deficit stress in raspberry canes, extensive diurnal measurements were carried out to further understand the timing of the responses.

Diurnal changes in g_s have been studied in other species, including apple (Lebese *et al*., 2011), rice (Shimono *et al*., 2010) and wheat (Zhang *et al*., 2019), with higher values of g_s measured in the morning and noon than in the afternoon, as was the case here (Figure 3-10 to 3-18).

When light is absorbed by chlorophyll, the energy is used to drive electrons from water to generate NADPH and drive protons across the membrane, which return through ATP synthase to make ATP, starting the light-dependent stage of photosynthesis (Berg *et al*., 2015). As the sun rises, light is no longer a limiting factor and can drive photosynthesis. Stomata open in response to light (Outlaw, 2003), hence the increase in stomatal conductance values as light increases in the morning and peaked around late morning/midday. In well-watered Pedunculate Oak seedlings, Bojović *et al*. (2017) reported an increase in g_s and Pn values in the morning to peaks around noon, and then a progressive decline towards the evening.

Stomatal closure limits water loss (Zhang & Davies, 1989) and when a rootzone water deficit was imposed here, diurnal changes in g_s were first evident as differences in measurements carried out in the evening. Following a thirty-day drought stress on Oak seedlings, measurements of g_s values were constantly low throughout the day, however, Pn values increased slightly at 06:00 before progressively falling before midday and remaining low (Bojović *et al*., 2017), with similar trends on Day 6 and 7 during the 7-day DD experiment (Figure 3-16), despite a much shorter drying duration.

Consecutive measurements of diurnal changes in g_s made over 26 days during and after the 10-day DD treatment indicated that g_s values in raspberry plants vary daily in response to environmental cues. While the development stage and substrate water conditions may affect the magnitude of changes in g_s, these factors do not affect g_s patterns (Zhang *et al*., 2019), and so environmental conditions in the glasshouse compartment were analysed to understand why different diurnal g_s response curves were noted (Table A-1).

In the 10-day DD experiment, WW plants exhibited different diurnal patterns on some days; this variability was absent in DD plants since coir drying caused g_s to decline gradually throughout the day. The single-peak curve is the main reported curve type in most plant species (Shimono *et al*., 2010) however, researchers are increasingly reporting the occurrence of non-single-peak curves in diurnal stomatal conductance patterns (Foster *et al*., 2013). Research that studied the effects of humidity and genotype on g_s diurnal changes found these to be important factors in determining g_s diurnal patterns (Zhang *et al*., 2019), therefore the mean RH values were calculated for this 10-day DD experiment, where the most variation in diurnal patterns were detected. When RH was lower compared to other days, the diurnal pattern was generally a constant drop throughout the day, and the single peak curves were formed on days with RH at 28% (Zhang *et al*., 2019). Similar findings were evident here (Table A-1), with single peak curves formed when RH was above 65%, but a gradual decline in g_s values were measured when RH fell below 60%. The midday depression in g_s values (seen as a double-peak diurnal pattern) may have resulted from high irradiance, as high light also affects the diurnal pattern; therefore, it is essential to evaluate the effects of changing diurnal light intensity separate from other factors when midday depression is visible (Koyama & Takemoto, 2014). Excess light, together with high leaf temperature, can also cause midday depression of photosynthesis (Valladares & Pearcy, 1997). In this experiment, the double peak g_s diurnal pattern was formed on days with high midday PAR values of 1200, 1350, and 1300 µmol m^{-2} s^{-1}, which is nearly double the light saturation point of 700 µmol m^{-2} s^{-1} of Malling™ Bella (Figure-A3).

3.4.3 Coir drying effects on berry yield and quality

Ripe fruit was harvested for the first five weeks with the aim of quantifying the effects of the coir drying treatment on Class 1 yield and berry quality attributes. Yields in the WW plants harvested in this experiment were similar to those of other experiments at NIAB East Malling and to those from other raspberry cultivars (Morales *et al*., 2013).

Reductions in fruit growth are a common response to drought stress (Ebel *et al*., 1993), as when a plant is subjected to a water deficit stress, plant growth is reduced due to inhibition of cell expansion (Hsiao *et al*., 1976). The 10-day DD treatment resulted in significant differences in total Class 1 yield, mean individual berry fresh weight, and SSC values. Class 1 berry yield per plant was reduced by 30% in DD plants compared to WW plants; this was due to a combination of effects on individual berry fresh weight and on the number of berries in the Class 1 category. Not only were lower yields recorded during the drying-down phase, but legacy effects were also evident a week after rewetting. Drought stress of varying severity and duration caused similar reductions in yield in other raspberry cultivars (Morales *et al*., 2013; Ortega-Farias *et al*., 2022), because of fewer fruit in those that experienced drought stress (Ortega-Farias *et al*., 2022). The mean berry fresh weight of Malling™ Bella was similar to other raspberry cultivars (Stephens *et al*., 2012; Darnell *et al*., 2008). Decreasing water availability in the coir increased the %BRIX in raspberry, likely due to the less dilution of sugars with less water available. Research on other fruiting plants also found that drought conditions can cause increases in %BRIX in pomegranates (Martínez-Nicolás *et al*., 2019), almonds (Lipan *et al*., 2019), olives (Goncalves *et al*., 2020) and some tomato cultivars (Klunklin & Savage, 2017).

The experiments in this chapter were designed to better understand the legacy effects of a temporary rootzone water deficit stress on leaf gas exchange in Malling™ Bella and to identify times when samples of xylem sap and leaves should be collected to help to identify the causal signals that regulate leaf gas exchange during the coir dying and subsequent recovery phases. Under more controlled environments, response times to coir drying were similar in the two experiments. However, the duration of the drying affected recovery response times. Experiments were carried out to investigate the causal signals that regulate this prolonged recovery in raspberry plants (see Chapter 4).

3.5 Conclusions

A transient rootzone water deficit stress again caused a reduction in stomatal conductance and photosynthesis and also impacted marketable yield and berry quality. Under a more controlled environment, changes in leaf gas exchange in response to the coir drying treatments were recorded within 4/5 days. The nature of the signals that might regulate the prolonged physiological recovery from a water deficit stress in raspberry plants were investigated in Chapter 4.

4 The role of chemical and hydraulic signalling in the recovery of leaf gas exchange following a transient rootzone water deficit stress

4.1 Introduction

Plants experience a variety of biotic and abiotic stresses which can affect growth, development, and functioning (Davies *et al*., 2002). However, as climate change occurs, droughts or floods are likely to increase (Godfray *et al*., 2010), thereby increasing the likelihood that crops will be subjected to abiotic stresses. Several studies have explored the effects of rootzone water deficits on leaf gas exchange and found that the longer the duration of the water deficit stress, the longer it takes leaf gas exchange values to return to pre-stress values (Vassileva *et al*., 2011; Romero *et al.,* 2017; Torres-Ruiz *et al*., 2015).

Limited research into the effects of rootzone water deficit stress on raspberry plants has been carried out (Morales *et al*., 2013); however, work on woody perennial fruit crops such as grapevine has shown that drought-stressed plants have much lower photosynthetic rates (Maroco *et al*., 2002). Since higher rates of photosynthesis can increase yield potential (Parry *et al*., 2011), lower rates of photosynthesis triggered by a root water deficit stress, can be expected to lower marketable yield and quality (Wenter *et al*., 2018).

During a rootzone water deficit stress, lower rates of photosynthesis are initially caused by stomatal closure which is the dominant limitation to photosynthesis; down-regulation or inhibition of metabolic processes limits photosynthetic CO_2 assimilation in more severe drought episodes (Flexas & Medrano, 2002). Many chemicals are involved in the signalling process that regulate stress-induced stomatal closure, but the research focus has centred on the plant hormone ABA (Davies *et al*., 2002; Wilkinson & Davies, 2002; Borel *et al*., 2001). A major role of ABA is to promote stomatal closure, which in turn reduces transpirational water loss (Comstock, 2002; Ng *et al*., 2014). Xylem ABA concentration correlates closely with lower stomatal conductance (Zhang & Davies, 1990; Dodd *et al*., 2006), suggesting that soil drying is communicated *via*. xylem-borne, root-to-leaf signalling, which promotes stomatal closure (Reddy *et al*., 2004).

The research presented in this chapter focused on studying the impact of a transient rootzone water deficit stress on leaf physiological parameters, and on the role of signalling mechanisms that may regulate leaf gas exchange responses and recovery in raspberry plants. Gaining insight into the signalling mechanism that govern these responses could provide valuable guidance on how best to mitigate legacy stress effects on photosynthesis in raspberry, thereby reducing the likelihood of lower marketable yield and sub-optimal berry quality in commercial production.

4.2 Materials and methods

4.2.1 Plant material and growing conditions

Twenty-four 2-year-old rooting blocks of the raspberry variety Malling™ Bella were grown in 7.5 L pots of Cocogreen™ coir substrate in a glasshouse compartment at NIAB East Malling, Kent. The Malling™ Bella plants were arranged in two rows of twelve plants, orientated north to south, with four cropping canes per pot and approximately two pots per linear metre. During the latter half of August, the Southeast of the UK has an average of 14 h of natural daylight. The glasshouse compartment was fitted with eight Attis LED lights (Lumatek Ltd., UK) at one-metre spacing and three and a half metres up from the ground, which provided supplemental light between 06:00 and 20:00 when natural PAR fell below 1150 μmol m^{-2} s^{-1}. The glasshouse compartment temperature was set to 22°C during the 14-hour photoperiod and 16 °C during the night, while RH was set at 60%. Temperature and RH set points were achieved by venting, heating pipes and fans already fitted into the compartment.

During establishment, all pots were fertigated (combined irrigation and fertiliser application) using an automated demand-driven system to achieve a target daily run-off volume of *ca*. 15%. Throughout these experiments, advice on crop husbandry, fertigation programmes, and pest and disease control was provided by a Berry Gardens Growers agronomist, NIAB's agronomy support, and all recommendations were implemented promptly by the CSPS technical team and NIAB East Malling's glasshouse facilities team.

4.2.2 Experimental design

The experiment was a complete randomised block design with two treatments and 12 experimental plants per treatment, arranged into 12 replicate blocks. Each block contained two pots, one of each treatment. Two irrigation treatments were applied: (1) WW with a target daily run-off volume of water of 15%, and (2) a DD treatment, where irrigation inputs were lowered so that CVMC values fell gradually to facilitate accurate measurements of the onset of plant physiological responses to limiting coir water availability. A 4-day DD treatment was imposed in this experiment, as previous experiments indicated that leaf gas exchange was first impacted four days after the imposition of the coir drying treatment. The DD treatment was imposed by reducing the irrigation set point for the DD plants from 64% initially, then by 5% each day, so that gradual coir drying was achieved.

At the end of the 4-day DD treatment, pots were re-wetted by raising the irrigation set point to 65%, and then the set point was adjusted further to achieve the target 15% run-off, as in WW controls.

4.2.3 Irrigation application and scheduling

A drip fertigation system was used in which each pot had two dripper stakes connected to Netafim CNL emitters (1.2 L h^{-1}). When CVMC values reached the pre-determined set points, irrigation was scheduled and supplied automatically using a sensor-based closed-loop system. In each treatment, five Delta-T SM150T (Delta-T Devices Ltd., Cambridge, UK) sensors were connected to a Delta-T GP2 Advanced Datalogger and Controller unit. The mean CVMC value from the SM150T sensors was calculated automatically by the GP2 unit using a preloaded script, and if the average CVMC value was equal to or less than the irrigation set point, the GP2 opened the solenoid valve. The irrigation duration at each event was adjusted to deliver the target average daily run-off volume of 15% of the input volume. This PI system was used in the WW treatment and also in the DD treatment outside of the coir drying treatment. The two GP2s were connected to a solar-powered Delta-T GPRS modem, which allowed remote access for daily monitoring and adjustment of the irrigation set points.

Fertiliser was added at each irrigation event (fertigation) using three Dosatrons attached to a 3-wire Galcon DC-4S unit (City Irrigation Ltd., Bromley, UK) connected to a manifold housing a DC-4S ¾" valve for each treatment. Fertiliser formulations were made up of three tanks: (i) Tank A – containing macronutrients, (ii) Tank B – containing micronutrients and (iii) Tank C – containing nitric acid. The EC and pH of the fertigation input were measured weekly and adjusted to keep within the recommended values.

4.2.4 Environmental conditions

The glasshouse compartment was fitted with GP2 (Delta-T Devices Ltd., Cambridge, UK) environmental sensors that recorded environmental conditions every 2 mins. Measurements included RH, coir moisture temperature, air temperature and PAR. Vapour-pressure deficit was calculated from the temperature and RH data using a pre-loaded script in the GP2. These environmental data sets were used to help to interpret the physiological data sets.

4.2.5 Coir volumetric moisture content, pore electrical conductivity and daily run-off volumes

Coir volumetric moisture content was measured using a hand-held WET-2 sensor connected to an HH2 meter (Delta-T Devices Ltd., Cambridge, UK). Measurements were conducted on all pots of each plot. Sampling holes were drilled at 60 and 160 mm from the top of each pot to allow insertion of the WET sensor prongs. Measurements of CVMC were made once a day starting between 10:00-10:15, ensuring at least 15 minutes had elapsed before starting measurements. The two CVMC readings were taken at the top and bottom of the pots, and a mean value was reported as the ratio of water volume in the coir to the total volume of coir (m^3 m^{-3}). The WET-2 sensor also measured coir pore EC and coir temperature.

Run-off volumes throughout the day were measured in each treatment, where run-off from a pot was channelled into a Decagon ECRN-50 rain gauge. The rain gauges measuring run-off and input volumes were connected to the Delta-T GP2, and at the end of each day, volumes of daily input to, and run-off from, each pot, and the mean daily % run-off, were calculated automatically using preloaded scripts. These values were uploaded onto DeltaLINK Cloud reports and dashboards to facilitate easy access and real-time monitoring.

4.2.6 Measurement of physiological parameters

Physiological measurements consisting of g_s, Pn, and midday SWP were carried out every day throughout the drying down and recovery phase. Midday SWP measurements for both experiments were carried out between 11:00 and 12:00 on leaves on nodes 14 to 19, as described in Section 2.2.5.

Leaf gas exchange measurements were collected using a LI-6800 Portable Photosynthesis system (LICOR Biosciences Inc., Lincoln, Nebraska, USA). An automatic leaf chamber was used (6 cm^2 leaf area, flow rate of 500 µmol s^{-1}, CO_2 at 400 µmol mol^{-1} and a 1,500 µmol saturation point m^{-2} s^{-1} PAR). Measurements of g_s and Pn were made four times a day between 07:00 and 16:00 on a fully expanded leaf that was exposed to sunlight on the eastern side of the canopy.

Leaf temperature was recorded on fully expanded leaves using a thermal camera (Teledyne FLIR TG167 Thermal Imaging IR Thermometer). Measurements were carried out on fully expanded leaves in all pots of each experimental plot.

4.2.7 Xylem sap sampling

Xylem sap was collected on Days 1, 4, 7 and 10 using the vacuum extraction method described by Bollard (1953). Four canes were cut near the coir surface from each experimental treatment. All leaves were removed prior to cutting the cane to reduce transpiration and subsequent loss of water from xylem elements (Bollard, 1953).

For each cane, a 5-cm-length of xylem from the proximal (root) end was exposed by removing the cortex and phloem layer with a scalpel (Chang *et al*., 2023) to prevent contamination of xylem sap samples with phloem sap (Zheng *et al*., 2020). This proximal end was passed through a hole cut through a silicon rubber bung, which was inserted into a metal cylinder with a narrow side tube that was connected to a vacuum pump. A screw cap plastic bottle was inserted into the bottom of the metal cylinder, and the tight seal formed ensured a strong vacuum. The side tube of the metal cylinder was connected to a vacuum pump with plastic tubing, and a vacuum was applied (Figure 4-1). A *ca*. 5 cm piece of cane was cut and discarded from the distal (top) of the cane every 20-30 seconds using sharp secateurs to release xylem tension; this allowed the xylem sap to drip from the proximal end of the cane into the plastic bottle. The xylem sap was collected on ice and frozen in liquid N_2 immediately before storage at -80°C until hormone analysis commenced.

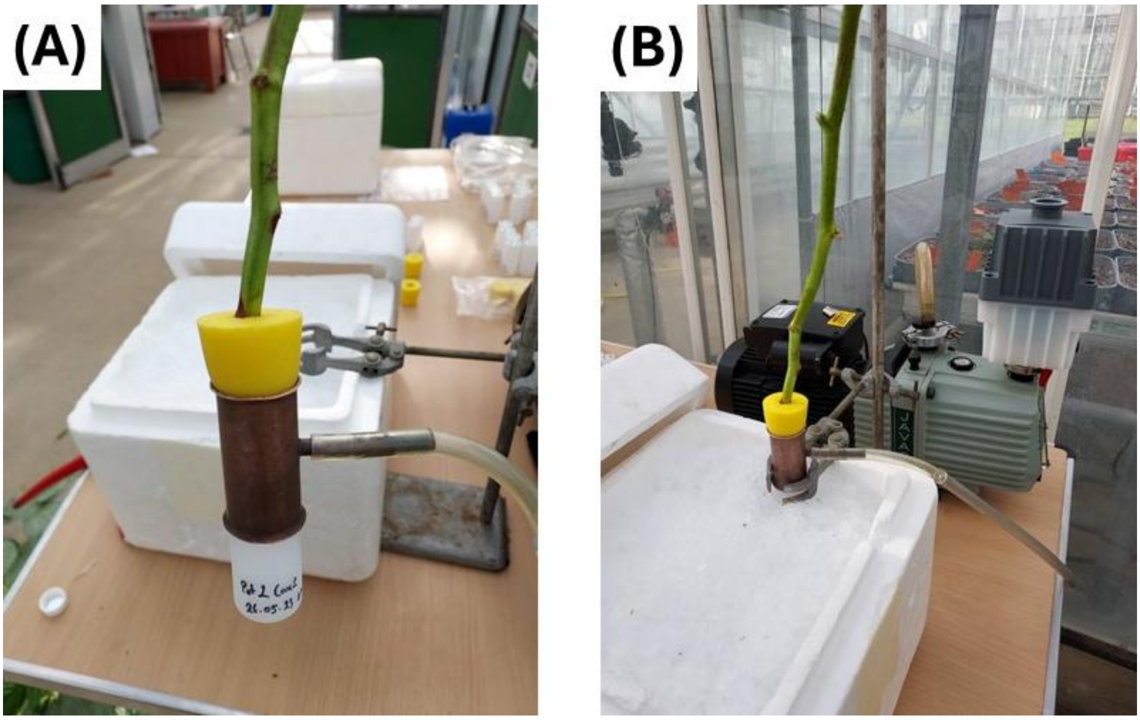

Figure 4-1. *(A) A cane with phloem removed and inserted in a rubber bung, which is inserted into a metal cylinder with a screw cap bottle attached for sap extraction The side tube is connected to a vacuum pump. (B) The set-up for sap extraction with the screw cap bottle collecting the sap sitting on ice. Photos were taken on 26/05/2023.*

4.2.8 Analysis of abscisic acid in xylem sap

To analyse xylem ABA concentrations one cubic millilitre aliquots of xylem sap were combined with acetic acid (AcOH) at a concentration of 5% (w/w) relative to the sample's total weight, and then 50 μL deuterium-labelled isotope of abscisic acid (D_6-ABA) at 1 ng/μL concentration was also added.

Discovery® DSC-18 SPE Tubes (Sigma-Aldrich, USA) were used for solid phase extraction to prepare the sap for hormone analysis. The Discovery® DSC-18 SPE Tubes were washed with 3 mL of 100% methanol (MeOH) and then primed with a 5% AcOH solution. The pre-prepared samples were then loaded into the AcOH-primed Discovery® DSC-18 SPE Tubes, followed by 1.5 mL of AcOH that had been used to rinse the sample tubes and was then loaded onto the columns - this step was carried out twice. Once samples had run through, 3 mL of a 10% MeOH solution was loaded into the Discovery® DSC-18 SPE Tubes. Newly-labelled glass test tubes were placed under the Discovery® DSC-18 SPE Tubes to collect the eluate once 2 x 1.5 mL of 80% MeOH were added to the tubes. The test tubes were then loaded onto a Genevac miVac Centrifugal Evaporator (ATS Scientific Products, USA) until *ca*. 100 μL of the elute remained. This, together with two washes of 100 μL of 100% MeOH of the sample tubes, was then transferred into SureSTART™ 0.3 mL Glass Screw Top Microvials (Thermo Fisher Scientific, USA). This was then loaded onto the Genevac miVac Centrifugal Evaporator (ATS Scientific Products, USA) and once fully dry, 20 μL of the derivatising agent N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) was added, and samples were injected into a gas chromatography coupled to mass spectroscopy

(GC-MS; Agilent GC/MS 6890N - 5973N MSD, Agilent Technologies, Santa Clara, CA, USA). On the GC-MS, the peaks for deuterated standard and endogenous ABA detection was at 194 and 190 mass-to-charge ratio (m/z).

4.2.9 Leaf collection and foliar ABA analysis

Leaf samples were collected on Days 1, 4, 7 and 10. Two leaf samples (terminal and adjacent leaf) between node 20 and the apex of the cane, were collected from each experimental treatment. The leaf samples were bagged and frozen in liquid N_2 immediately, before storage at -80°C until hormone analysis commenced.

To analyse foliar ABA concentrations, 55 mg of dry leaf material was combined with 200 ng of D6-ABA and 5 mL of 80% methanol (MeOH) containing Butylated hydroxytoluene (at a concentration of 0.02 g/l). Once the samples were vortexed for a minute, the samples were placed on a shaker in the cold cabinet overnight.

The samples were centrifuged, and the supernatant was put onto the Genevac miVac Centrifugal Evaporator (ATS Scientific Products, US). The remaining leaf material was combined with 1 mL of 80% MeOH containing Butylated hydroxytoluene and placed on the centrifuge again. The supernatant was added to the previous sample and added back onto the Genevac miVac Centrifugal Evaporator until 1 mL of aqueous solution was left. To the aqueous solution, 1 mL of ammonium acetate ($NH_4CH_3CO_2$) at pH 6-7 was added, ready to be put through the Discovery® DSC-SAX SPE Tube (Sigma-Aldrich, US).

Both Discovery® DSC-SAX SPE Tubes and Discovery® DSC-18 SPE Tubes (Sigma-Aldrich, US) were used for extraction to prepare the sample for hormone analysis. The Discovery® DSC-SAX SPE Tubes were washed with 3 mL of 100% MeOH and then primed with 6 mL of $NH_4CH_3CO_2$ at pH 6-7. The pre-prepared samples were then loaded into the $NH_4CH_3CO_2$-primed Discovery® DSC-SAX SPE Tubes, followed by 3 mL of $NH_4CH_3CO_2$, and washed with 6 mL of MeOH. Newly-labelled glass test tubes were placed under the Discovery® DSC-SAX SPE Tubes to collect the eluate once 2 x 1.5 mL of 0.4M formic acid in MeOH were added to the tubes - this step was repeated twice. The two samples were then joined together and the test tubes were then loaded onto a Genevac miVac Centrifugal Evaporator (ATS Scientific Products, US) until ca. 1 mL of the elute remained. Once 1 mL of aqueous solution was left, 1 mL of 0.4M formic acid in H_2O was added to the sample ready to be loaded to Discovery® DSC-18 SPE Tubes.

The Discovery® DSC-18 SPE Tubes were washed with 3 mL of 100% MeOH and then primed with 3 mL 0.4M formic acid in H_2O. The pre-prepared samples were then loaded into the primed Discovery® DSC-18 SPE Tubes, followed by 3 mL of pH3 H_2O. Newly-labelled glass test tubes were placed under the Discovery® DSC-18 SPE Tubes to collect the eluate once 2 x 1.5 mL of 0.4M formic acid in MeOH were added to the tubes - this step was repeated twice. The two samples were then joined together and the test tubes were then loaded onto a Genevac miVac Centrifugal Evaporator (ATS Scientific Products, US) until *ca.* 100 µL of the elute remained. This, together with two washes of 100 µL of 100% MEOH of the sample tubes, was then transferred into SureSTART™ 0.3 mL Glass Screw Top Microvials (Thermo Fischer Scientific, USA). This was then loaded onto the Genevac miVac Centrifugal Evaporator (ATS Scientific Products, US) and once fully dry, 30 µL of the derivatising agent MTBSTFA with 1% tert-butyldimethylchlorosilane was added, and samples were injected into a GC-MS (Agilent GC/MS 6890N – 5973N MSD, Agilent Technologies, Santa Clara, CA, USA). On the GC-MS, the quantification of endogenous ABA was determined using the peaks for the deuterated standard and endogenous ABA at 194 and 190 mass-to-charge ratio (m/z).

4.2.10 Leaf hydraulic conductance

Measurements of leaf hydraulic conductance (Figure 4-2) were measured using the evaporative flux method and were carried out between 06:00 and 10:00 on terminal leaves situated between nodes 10 and 20, according to the protocol described by Sack and Scoffoni (2012).

Figure 4-2. *The set-up for leaf hydraulic conductance measurements. Photo was taken on 23/05/2024.*

4.2.11 Statistical analyses

Statistical analyses were carried out using RStudio (version 2023.06.0). To determine whether differences between irrigation treatments were statistically significant, one-way ANOVA tests were carried out, and Tukey HSD values for $p < 0.05$ were calculated.

4.3 Results

4.3.1 Coir volumetric moisture content

The mean CVMC value in the WW pots for the duration of the experiment was 0.62 $m^3 m^{-3}$. In the DD treatment, the imposition of substrate drying began on 16 August 2023, when the terminal leaf on node 20 was fully expanded in all pots. The GP2 irrigation trigger set point was reduced from 64% to 59%; thereafter, the irrigation set point was reduced by 5% each day until the final reduction was imposed on 19 August 2023 (Figure 4-3). The drying of coir caused CVMC values to decrease in the DD plants, which became statistically significant ($p < 0.001$) from WW values on Day 1. From 16 to 19 August 2023, the corresponding mean CVMC value determined from "spot" measurements made with the WET sensor fell from 0.58 to 0.31 $m^3 m^{-3}$ in the DD plants, a decrease of approximately 46%.

On 20 August 2023, the pots in the DD treatments were re-wetted to a point at which the mean daily run-off volume was approximately 15% of input. After re-wetting, the CVMC value for the previous DD pots was restored to pre-stress values four days after the rewetting commenced on 23 August 2023.

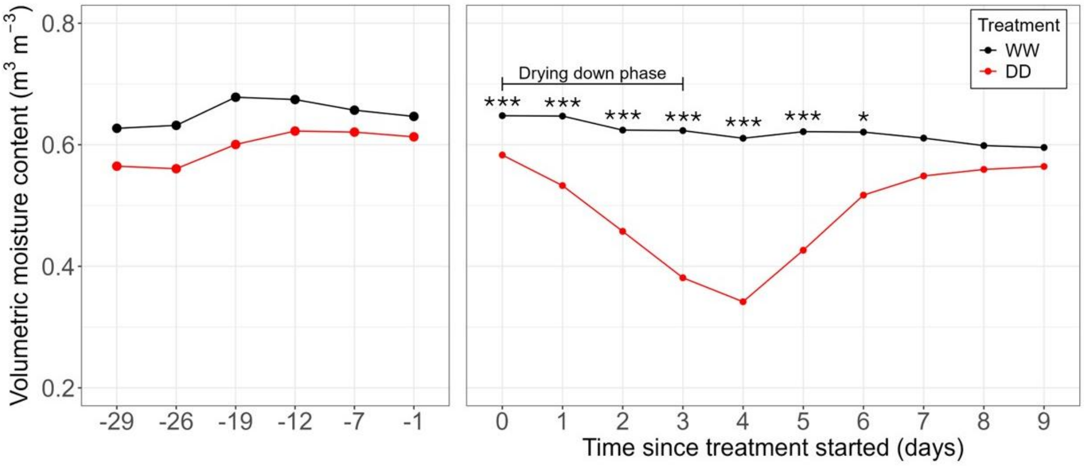

Figure 4-3. *The effects of the DD treatment on coir volumetric moisture content of the twelve Malling™ Bella, made by carrying out "spot" measurements using a Delta-T WET sensor. Each point represents the mean CVMC value from the six pots in each block. The duration of the drying-down treatment is shown for information. Asterisks indicate when statistically significant differences (p < 0.05) between treatments were first measured.*

4.3.2 Plant physiological responses to irrigation treatments

The first detectable physiological response to coir drying in DD plants was a difference in midday SWP, values were significantly different ($p < 0.01$) to those recorded in WW plants on Day 2 (17 August 2023; Figure 4-4).

Rewetting of the coir commenced at 06:00 on Day 5, 20 August 2023, and by Day 6, the mean midday SWP value in previously DD plants had recovered ($p = 0.811$) to pre-stress WW values (Figure 4-4).

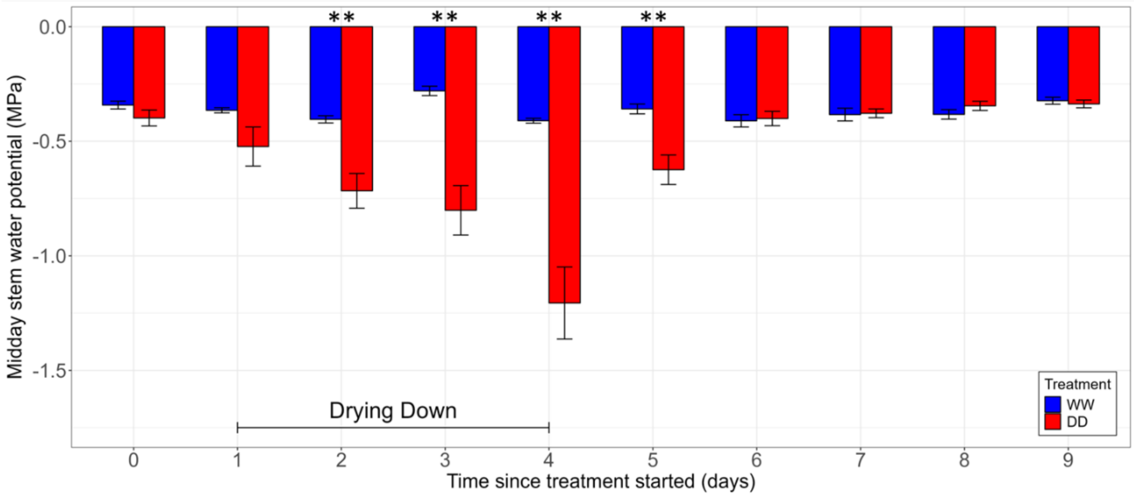

Figure 4-4. *The effects of the DD treatment on midday stem water potential. The results are mean values from six experimental blocks. Error bars are ±SE, where n = 10. The duration of the drying-down treatment is shown for information. Asterisks indicate statistically significant differences (p < 0.05) between treatments.*

On Day 2, statistically significant differences in midday gs ($p < 0.05$) and midday Pn ($p < 0.05$) values between WW and DD plants (Figure 4-5) were also detected. As the coir dried, g_s and Pn values continued to decrease. Once coir rewetting commenced on Day 5, 20 August 2023, midday g_s and Pn values remained significantly lower for the next 3 days. On Day 8, these differences in g_s and Pn values persisted, although they were just outside of statistical significance ($p = 0.068$ and $p = 0.09$, respectively; Figure 4-5), and by Day 9, both parameters had recovered to pre-stress values.

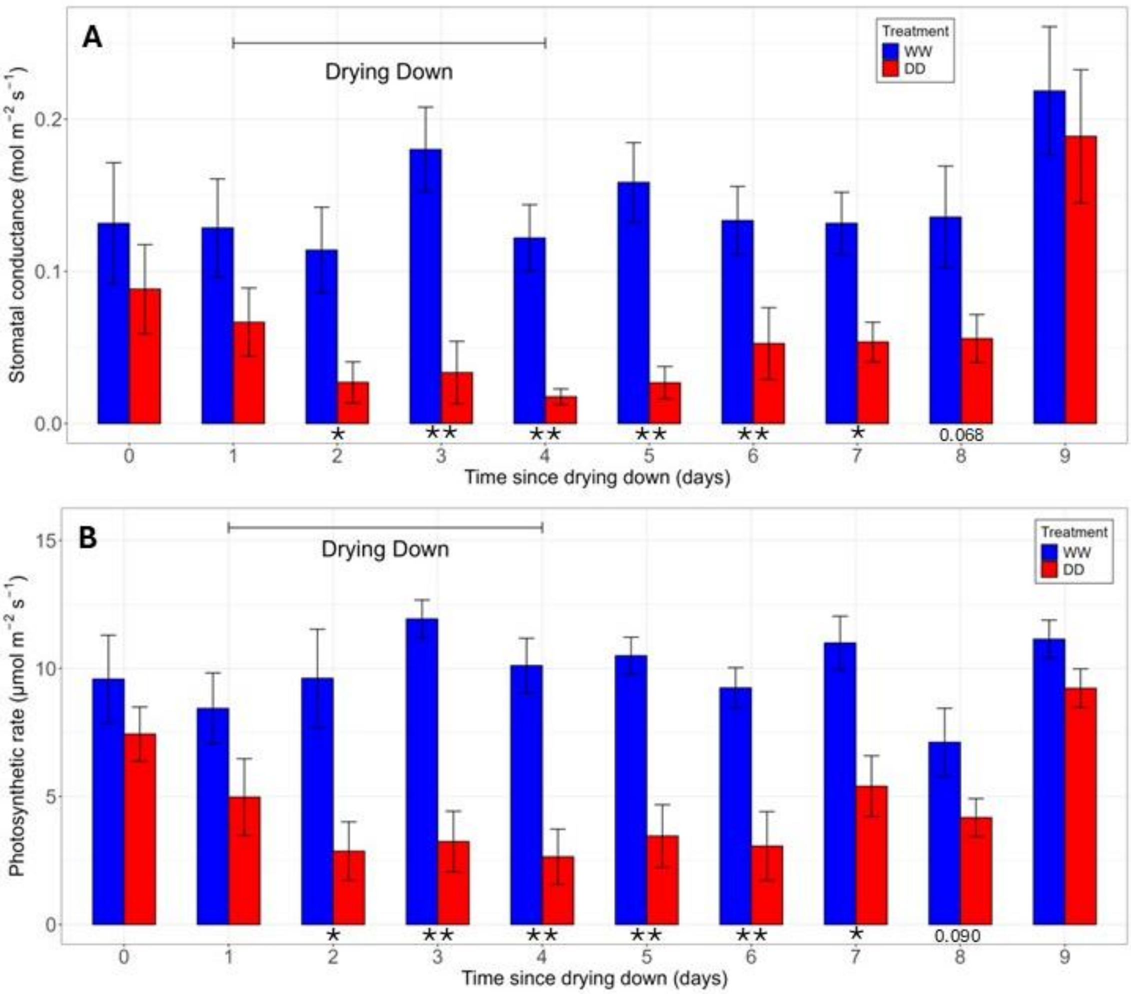

Figure 4-5. *The effects of the DD treatment on A) midday stomatal conductance and B) midday photosynthetic rate. The results are an average value from six blocks. Error bars are ±SE, where n = 12. The duration of the drying-down treatment is shown for information. Asterisks indicate statistically significant differences ($p < 0.05$) between treatments. On Day 8, values just outside of statistical significance are shown for information.*

To better understand the dynamics of these adaptive responses during the drying-down and subsequent recovery phases, measurements of g_s and Pn were made at four time points during the light period. On Day 0, Pn (Figure 4-6A) and g_s (Figure 4-6B) values were similar in WW plants and those that were to be dried down at all four time points. By Day 2, the effects of the transient rootzone water deficit stress were first evident at midday, with a significant decrease in Pn ($p <$ 0.05) and g_s ($p < 0.05$) values measured in DD plants (Figure 4-6C and D). On the last day of drying down, Day 4, low Pn and g_s values persisted throughout the day in DD plants compared to WW counterparts.

On Day 5 following coir rewetting, Pn values in the previously DD plants were significantly different ($p < 0.05$) at all four measurement times (Figure 4-7A), and although g_s values were similar to WW values at 07:00, lower values were measured at all other time points (Figure 4-7B). By Day 7, the recovery of Pn and g_s values was similar (Figure 4-7C & D), but complete recovery to WW values at all four time points occurred on Day 9 (Figure 4-7E & F).

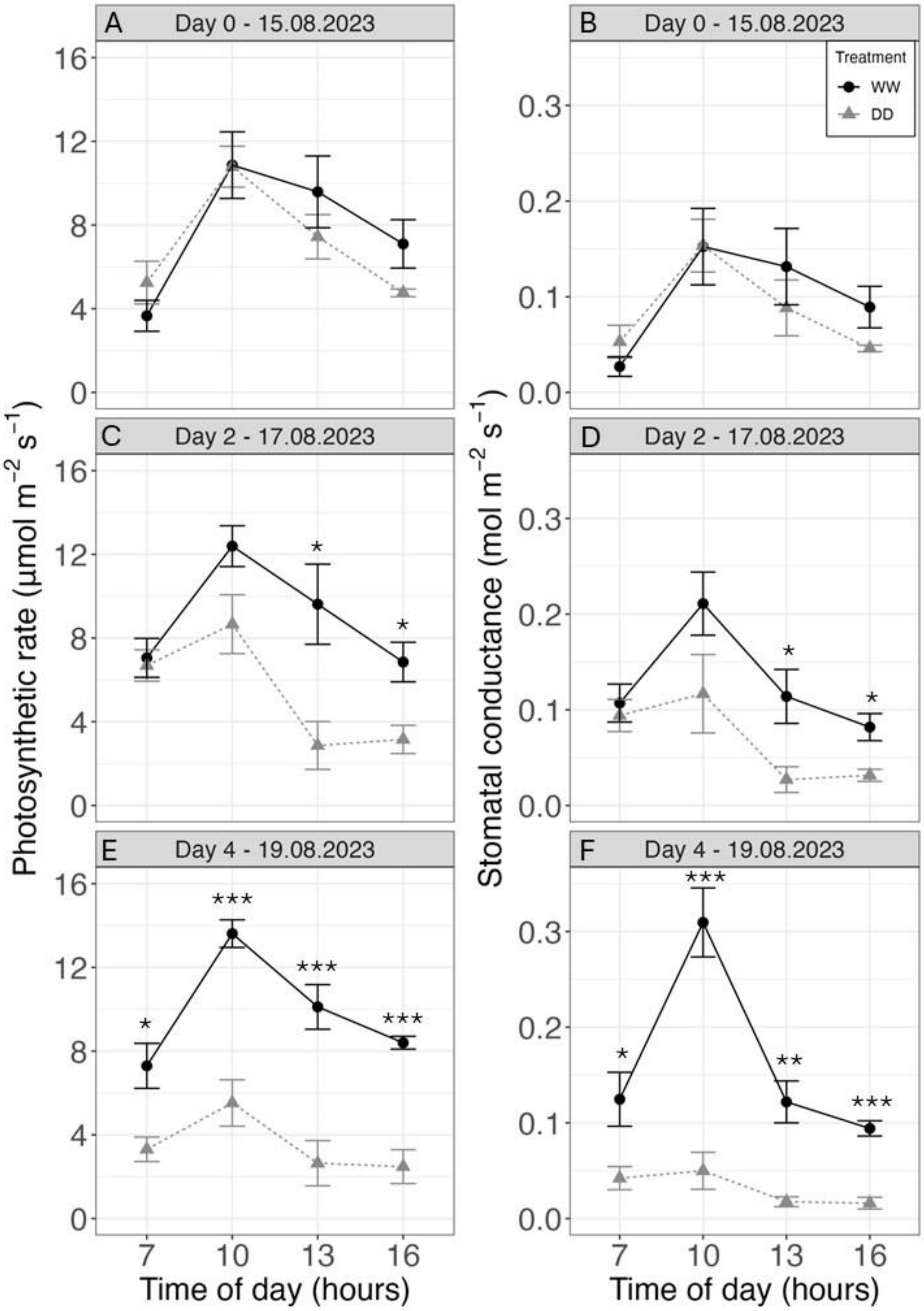

Figure 4-6. *The effects of the DD treatment on diurnal photosynthesis (A, C, E) and diurnal stomatal conductance (B, D, F) values during the drying down phase. Data points at each time are mean values from six blocks. Error bars are ±SE, where n = 12. Asterisks indicate statistically significant differences (p < 0.05) between treatments. Measurements were carried out four times a day (07:00, 10:00, 13:00 and 16:00).*

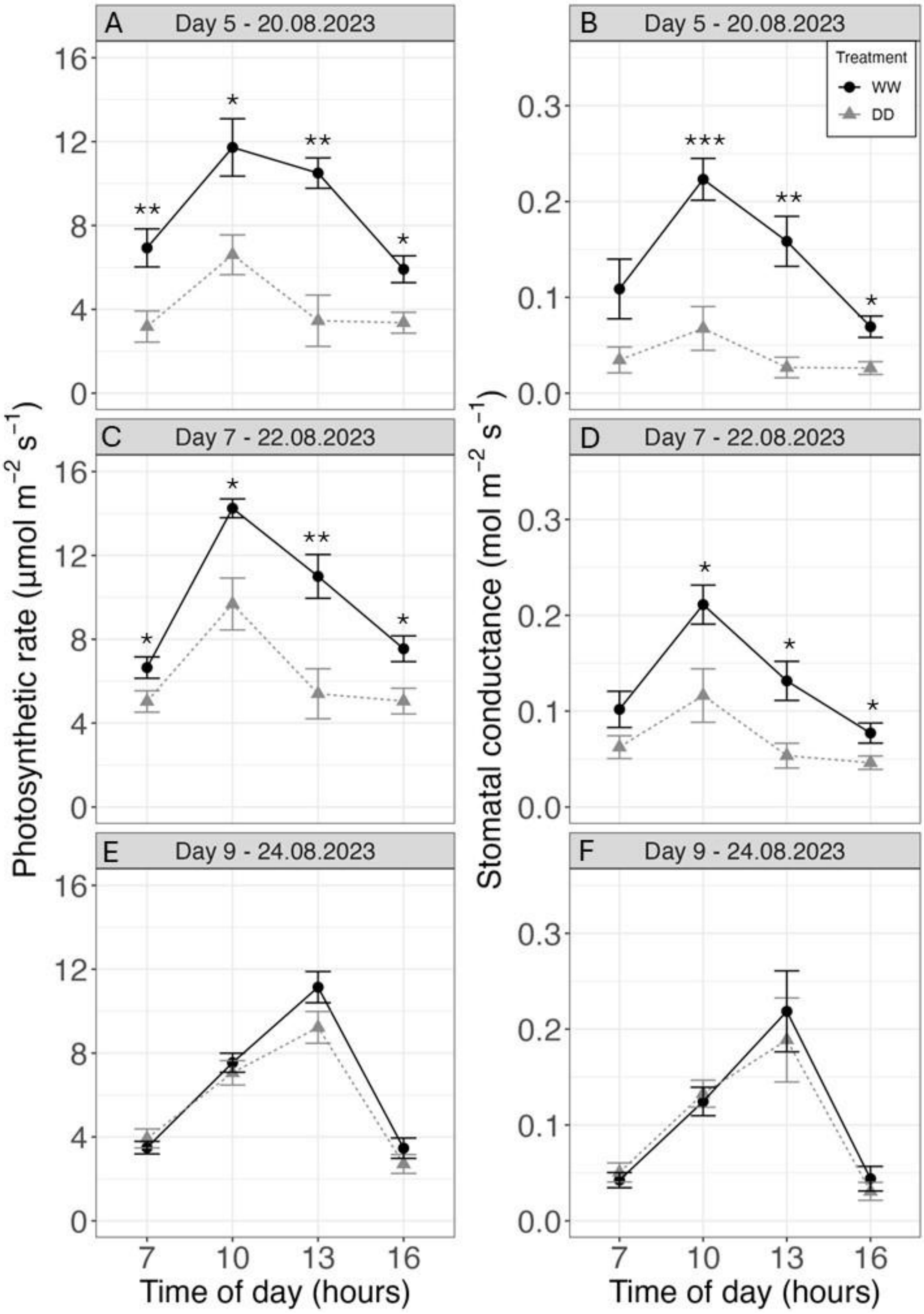

Figure 4-7. *The effects of the DD treatment on diurnal photosynthesis (A, C, E) and diurnal stomatal conductance (B, D, F) values during the recovery phase. Data points at each time are mean values from six blocks. Error bars are ±SE, where n = 12. Asterisks indicate statistically significant differences ($p < 0.05$) between treatments. Measurements were carried out four times a day (07:00, 10:00, 13:00 and 16:00).*

Although leaf temperature in the DD plants was higher between days 1 to 6, these increases were not statistically significant (*p* > 0.05). On days 7, 8 and 9, mean leaf temperatures in both treatments were similar (Figure 4-8).

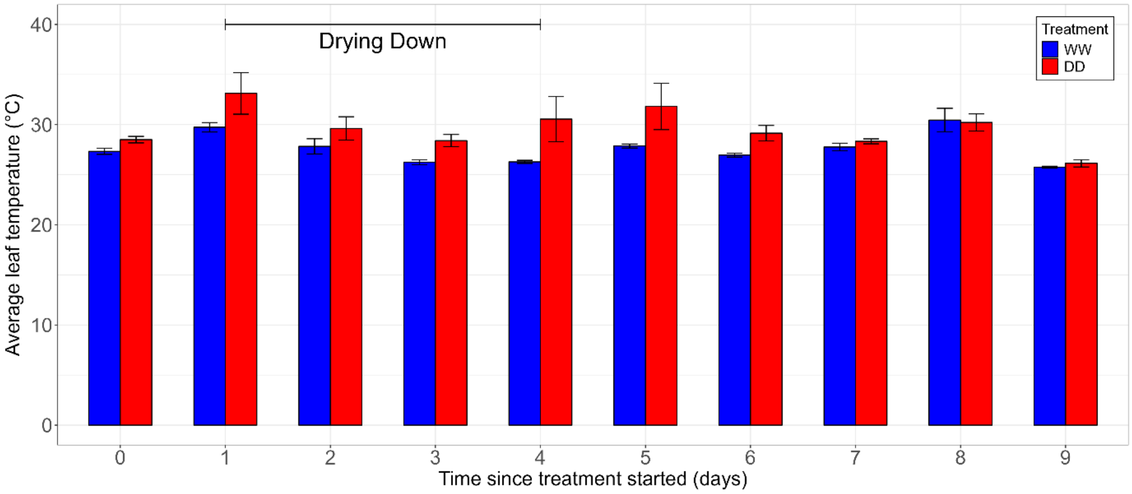

Figure 4-8. *The effects of the DD treatment on leaf temperature. The results are an average value from six blocks. Error bars are ±SE, where n = 12. The duration of the drying-down treatment is shown for information.*

4.3.3 Xylem abscisic acid changes during the drying down experiment

Abscisic acid was detected in xylem sap samples collected on all four dates. Xylem-borne ABA in WW plants remained similar throughout the experiment; however, changes in ABA were evident in DD plants (Figure 4-9). On Day 1, xylem-borne ABA was significantly different (*p* < 0.001) between WW and DD plants, on this day significant differences were also measured in CVMC values (*p* < 0.001; Figure 4-3). In xylem sap samples collected on the last day of drying down (Day 4), there was more than a 50-fold increase in xylem-borne ABA at 151.09 nM in DD plants compared to WW plants at 2.22 nM.

Following the rewetting of the coir on Day 5, xylem sap samples were collected on Day 7 and 10. On Day 7, when midday SWP had recovered to pre-stress values (p = 0.874), xylem-borne ABA in WW and DD plants was similar (p = 0.133), but significant differences in midday g_s and Pn values in WW and DD plants remained ($p < 0.05$ and $p < 0.01$, respectively).

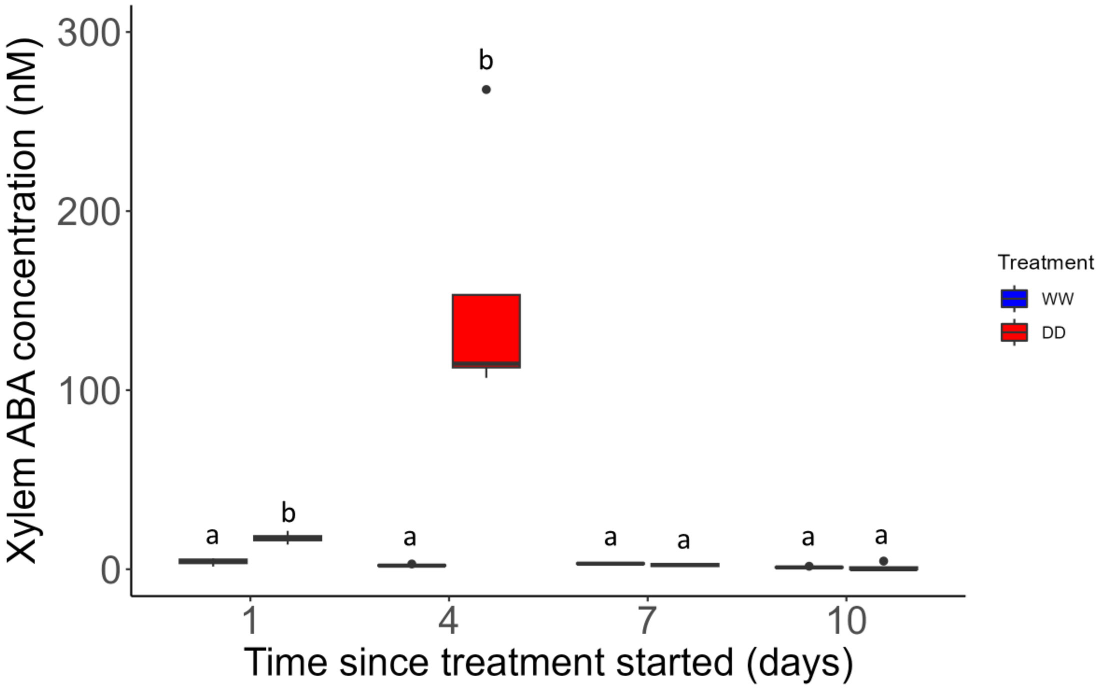

Figure 4-9. *The effects of the DD treatment on xylem-borne ABA concentrations. The results are a mean value from four sampling canes for each treatment. Letters denote significant differences between treatments on each sampling day.*

4.3.4 Foliar ABA changes following the drying of the substrate

In all the collected leaf samples, ABA was detected on all the days. Foliar ABA levels in WW plants were similar on Days 1 and 4, but increased slightly on Days 7 and 10 (Figure 4-10). In DD plants, foliar [ABA] increased until the end of the drying-down phase with significant differences between the WW and DD plants on Days 1 and 4 ($p < 0.005$). On the last day of the drying-down treatment, on Day 4, foliar [ABA] were just over 1.5 times greater in the DD plants than in the WW plants. Foliar [ABA] then returned to pre-stress values by Day 7 and remained at pre-stress values for the duration of the experiment.

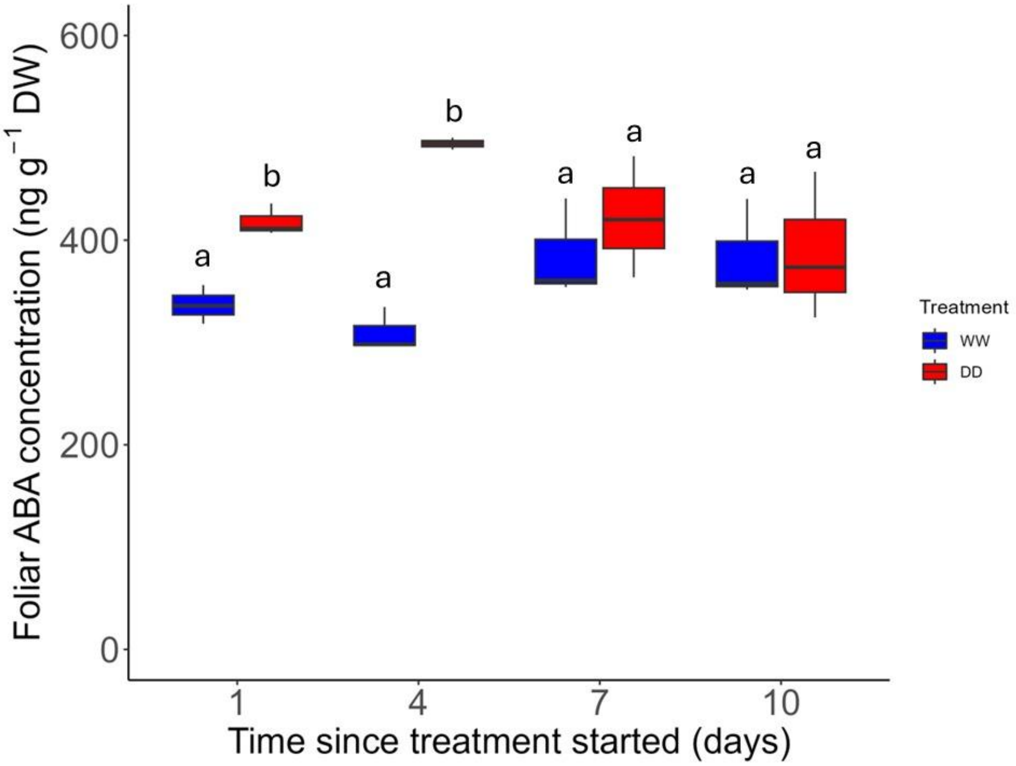

Figure 4-10. *The effects of the DD treatment on foliar ABA concentrations. The results are a mean value from three samples pots for each treatment. Letters denote significant differences between treatments on each sampling day.*

4.3.5 Leaf hydraulic conductance responses to the drying-down treatment

Leaf hydraulic conductances were not significantly different ($p > 0.05$) between the WW and DD plants during the drying-down or the subsequent recovery phase (Figure 4-11).

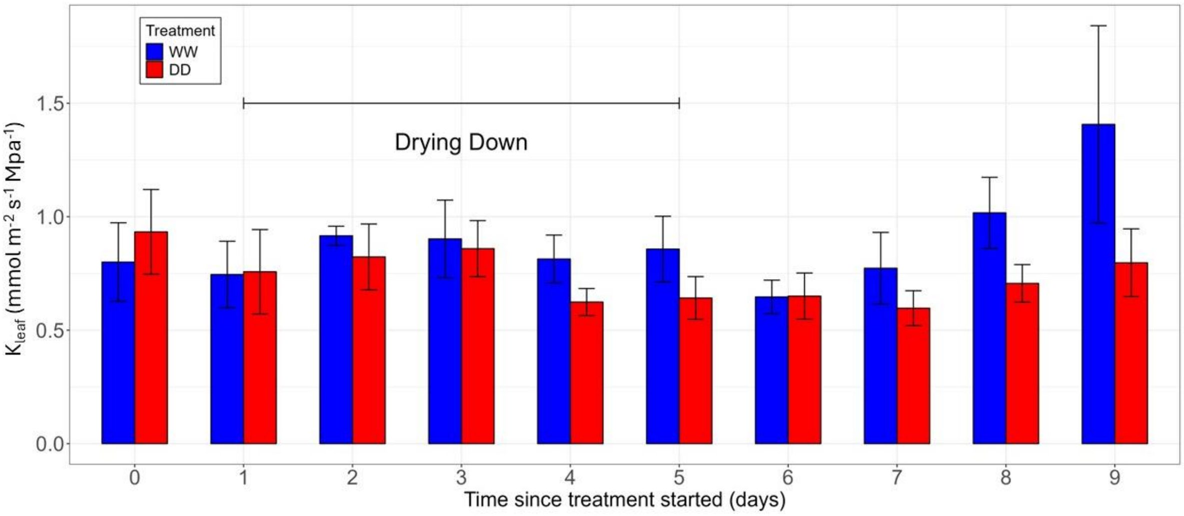

Figure 4-11. *The effects of the DD treatment on leaf hydraulic conductance (K_{leaf}). The results are a mean value from four samples pots for each treatment.*

4.4 Discussion

4.4.1 Using a sensor-based automated irrigation system to optimise fertigation to plants

Water deficit stress causes many physiological responses, but responses vary with the severity and duration of the stress (Bradford & Hsiao, 1982). However, over-watering or even flooding plants to prevent water deficit stress is not ideal either, as flooding can also affect plant physiology by reducing stomatal conductance and slowing transpiration (Else *et al*., 1995). With raspberry canes grown in pots of coir, irrigation control is even more important, as water drains well in coir, so the frequency and volume must be controlled to ensure that plants receive enough irrigation and fertilisers without water running through the pots.

Using a sensor-based automatic irrigation scheduling system ensured that CVMC of WW plants was maintained consistently within an optimum range, which provided confidence that the differences in leaf physiological parameters in WW and DD plants were due to the imposed rootzone water deficit. Automated irrigation has been used in other experiments, for example, this approach helped to provide a reliable threshold to quantify the growth and water use of *Cymbidium* grown in coir dust (An *et al*., 2020). The gradual imposition of the DD treatment was also controlled by the automated irrigation system; the accurate and precise control of CVMC during the drying and recovery phases helped to ensure that the degree and rate of imposed coir drying was similar and repeatable in the experiments reported in Chapter 3. Since the sampling dates here were chosen based on the timing of the physiological responses noted in Chapter 3, similar rates of drying and rewetting were essential in efforts to identify the causal signals that might regulate the response and recovery following a rootzone water deficit stress in the raspberry variety Malling™ Bella.

4.4.2 Plant physiological responses to the transient water deficit stress

In this experiment, leaf physiological responses of Malling™ Bella to a short and tightly regulated transient root water deficit stress were explored. Adaptive responses to coir drying were first detected as changes in midday SWP, a response that has been noted many times in the literature in many crops, including raspberry (Percival *et al*., 1998), grapevines (Romero *et al*., 2017; Tombesi *et al*., 2015) and peach trees (Marsal *et al*., 2015). For many years, SWP has been used as an indicator of when plants perceive water deficit stress (McCutchan & Shackel, 1992). The timing and extent of changes in midday SWP during the drying down and recovery phases here were similar to those reported in Chapter 3. This was important in ensuring that sampling times could be kept as close to the time when a change in midday SWP was detected.

Analysis of xylem-borne ABA in samples collected during the DD and recovery phases suggests that there is a correlation between xylem-borne ABA changes and midday SWP values of raspberry plants. Research in *Arabidopsis* found xylem-fed ABA can affect leaf hydraulic conductivity, where hydraulic conductivity within the plant decreases when ABA increases in the xylem (Shatil-Cohen *et al*., 2011). Changes in hydraulic conductivity have also been shown to be mediated by aquaporin activity (Martre *et al*., 2002) and studies have demonstrated that

ABA affects aquaporin activity (Morillon & Chrispeels, 2001; Siefritz *et al*., 2001). Aquaporins are membrane channels that play a role in root and shoot hydraulic conductivity (Javot *et al*., 2003), which can adjust water potential gradients within the plant (Scharwies & Dinneny, 2019). Morillon and Chrispeels (2001) suggested that ABA acts at the plasma membrane by activating aquaporins, which enables water uptake by the roots during non-transpiring conditions. This suggests that increases in xylem ABA can affect hydraulic conductivity, hence, affecting midday SWP values. However, following rewetting of the coir, in the absence of xylem-borne ABA, hydraulic conductivity would be restored to pre-stress conditions reducing tension within the plant with greater water uptake, hence the midday SWP values of DD plants returning to pre-stress values.

Environmental conditions can alter the relative timing of leaf physiological responses. When analysing the environmental conditions in the glasshouse compartment at the time, high air temperature (Figure-A4) and VPD values (Figure-A5) on Day 1, 16 August 2023, may have resulted in a more rapid rate of coir drying, hence differences in leaf gas exchange were detected on Day 2. High VPD values can affect transpirational water loss, and research has shown that differences between tolerant and sensitive line in *Vigna unguiculata* for canopy conductance was most evident when VPD was above 3.5 kPa (Belko *et al*., 2012). Increases in VPD above 2 kPa reduced stomatal conductance in *Umbellularia californica* (Buckley, 2016; Grossiord *et al*., 2020), and on Day 1, environmental sensors recorded VPD above 4 kPa in the glasshouse compartment, which would have affected the rate of water loss from leaves.

Increasing VPD can result in increased water loss from the leaves (Grossiord *et al.,* 2020). Along with high VPD, fluctuations in PAR values (Figure-A6) throughout the experiment could also have affected the leaf physiological responses. Studies have shown that fluctuating light can limit photosynthesis (Sassenrath-Cole & Pearcy, 1994), as slight fluctuations in light intensity can reduce the electron transport rate, reducing photosynthetic efficiency (Powles, 1984). Plant species respond to increases in leaf temperature differently, for example in rice, photosynthesis increases with temperature up to 30°C after which photosynthesis drops; however, in wheat, photosynthetic rates drop at temperatures greater than 20°C (Yang *et al*., 2020). Increases in ambient temperature above 30°C led to a decrease photosynthetic rate and stomatal conductance in two raspberry cultivars, ‘Reveille’ and ‘Autumn Bliss’, also causing increases in leaf temperature (Stafne *et al*., 2001). Yang *et al*. (2020) also mentioned that leaf surface temperatures were similar to air temperatures, and in the reported experiment in this chapter, measurements of leaf temperature on Days 1 and 8 were higher in the WW plants compared to other days, and on those days, air temperature values in the glasshouse compartment were much higher than other days (Figure-A4).

Measurements of xylem-borne ABA were constantly low in all WW plants, suggesting that the control plants were not under stress. Foliar ABA concentrations were also consistent on all four days of measurements. This consistency in xylem-borne and foliar ABA in WW plants was achieved from the steady and consistent CVMC values achieved using the PI system. While there were no Day 0 xylem or foliar ABA values due to the limited number of plants available for destructive measurements, the elevated xylem and foliar ABA levels in DD plants on Day 1 serve as an indication that concentrations had only started to increase. As the coir dried, higher xylem-borne ABA was measured, this is a result of ABA synthesised in the roots, which is then transported to the leaves *via*. the xylem sap (Davies & Zhang, 1991), which then caused increases in foliar ABA.

Elevated xylem-borne ABA on Day 1 could be the causal signal that promotes stomatal closure on Day 2. As the sap flow rate increases, decreases in ABA concentration have been measured (Else *et al*., 1994) and this should be taken into consideration when analysing xylem sap data. In an experiment where partial rootzone drying was used as an irrigation technique, plants that received 50% less water than the control plants had increased xylem ABA and decreased g_s values (Dodd *et al*., 2006). The importance of ABA in the regulation of gas exchange response to drying soils has been highlighted in reviews since the ‘90s (Davies *et al*., 1994, Dodd *et al*., 1996), and several studies have shown a correlation between ABA and leaf gas exchange (Socias *et al*., 1997, Tombesi *et al*., 2015, Shatil-Cohen *et al*., 2011). Modern work still centres around signalling molecules involved in drought responses to be able to improve drought resistance in crops (Liu *et al*., 2022). In a review by Liu *et al.* (2022), it was stated that ABA was a core signal molecule in drought-induced stomatal closure. ABA plays a major role in promoting stomatal closure when plants experience rootzone water deficits, which helps to reduce transpirational water loss (Comstock, 2002; Ng *et* *al*., 2014). When a plant is subjected to a water deficit stress, ABA is synthesised in the roots, transported in the xylem and then to the guard cells (Liu *et al*., 2022), and as ABA is a weak acid, it will accumulate in more alkaline compartments of the leaf (Wilkinson & Davies, 1997). Schroeder *et al.* (2001b) described how increasing ABA concentration can cause stomatal closure by promoting guard cell turgor loss. The pathway summarised in Schroeder *et al*., (2001b) describes ABA being detected by guard cells, inducing cytosolic Ca^{2+} elevations, mediating anion release causing depolarisation, which activates K^+ channels resulting in a K^+ efflux from guard cells. The long-term efflux of anion and K^+ from guard cells contributes to the loss of turgor in the guard cells, leading to stomatal closure (Schroeder *et al*., 2001b). As well as the effects of xylem ABA on stomatal closure, research has shown that an increase in xylem sap pH induced by drought can also create a root-sourced signal to the leaf, which can cause stomatal closure (Wilkinson & Davies, 2002).

Diurnal measurements of g_s and Pn were carried out to better understand the legacy effect on photosynthesis. Even though a shorter rootzone water deficit stress duration was imposed here, results were similar to those reported in Chapter 3. With the changing environmental conditions described above, Pn and g_s curves differed daily from 13:00 onwards. However, measurements at 10:00 were more consistent, perhaps due to the more muted fluctuations in air temperature and VPD in the late morning. The regulation of stomatal aperture is a compromise between maintaining the rate of photosynthesis at a level dependent on the intrinsic capacity of carbon fixation and the need to conserve water (Wong *et al*., 1979), and so stomata respond to environmental cues accordingly. In a review by Tallman (2004), ABA was linked to diurnal stomatal movements, whereby early morning, at first light, activation of a cytochrome P450 mono-oxygenase would deplete endogenous guard cells of ABA, allowing guard cells to accumulate water and ions to increase guard cell turgor and hence favour stomatal opening. From around midday, stomatal apertures can be affected by the intensity of xylem-borne ABA signalling (Tallman, 2004); this may explain the differences in g_s and Pn between DD and WW plants, as DD treatments increased xylem ABA (Figure 4-9), hence affecting stomatal aperture. Tombesi *et al.* (2015) mentioned that foliar ABA only rose in the late afternoon when stomatal closure was observed late in the morning. This would suggest that other signalling mechanisms would be involved in triggering early stomatal closure. However, in that experiment, when stomata were closed throughout the day, foliar ABA was steadily higher (Tombesi *et al*., 2015), suggesting that increased foliar ABA could be a constant signal that maintains stomatal closure and allows partial stomatal opening in plants recovering from a root water deficit.

Research on *Eucalyptus tetrodonta* found as drought progressed, increases in foliar ABA were measured and the decline in maximum g_s correlated with increases in foliar ABA (Thomas and Eamus, 1999). When subjected to a water deficit stress, foliar ABA concentration more than tripled in some genotypes of tomato, but a range of values were recorded for different genotypes (Gao *et al.*,2022). The increases in the experiment reported in this chapter were not as significant, although values increased by more than 1.5 times after a four-day DD treatment. Temperature can also affect foliar ABA levels as Qiu *et al*. (2017) demonstrated an increase by 67% when temperature increased from 20 to 30°C in raspberry. However, the foliar ABA concentrations of the unstressed raspberry plants were higher than those quantified in Malling™ Bella leaves. As this is the first time foliar ABA concentrations are being reported in Malling™ Bella, it is not possible to compare these values with available literature.

Wang *et al.* (2018) demonstrated that when rice was subjected to a drought period, stomatal closure could be best explained by hydraulic signals, even though hormonal signals also played a role. Much of the available research have suggested that hydraulic signals and “possible” chemical signals are the cause of regulating stomatal conductance that can later limit photosynthetic rate. Although the response to water deficit stress has been tried to be explained by either hydraulic or chemical processes, research shows that they are not separate pathways but, in fact, coupled (Brunetti *et al*., 2019).

Leaf hydraulic conductance is a representation of the ability of the transport system to supply water that then enables stomata to stay open for photosynthesis (Scoffoni *et al*., 2011). Comstock (2002) reported a positive response between leaf hydraulic conductance and stomatal conductance, however this was not represented in the experiment described in this chapter. Values of leaf hydraulic conductance are out by a factor of 10 compared to that in the ranges described for a leaf of that dimension (Scoffoni *et al*., 2011; Wang *et al*., 2022). However, environmental factors, like air temperature, airflow around the leaf and PAR can all effect K_{leaf} estimation when using the evaporative flux method (Wang *et al*., 2022). However, evaporative flux method is preferred when estimating K_{leaf} as it mimics the natural transpiration pathways of water through the leaf (Wang *et al*., 2002).

The potential signalling mechanisms that regulate the recovery from a short-term water deficit stress in raspberry are reported here for the first time. Much research has been conducted to understand how signalling pathways combine to regulate adaptive responses when a plant experiences stress; however, there is no published research explaining why raspberry canes experience a legacy effect on photosynthesis after experiencing water deficit stress as short as four days. The data presented in this chapter suggests that ABA regulates stomatal behaviour, and this can be either directly (presence or absence of ABA) or indirectly (changes in pH and affecting hydraulic conductivity).

4.5 Conclusions

In this experiment, a short duration of a rootzone water deficit stress caused increases in xylem-borne ABA, followed by reductions in leaf gas exchange. Once the coir was rewetted, CVMC and shoot water balance was restored, and xylem-borne and foliar ABA quickly returned to pre-stress values, but g_s and Pn values remained low for another four days. The relative timing of these changes suggests that an increase in xylem-borne and foliar ABA may promote stomatal closure during the drying-down phase, but that the legacy effects on stomatal conductance and photosynthesis measured following a rootzone water deficit stress are mediated by a different signal(s). Further work is needed to identify possible signalling mechanisms.

5 Examining the impact of rooting volume on leaf physiological parameters following a temporary rootzone water deficit stress

5.1 Introduction

Given the unpredictable nature of weather conditions, it is crucial for growers to understand how crops respond to abiotic stresses to ensure yield and quality remain uncompromised. These environmental conditions vary from year to year and within the growing season. Therefore, it is imperative for growers to adjust the growth environment to provide optimal growing conditions to maximise crop yield and quality.

Photosynthesis is highly regulated (Tanaka & Makino, 2009) and is a key process in plants, whereby light energy is converted into chemical energy for all plant processes. Low water availability can reduce photosynthesis through decreased stomatal conductance; the resulting diffusional constraint on CO_2 uptake inevitably limits photosynthetic rate (Medrano *et al*., 1997).

Research by Maroco *et al*. (2002) has shown that water deficit stress causes a drop in photosynthetic rates, stomatal conductance, and photosystem II activity; photosynthetic rates under these conditions can be up to 70% lower compared to WW plants (Maroco *et al*., 2002). Research has shown that increasing photosynthesis can raise yield potential (Parry *et al*., 2011); however, it is also critical to examine how photosynthetic rate is affected when subjected to stress, particularly given the predicted changes to climatic conditions.

To date, the majority of research on understanding leaf gas exchange responses to abiotic stresses has focused on field crops, and limited attention has been given to protected crops such as tomato (Dodd *et al*., 2006; Else *et al*., 1995) and strawberry (Dodds *et al*., 2007; Grant *et al*., 2010). More growers are shifting to growing raspberries in pots or containers in substrate and moving away from growing in the soil (Dolan *et al*., 2018) to: (1) circumvent issues with soil-borne diseases; (2) having the flexibility to schedule cropping over the season by bringing in successive crops from cold store, and (3) facilitate the ease of growing crops without having to dig out the plants at the end of each growing season. Raspberry plants grown in the soil are dug out to minimise their exposure to pathogens and to shorten the duration of their cultivation in commercial production (Dolan *et al*., 2018).

A recent trend to grow commercial raspberry crops in 4.7 L pots rather than replanting them into 7.5 L pots is a practice that is likely to result in yield penalties under conditions of high evaporative demand if irrigation is not optimised. Growers are changing 7.5 L pots to 4.7 L pots, mainly for economic reasons. The utilisation of smaller pots requires less coir and concurrently diminishes labour costs, as growers are inclined to avoid potting on the 4.7 L pots that were previously used in cane production in the nursery. Also, growers are using smaller pots to reduce the chances of root rot (caused by *Phytophthora* spp.) by protecting plants from over-watering, as the disease is more prone in water-saturated substrates (Dolan *et al*., 2018). With smaller pots able to hold less water, irrigation needs to be scheduled depending on plants' needs, as the longer the interval between irrigation times, smaller pots would have less water available per plant compared to those in larger pots (Targino *et al*., 2019). Inefficient and ineffective irrigation scheduling can rapidly lead to transient rootzone water deficits, and therefore understanding the possible impacts on photosynthesis rates, as well as fruit size and marketable yield when using different rooting pot sizes, is vital.

In this research, experiments were designed to identify the response and recovery to a rootzone water deficit stress in Malling™ Bella using two commercial pot sizes. This research provides insights into the impact of rooting volume on plant physiological parameters following temporary rootzone water deficit stress, which are needed to help growers to make informed decisions about their cultivation practices.

5.2 Materials and methods

5.2.1 Plant material

Malling™ Bella plug plants supplied by van der Avorid (Holland) were potted on into two different rooting volume pots, 7.5 L and 4.7 L. In each pot, two plug plants were potted at opposite corners in Cocogreen™ coir substrate on 10 May 2023. The plants were positioned in the outer row on the west side of BGG T2 at NIAB, East Malling with approximately two pots per linear metre (Figure 5-1). The pots were left in the outer row of the west side of BGG T2 for two consecutive years, harvesting berries in both years.

Figure 5-1. *Malling™ Bella plants in row 3 of polytunnel BGGL T2. Photo taken on 30 September 2023.*

During establishment, all pots were well-watered with a target daily run-off volume of *ca*. 15%. Precision Irrigation was first implemented on 15 May 2023. A drying-down treatment was imposed on half of the plants beginning on 22 September 2023, when the primocane plants were approaching full crop load. This experiment was designed to identify whether the two rooting volumes differentially affected plant physiological parameters when coir water availability was limited.

Throughout these experiments, advice on crop husbandry, fertigation programmes, and pest and disease control was provided by the Berry Garden Growers agronomy team, and all recommendations were implemented promptly by the CSPS technical team and NIAB East Malling's glasshouse facilities team.

5.2.2 Experimental design

The experiment was a complete randomised design with four treatments and 20 experimental plants per treatment, arranged in four replicate blocks. Each block contained one plot for each treatment, with five plants per plot (Figure 5-2). Two irrigation treatments were applied to the two different rooting volume pots: (i) WW with a target daily run-off volume of water (and fertilisers) of 15%: (ii) a DD treatment, where irrigation was withheld so that the CVMC fell gradually to facilitate accurate measurements of the onset of plant physiological responses to limited coir water availability. Therefore, the notations for the four treatments were: (i) Well-watered 7.5 L pots – WW 7.5, (ii) drying down 7.5 L pots – DD 7.5, (iii) well-watered 4.7 L pots – WW 4.7, and, (iv) drying down 4.7 L pots – DD 4.7.

Figure 5-2. *The two different pot sizes used for the two irrigation treatments (7.5 L on the left and 4.7 L on the right), with two cropping canes per pot. Photo taken on 08 August 2023.*

5.2.3 Irrigation application and scheduling

The timing and duration of irrigation events were controlled using a Galcon DC-4S unit (City Irrigation Ltd., Bromley, UK) connected to a manifold housing a DC-4S ¾” valve for each treatment. Water was sourced from the rainwater harvesting tank at the WET Centre to ensure a reliable supply throughout the experiment.

Irrigation water and fertigation to Malling™ Bella was delivered to each pot via two dripper stakes, each connected to a 1.2 L h^{-1} Netafim non-return dripper.

Irrigation was scheduled using PI control where changes in CVMC were monitored using Delta-T SM150T sensors (Delta-T Devices Ltd., Cambridge, UK). In each treatment, three sensors were connected to a Delta-T GP2 Advanced Datalogger and Controller unit. The average value from the SM150T sensors was calculated automatically by the GP2 unit using a preloaded script, and if the average CVMC value was equal to or less than the irrigation set point, the GP2 opened the solenoid valve. The duration of irrigation at each event was adjusted to deliver the target average daily run-off volume of 15% of the input volume. This PI system was used in both WW treatments and the two DD treatments outside of the period of coir drying. In each polytunnel, the GP2s were connected in series to a solar-powered Delta-T GPRS modem, which allowed remote access for daily monitoring and adjustment of the irrigation set points.

5.2.4 Coir volumetric moisture content, pore electrical conductivity and daily run-off volumes

Weekly "spot" measurements of CVMC and coir pore EC were made with a Delta-T "WET" sensor calibrated for coir. During the drying down phase, these measurements were taken three times each week.

Measurements were conducted on the first, third and fifth pot of each plot. For the 7.5 L pots, sampling holes were drilled at 60, 110 and 160 mm from the top of each pot, and for the 4.7 L pots, sampling holes were drilled at 60 and 110 mm from the top of each pot to allow insertion of the WET sensor prongs. Volumes of run-off throughout the day were measured in each treatment, where run-off from a pot was channelled into a Decagon ECRN-50 rain gauge.

The number of irrigation events and volume of water applied to each treatment each day were recorded with a Decagon ECRN-50 rain gauge. The rain gauges measuring run-off and input volumes were connected to the Delta-T GP2, and at the end of each day, volumes of daily input to, and run-off from, each pot, and the average daily % run-off, were calculated automatically using preloaded scripts. These values were uploaded onto DeltaLINK Cloud reports and dashboards for easy access and real-time monitoring on mobile devices.

5.2.5 Measurements of physiological parameters

Between 20 September and 03 October 2023, physiological measurements were taken three times a week during the drying down phase of the experiment, consisting of g_s, Pn and midday SWP. In the recovery phase, after coir rewetting on 03 October 2023, these measurements were initially taken every 1-2 days until 06 October 2023 and then weekly from 11 October 2023.

Midday SWP measurements were carried out between 11:00 and 12:30, as described in Section 2.2.5.

Leaf gas exchange parameters were also collected using the LI-6800 Portable Photosynthesis system (LICOR Biosciences Inc., Lincoln, Nebraska, USA; Figure 5-3). An automatic leaf chamber was used with the same conditions in each of the systems (6 cm^2 leaf area, flow rate of 500 µmol s^{-1}, CO_2 at 400 µmol mol^{-1}

and a 1,500 µmol saturation point m^{-2} s^{-1} PAR). Measurements were carried out between 10:30 and 12:00 using a fully expanded leaf that was exposed to sunlight on the eastern side of the canopy.

Figure 5-3. *The Li-Cor infra-red gas analyser used to quantify rates of stomatal conductance and photosynthesis in Malling™ Bella. Photo taken on 30 September 2023.*

During this experiment, two leaves from newly emerging spawn (new shoot growth from the established roots in the pot) growth from each plot were labelled, and the leaf length along the midrib measured carefully with a ruler and daily leaf elongation rates (LER) were calculated. Two nodes on new spawn growth were also labelled from each plot, and the internode length was measured carefully with a ruler, and daily stem elongation rates (SER) were calculated.

Leaf temperature was recorded on fully expanded leaves using a thermal camera (Teledyne FLIR TG167 Thermal Imaging IR Thermometer). Measurements were carried out on fully expanded leaves on the first, third, and fifth pot of each experimental plot.

5.2.6 Fruit yield and quality

Ripe berries from each experimental plot were harvested three times a week from 01 September to 09 November 2023. All berries were graded into Class 1 and waste, and the number and fresh weight of berries in each category were recorded.

Berry soluble solids content (SSC or %BRIX) was measured with a digital refractometer (Palett 100, Atago & Co. Ltd., Tokyo, Japan) using a pooled sample of juice expressed from five individual berries collected from each experimental plot. These measurements were taken three times a week during the DD treatment and weekly thereafter.

In the following cropping season (2024), ripe berries were harvested three times a week from 07 June to 30 August 2024. All berries were analysed as previously mentioned.

5.2.7 Statistical analyses

Statistical analyses were carried out using RStudio (version 2023.06.0). To determine whether differences between irrigation treatments and pot sizes were statistically significant, two-way analysis of variance (ANOVA) tests were carried out, and Tukey HSD values for $p < 0.05$ were calculated.

5.3 Results

5.3.1 Coir volumetric moisture content

Controlled coir moisture deficits were imposed to identify the CVMC values at which physiological responses were triggered in Malling™ Bella in two different rooting volumes. The averaging function of the GP2 ensured that the frequency of irrigation events was adjusted continually during and between days to maintain the CVMC within a narrow range in all four treatments.

In the DD treatment, the imposition of substrate drying began on 20 September 2023, when the first berries were beginning to ripen. The GP2 irrigation trigger set point for the 4.7 L and 7.5 L treatments was reduced from 71% to 66%, and from 67% to 62% respectively, thereafter the irrigation setpoint was reduced by 5% three times a week until the final adjustment was imposed on 02 October 2023 (Figure 5-4).

During the period from 20 September to 03 October 2023, the corresponding average CVMC determined from “spot” measurements with the WET sensor, taken from every DD 4.7 and DD 7.5 plot within the experiment, fell from 0.54 to

0.37 m^3 m^{-3} and 0.53 to 0.34 m^3 m^{-3}, respectively, with similar drying rates in the different rooting volumes. The differences in CVMC values were generally a drying down treatment effect (Table 5-1). The CVMC in the WW 4.7 and WW 7.5 treatment averaged 0.53 m^3 m^{-3} and 0.54 m^3 m^{-3}, respectively, from 17 July 2023 to the end of cropping, with an average run-off volume of 17.9% and 14.7%, respectively.

As the drying down phase commenced, significant differences between WW and DD plants in the different rooting volumes were apparent in physiological parameters and berry yield and quality (Figure 5-4). While significant differences mainly occurred during the drying-down phase, some effects of the drying-down were apparent once the rewetting of the coir had already commenced. The first significant difference was noted in Class 1 yield between WW 4.7 and DD 4.7, followed by physiological differences in g_s, Pn and SWP during the drying-down phase (Figure 5-4).

On 03 October 2023, the pots in both DD treatments were re-wetted to achieve a pre-stress CVMC value that maintained the mean daily run-off volume at approximately 15% of input. After re-wetting the pots in the DD treatment, the CVMC for DD 4.7 averaged 0.52 m^3 m^{-3} and for DD 7.5 averaged 0.50 m^3 m^{-3} for the remainder of the experiment.

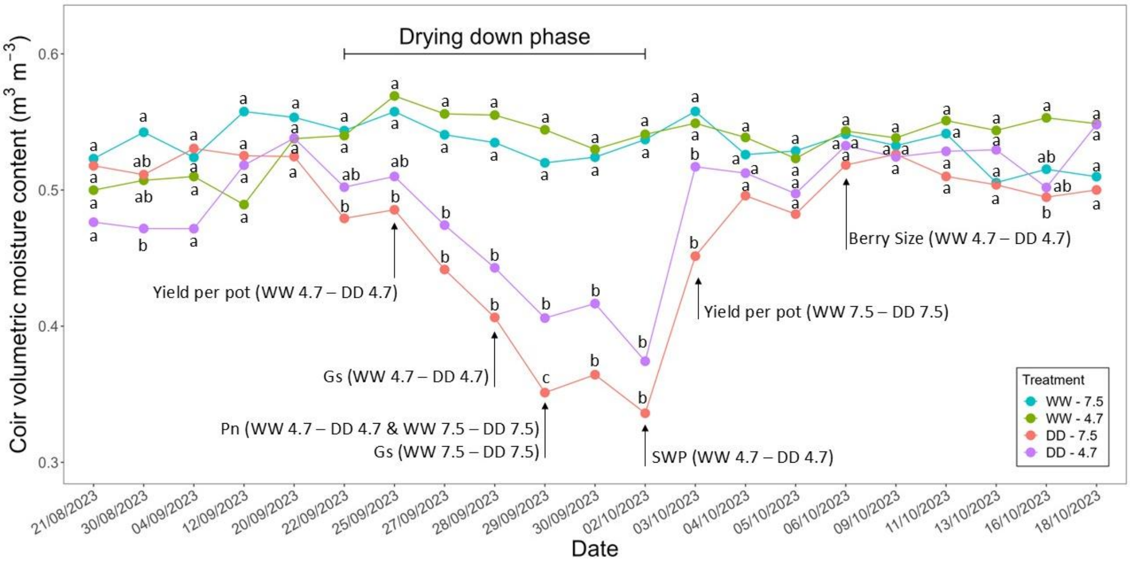

Figure 5-4. *"Spot" measurements of CVMC made using a WET sensor. Letters indicate significant differences (F.prob < 0.05) between WW 4.7, DD 4.7, WW 7.5 and DD 7.5.The information in the bracket next to the response indicates the comparison in which the significant difference was noted.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
21/08/2023	0.456	0.096	0.634
30/08/2023	0.056	0.032	0.901
04/09/2023	0.391	0.051	0.227
12/09/2023	0.932	0.067	0.135
20/09/2023	0.225	0.942	0.312
22/09/2023	<0.0001***	0.476	0.327
25/09/2023	<0.0001***	0.242	0.674
27/09/2023	<0.0001***	0.135	0.589
28/09/2023	<0.0001***	0.066	0.596
29/09/2023	<0.0001***	0.008**	0.298
30/09/2023	<0.0001***	0.065	0.139
02/10/2023	<0.0001***	0.217	0.312
03/10/2023	<0.0001***	0.110	0.038*
04/10/2023	0.053	0.330	0.895
05/10/2023	0.012	0.754	0.502
06/10/2023	0.258	0.608	0.713
09/10/2023	0.578	0.912	0.824
11/10/2023	0.098	0.413	0.794
13/10/2023	0.655	0.037*	0.678
16/10/2023	0.025*	0.131	0.301
18/10/2023	0.704	0.009**	0.784

Table 5-1. *The tabulated P-values from the "Spot" measurements of CVMC made using a WET sensor. Significance is denoted with asterisks.*

5.3.2 Effects of rooting volume on leaf physiological responses to irrigation treatments

Differences in midday SWP were only detected twice during the drying down phase, both of which were in the 4.7 L pots (Figure 5-5), and the anticipated response of a gradual negative decline in those pots that were dried down was not observed. Although a statistical difference was noted on 27 September 2023 ($p < 0.05$), this was not between WW 4.7 and DD 4.7 nor between WW 7.5 and DD 7.5, but between smaller (4.7 L) and larger pots (7.5 L), with midday SWP lowered in the former, as the difference was due to the effect of the pot size (Table 5-2). On 02 October 2023, when the average CVMC in DD 4.7 had reached 0.37 $m^3\ m^{-3}$, values of midday SWP were significantly lower ($p < 0.001$) in the DD 4.7 treatment compared to WW 4.7, but the difference between the 7.5 L WW and DD treatments was not significant.

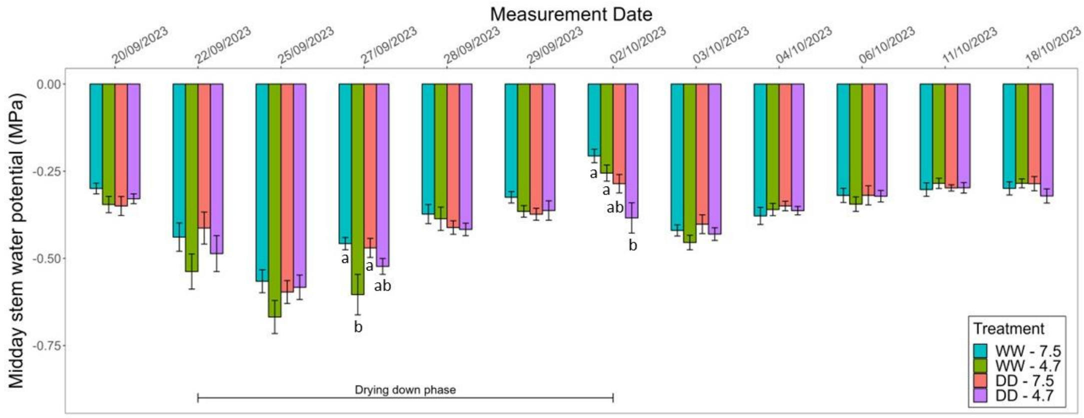

Figure 5-5. *Effects of the four treatments on stem water potential of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
20/09/2023	0.423	0.546	0.116
22/09/2023	0.410	0.072	0.786
25/09/2023	0.471	0.240	0.128
27/09/2023	0.322	0.006**	0.180
28/09/2023	0.184	0.716	0.878
29/09/2023	0.257	0.462	0.209
02/10/2023	<0.0009***	0.016*	0.407
03/10/2023	0.327	0.143	0.883
04/10/2023	0.463	0.885	0.361
06/10/2023	0.600	0.519	0.612
11/10/2023	0.793	0.555	0.569
18/10/2023	0.514	0.594	0.173

Table 5-2. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on stem water potential of Malling™ Bella. Significance is denoted with asterisks.*

Leaf expansion rate in all plants began to slow after 25 September 2023, but no significant differences were noted during the drying-down phase (Figure 5-6A; Table 5-3). Stem expansion rate in all plants also began to slow after 25 September 2023, and significant differences with a drying down effect (p = 0.006) and pot size effect (p = 0.003) between the WW 7.5 and the other three treatments (Figure 5-6B; Table 5-4).

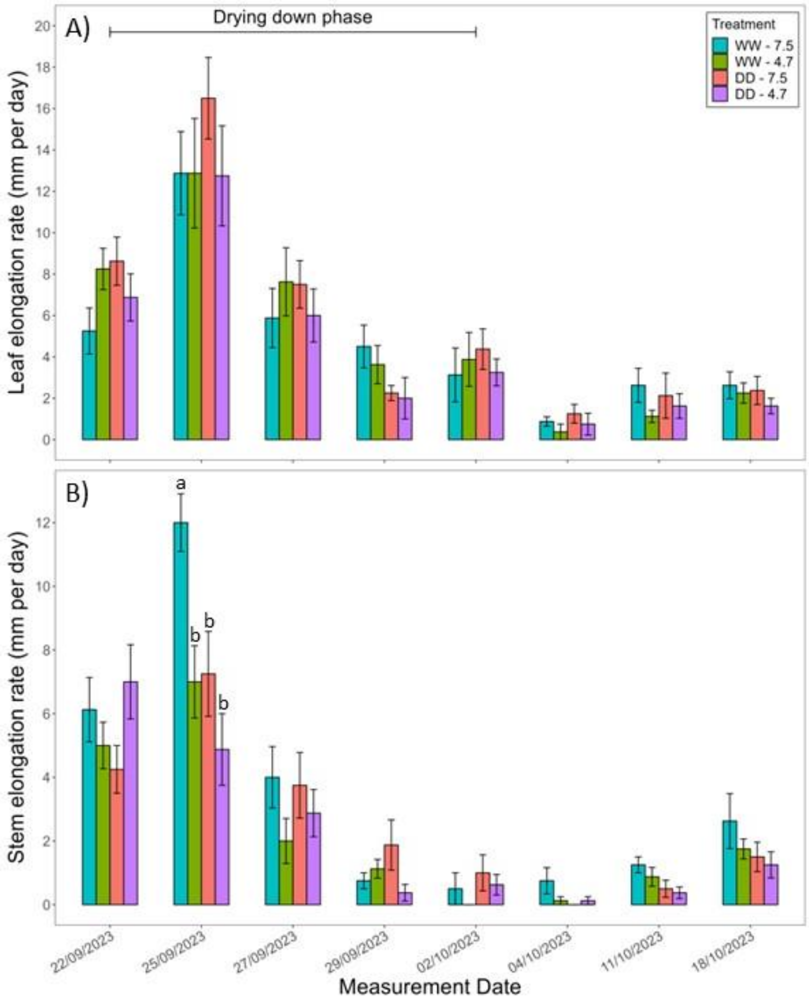

Figure 5-6. *Treatment effects on A) leaf elongation rate and B) stem elongation rate of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
22/09/2023	0.339	0.547	0.029*
25/09/2023	0.425	0.393	0.393
27/09/2023	1.000	0.926	0.232
29/09/2023	0.038*	0.532	0.728
02/10/2023	0.770	0.860	0.382
04/10/2023	0.374	0.238	1.000
11/10/2023	1.000	0.195	0.512
18/10/2023	0.452	0.335	0.746

Table 5-3. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on LER of Malling™ Bella. Significance is denoted with asterisks.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
22/09/2023	0.948	0.399	0.051
25/09/2023	0.006**	0.003**	0.260
27/09/2023	0.724	0.112	0.526
29/09/2023	0.676	0.216	0.044*
02/10/2023	0.138	0.245	0.867
04/10/2023	0.102	0.270	0.102
11/10/2023	0.021*	0.337	0.629
18/10/2023	0.123	0.280	0.545

Table 5-4. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on SER of Malling™ Bella. Significance is denoted with asterisks.*

During the drying-down phase, there were significant differences in leaf temperature on two occasions, where a treatment effect ($p < 0.05$; Table 5-5) was evident. On 03 October 2023, differences were apparent between WW 4.7 and DD 4.7, while the difference on 11 October 2023 was between WW 7.5 and DD 7.5 (Figure 5-7).

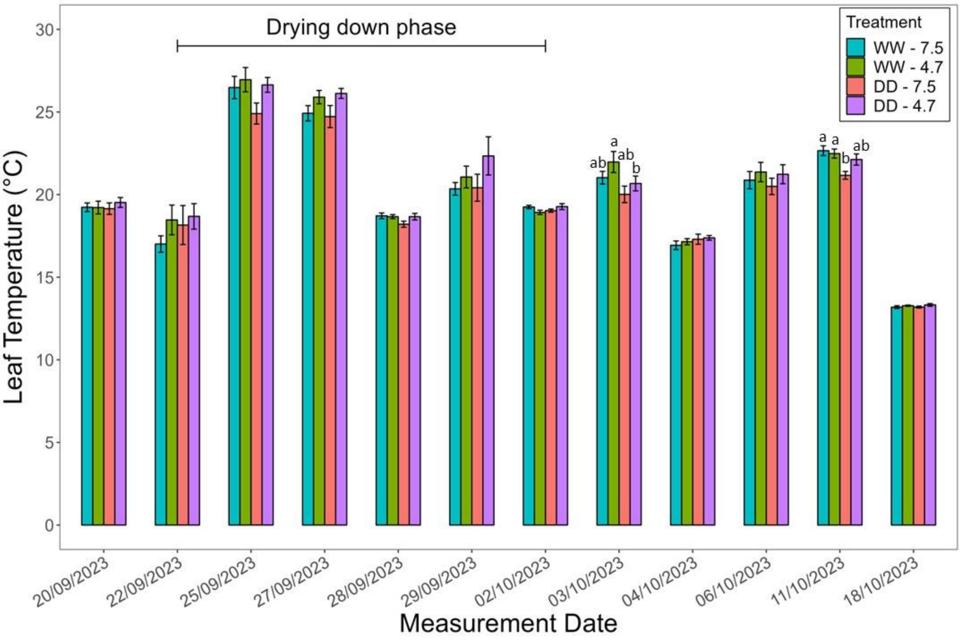

Figure 5-7. *Treatment effects on leaf temperature of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
20/09/2023	0.579	0.379	0.336
22/09/2023	0.351	0.178	0.523
25/09/2023	0.147	0.092	0.332
27/09/2023	0.979	0.018*	0.661
28/09/2023	0.128	0.219	0.128
29/09/2023	0.389	0.094	0.438
02/10/2023	0.596	0.740	0.028*
03/10/2023	0.027*	0.117	0.773
04/10/2023	0.149	0.467	0.746
06/10/2023	0.518	0.124	0.758
11/10/2023	0.001**	0.158	0.043*
18/10/2023	0.717	0.121	0.717

Table 5-5. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on leaf surface temperature of Malling™ Bella. Significance is denoted with asterisks.*

A reduction in the rate of Pn in response to coir drying was first seen on 27 September 2023, when the average CVMC in DD 4.7 and DD 7.5 had reached 0.47 m^3 m^{-3} and 0.44 m^3 m^{-3}, respectively (Figure 5-8A). On 29 September 2023, when CVMC had reached 0.41 m^3 m^{-3} for DD 4.7, and 0.35 m^3 m^{-3} for DD 7.5, Pn values in the DD-treated plants had dropped significantly ($p < 0.05$), however, there were no statistically significant differences between pot sizes. When Pn values were measured on 29 September 2023, the light settings were at 600 μmol m^{-2} s^{-1}, rather than the usual saturating light at 1500 μmol m^{-2} s^{-1}, an error not picked up during the time of recording. Therefore, the following day, the measurements were repeated using saturating light intensity, which confirmed results recorded on 29 September 2023, *i.e*. a significant impact of the drying-down treatment on Pn.

The first statistically significant reduction in g_s occurred on 28 September 2023 ($p < 0.05$), when the mean CVMC in DD 4.7 was 0.44 m^3 m^{-3}. The drying-down treatment reduced g_s on 28 September 2023, with a significant difference between 4.7 L WW and DD ($p < 0.05$) but no difference between 7.5 L WW and DD (Figure 5-8B). On 29 September 2023, g_s was significantly reduced in both rooting volumes subjected to the DD treatment ($p < 0.05$).

All DD pots were re-wetted at 06:00 on 03 October 2023: when midday SWP was measured later that day, values in both rooting volume pots in the DD treatment returned to WW values (Figure 5-5). However, Pn and g_s values remained significantly reduced in the previously DD-treated plants for different durations depending on pot volume ($p < 0.05$; Table 5-6 & 5-7). The recovery of Pn and g_s

values varied from day-to-day and were likely influenced by environmental conditions. Leaf gas exchange parameters in the DD treated different rooting volumes recovered at different rates, where Pn in the DD 4.7 recovered two days later than DD 7.5, and g_s recovered four days later in the DD 4.7 than DD 7.5 (Figure 5-8B). These results indicate that the legacy effects of transient coir drying and associated root water deficits on g_s and Pn in Malling™ Bella differed between pot sizes, with those in the smaller 4.7 L pots taking four days longer to recover fully than those plants experiencing limited water availability in the 7.5 L pots. Although CVMC values increased in both pots, values of g_s and Pn took longer to recovery (Figure 5-9 & 5-10). Positive correlation between g_s and Pn was measured in all pots during all phases of the experiment (Figure 5-11).

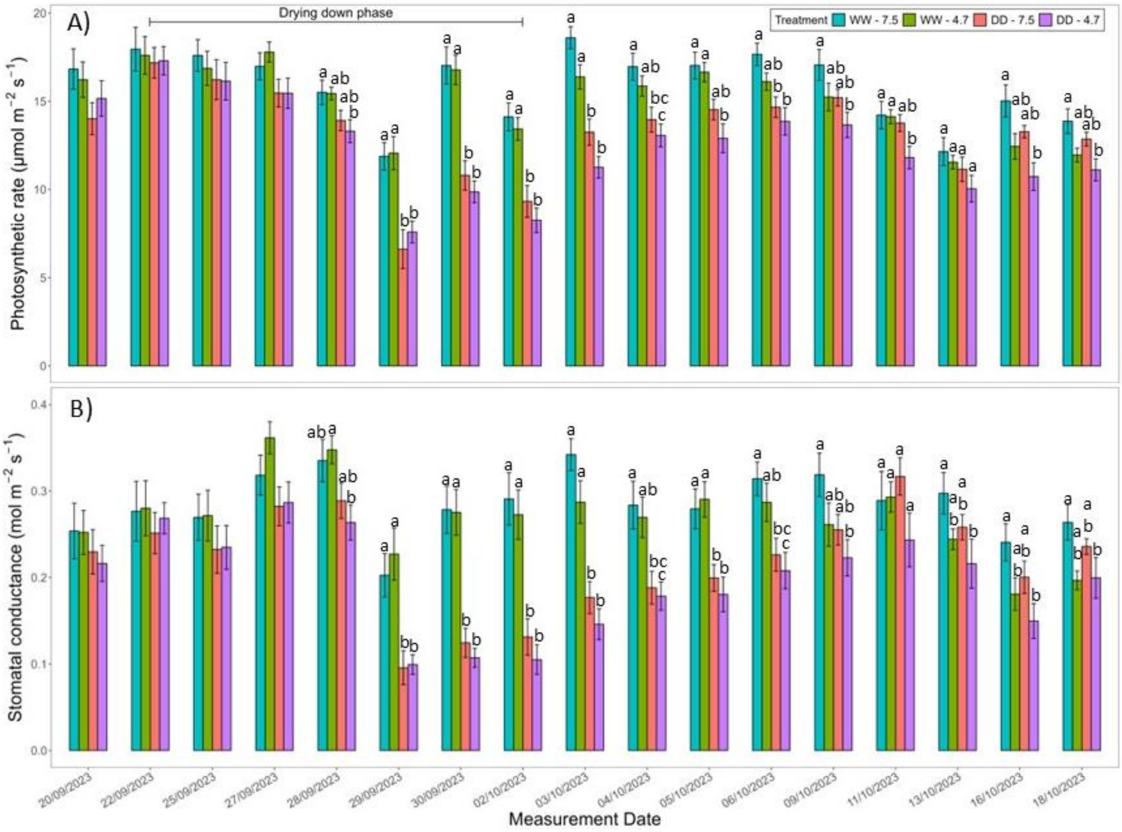

Figure 5-8. *Treatment effects on A) photosynthetic rate and B) stomatal conductance of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
20/09/2023	0.065	0.794	0.399
22/09/2023	0.600	0.907	0.820
25/09/2023	0.315	0.694	0.756
27/09/2023	0.012*	0.586	0.579
28/09/2023	0.002**	0.564	0.646
29/09/2023	<0.0001***	0.519	0.658
30/09/2023	<0.0001***	0.477	0.680
02/10/2023	<0.0001***	0.261	0.805
03/10/2023	<0.0001***	0.003**	0.869
04/10/2023	0.0001***	0.149	0.881
05/10/2023	<0.0001***	0.148	0.365
06/10/2023	0.0001***	0.071	0.559
09/10/2023	0.020*	0.023*	0.848
11/10/2023	0.021*	0.082	0.112
13/10/2023	0.073	0.217	0.715
16/10/2023	0.017*	0.0006***	0.980
18/10/2023	0.083	0.001**	0.866

Table 5-6. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on photosynthetic rate of Malling™ Bella. Significance is denoted with asterisks.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
20/09/2023	0.265	0.780	0.824
22/09/2023	0.513	0.712	0.807
25/09/2023	0.186	0.938	0.992
27/09/2023	0.016*	0.288	0.384
28/09/2023	0.002**	0.751	0.345
29/09/2023	<0.0001***	0.537	0.651
30/09/2023	<0.0001***	0.631	0.738
02/10/2023	<0.0001***	0.379	0.875
03/10/2023	<0.0001***	0.039*	0.554
04/10/2023	0.0001***	0.597	0.922
05/10/2023	<0.0001***	0.838	0.460
06/10/2023	0.0002***	0.273	0.830
09/10/2023	0.030*	0.053	0.584
11/10/2023	0.691	0.207	0.160
13/10/2023	0.106	0.024*	0.786
16/10/2023	0.062	0.005**	0.809
18/10/2023	0.475	0.005**	0.372

Table 5-7. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on stomatal conductance of Malling™ Bella. Significance is denoted with asterisks.*

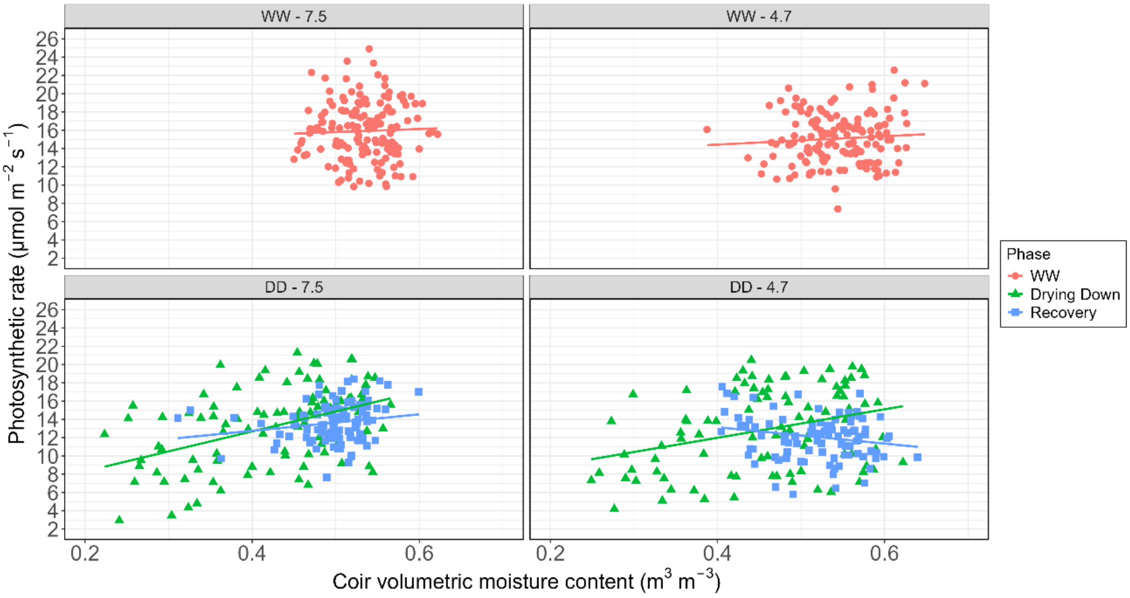

Figure 5-9. *Relationship between the Pn and CVMC of Malling™ Bella during the drying down and recovery phase. The panel are separated according to treatment and pot size, and the different colours highlight the experimental phase.*

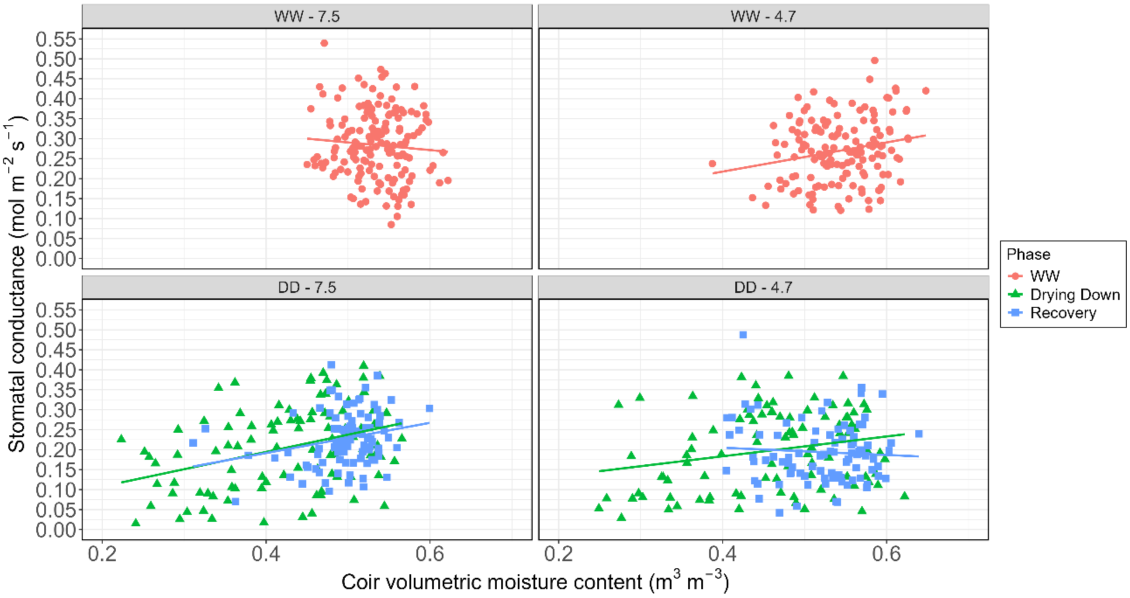

Figure 5-10. *Relationship between the g_s and CVMC of Malling™ Bella during the drying down and recovery phase. The panel are separated according to treatment and pot size, and the different colours highlight the experimental phase.*

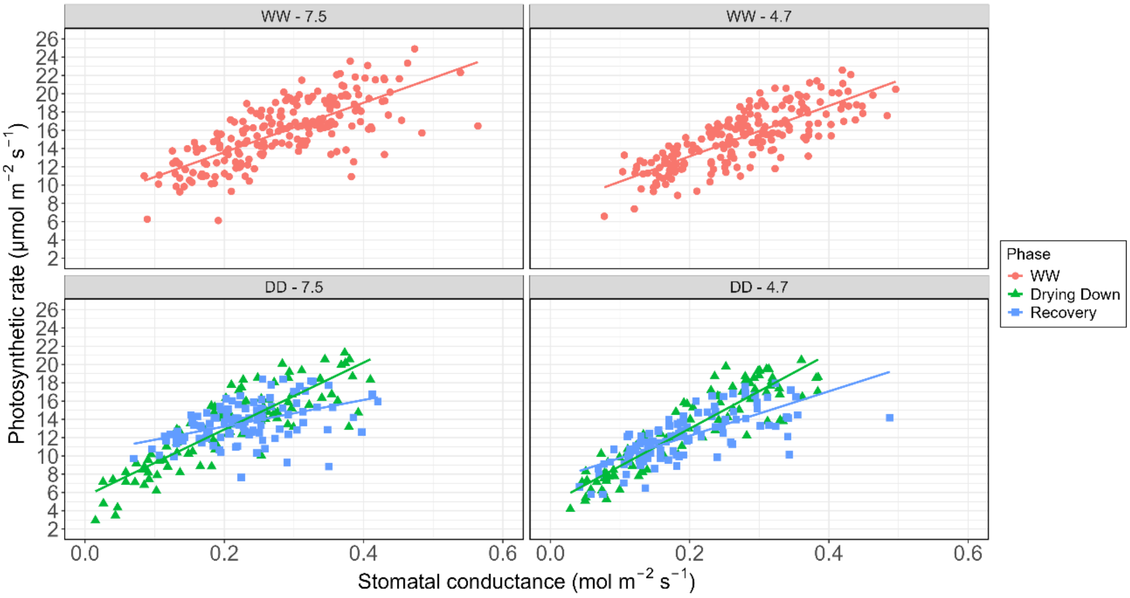

Figure 5-11. *Relationship between the g_s and Pn of Malling™ Bella during the drying down and recovery phase. The panel are separated according to treatment and pot size, and the different colours highlight the experimental phase.*

5.3.3 Yield and berry quality responses to substrate drying and rooting volume

The primocane Malling™ Bella cropped from 01 September to 09 November 2023. The mean Class 1 yield for WW 7.5 was 2.2 kg/pot compared to 1.5 kg/pot for DD 7.5, whilst for WW 4.7, the mean Class 1 yield was 2.4 kg/pot and 1.3 kg/pot for DD 4.7 (Figure 5-12A); the reduction in Class 1 yield due to the imposed DD treatment was statistically significant ($p < 0.05$). Total Class 1 berry numbers per pot were significantly lower in both DD treatments, 304 and 266 berries per pot for DD 7.5 and DD 4.7, respectively, compared to 416 and 473 berries per pot for WW 7.5 and WW 4.7, respectively (Figure 5-12B).

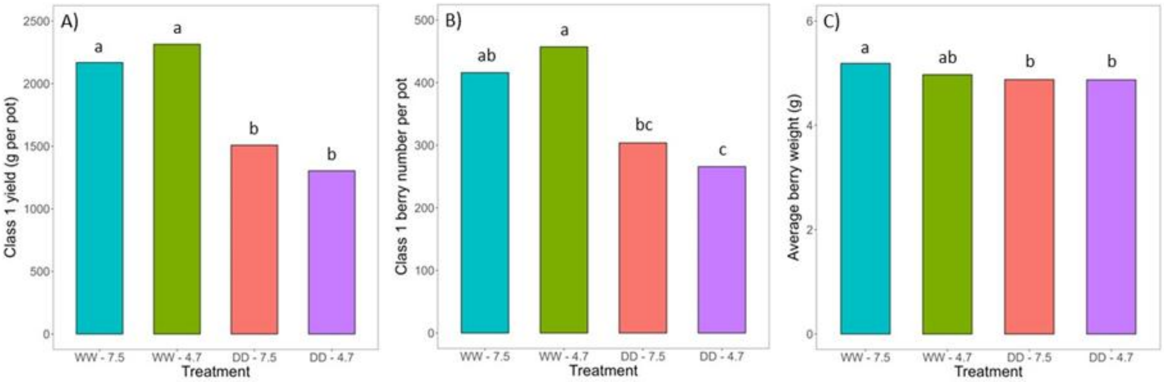

Figure 5-12. *Effect of coir drying on A) overall Class 1 yield per pot, B) total Class 1 berry number per pot, and C) mean individual berry fresh weight of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

	Class 1 yield per pot	Total Class 1 berry number per pot	Mean individual berry fresh weight
Drying down p-value	<0.0001***	0.0002***	0.008**
Pot size p-value	0.962	0.744	0.084
Interaction p-value	0.158	0.124	0.276

Table 5-8. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on Class 1 yield per pot, total Class 1 berry number per pot and mean individual berry fresh weight of Malling™ Bella (Figure 5-12). Significance is denoted with asterisks.*

A reduction in Class 1 yield in the DD treatments compared to WW plants was first noted on 25 September 2023 for plants in the DD 4.7 treatment, and on 29 September 2023 for the DD 7.5 treatment (Figure 5-13), when CVMC was 0.51 m^{3} m^{-3} and 0.35 m^{3} m^{-3} for the DD 4.7 and DD 7.5 respectively (Figure 5-4). Class 1 yield remained significantly lower in the DD pots at each pick between 7.5 L WW and DD pots until 06 October 2023 (*p* < 0.05), and between 4.7 L WW and DD pots until 16 October 2023 (*p* < 0.05), with DD pots having a lower Class 1 yield (Table 5-9).

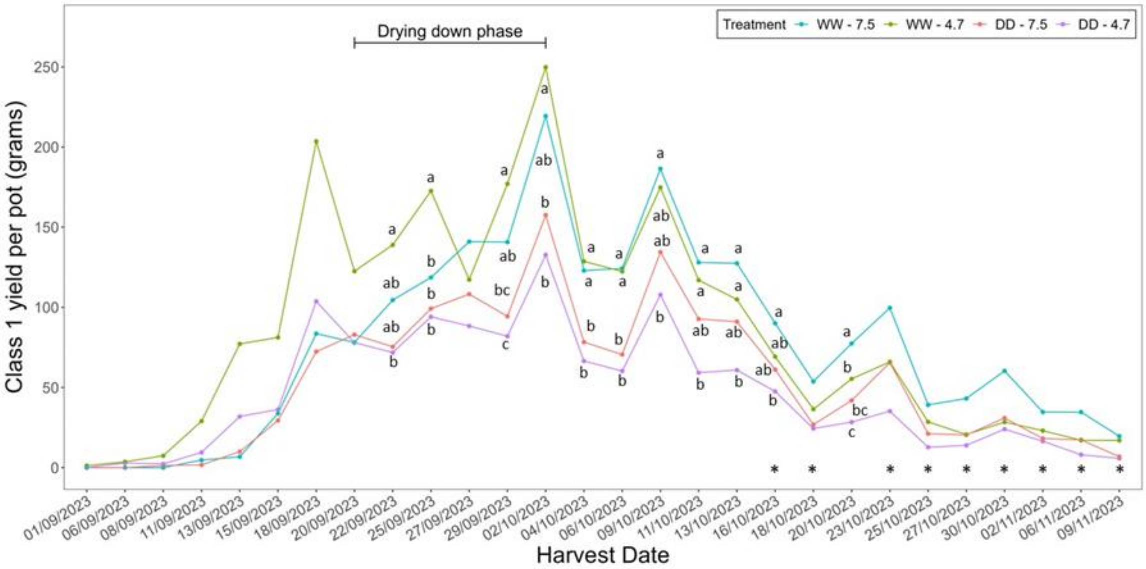

Figure 5-13. *Effects of the four treatments on Class 1 yield harvested per pot from Malling™ Bella on each pick from 06 September to 09 November 2023. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments. Asterisks on the later section of the graph indicate a significant difference (p < 0.05) on those dates as well, however the details could not be included due to the minimal space.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
06/09/2023	0.830	0.070	0.830
08/09/2023	0.230	0.018*	0.060
11/09/2023	0.055	0.011*	0.143
13/09/2023	0.064	0.0009***	0.037*
15/09/2023	0.128	0.097	0.203
18/09/2023	0.043*	0.001**	0.095
20/09/2023	0.185	0.188	0.107
22/09/2023	0.009**	0.329	0.234
25/09/2023	0.0001***	0.047*	0.021*
27/09/2023	0.173	0.325	0.929
29/09/2023	0.0003***	0.396	0.099
02/10/2023	0.001**	0.893	0.203
04/10/2023	0.0002***	0.756	0.377
06/10/2023	0.0001***	0.551	0.680
09/10/2023	0.003	0.255	0.647
11/10/2023	0.0009***	0.054	0.301
13/10/2023	0.002**	0.021*	0.705
16/10/2023	0.014*	0.073	0.687
18/10/2023	0.001**	0.050	0.124
20/10/2023	<0.0001***	0.005**	0.412
23/10/2023	0.003**	0.003**	0.844
25/10/2023	0.001*	0.032*	0.785
27/10/2023	0.002**	0.002**	0.048*
30/10/2023	0.013*	0.006**	0.051
02/11/2023	0.047*	0.223	0.358
06/11/2023	0.003**	0.003**	0.262
09/11/2023	0.0008***	0.491	0.783

Table 5-9. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on Class 1 yield per pot. Significance is denoted with asterisks.*

Total waste fruit was 93.1 g/pot from WW 7.5, 63.6 g/pot from DD 7.5, 124.7 g/pot from WW 4.7 and 79.6 g/pot from DD 4.7; differences were significantly lower in DD pots ($p < 0.05$). It should also be noted that flowers and, therefore, fruit developed more rapidly in the 4.7 L pots, such that fruit ripened over a week earlier than in the 7.5 L pots, and this could have affected the total Class 1 yield as well as the total berry number due to the reduction in thermal time and light intensity towards the end of the experiment.

Mean individual berry fresh weight was lower in the WW 4.7 treatment compared to WW 7.5 values on all measurement dates from 18 September 2023, and differences were statistically significant on several occasions ($p < 0.05$; Figure 5-14; Table 5-10). Between 18 September and 09 November 2023, mean individual berry fresh weight was 4.6 g/berry for WW 4.7 plants and 5.1 g/berry for WW 7.5 plants (Figure 5-12), a 10% reduction in berry fresh weight for plants grown in the smaller pots, although this difference was just outside statistical significance ($p = 0.06$). Individual berry fresh weight remained lowered in plants previously exposed to drying coir for up to 3 weeks after rewetting the DD pots.

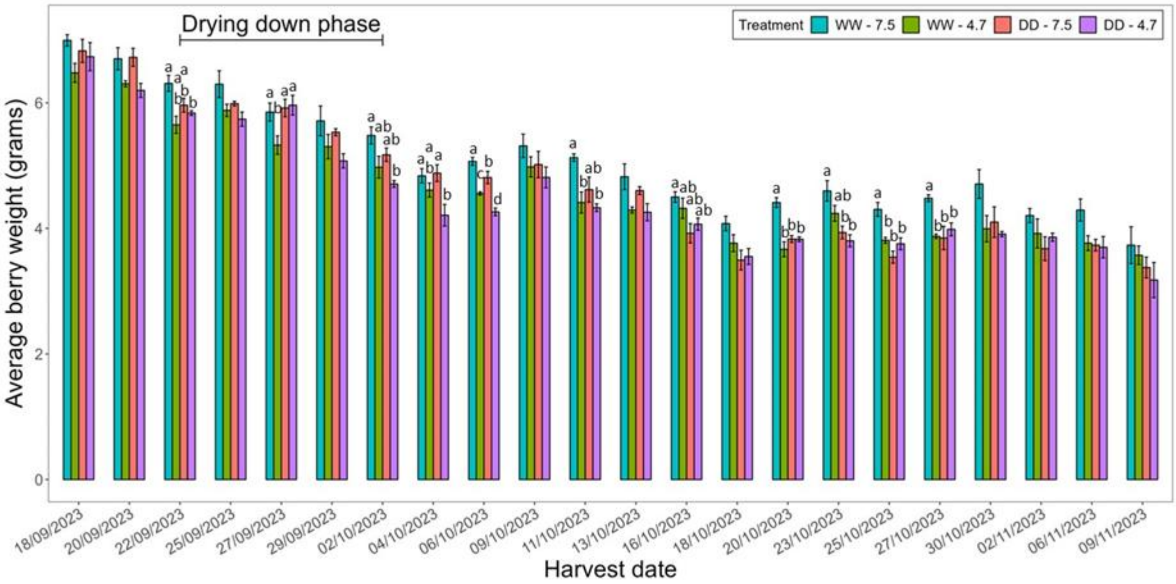

Figure 5-14. *Effects of the four treatments on average berry weight of Malling™ Bella from 18 September to 09 November 2023. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
18/09/2023	0.787	0.088	0.219
20/09/2023	0.756	0.005**	0.635
22/09/2023	0.500	0.006**	0.040*
25/09/2023	0.131	0.034*	0.550
27/09/2023	0.008**	0.047*	0.022*
29/09/2023	0.251	0.026*	0.890
02/10/2023	0.049*	0.003**	0.880
04/10/2023	0.226	0.008**	0.141
06/10/2023	0.0006***	<0.0001***	0.754
09/10/2023	0.161	0.108	0.682
11/10/2023	0.061	0.005**	0.160
13/10/2023	0.358	0.007**	0.501
16/10/2023	0.010**	0.897	0.246
18/10/2023	0.015*	0.377	0.209
20/10/2023	0.026*	0.0008***	0.0009***
23/10/2023	0.001**	0.085	0.404
25/10/2023	0.001**	0.164	0.003**
27/10/2023	0.041*	0.062	0.007**
30/10/2023	0.116	0.048*	0.224
02/11/2023	0.110	0.767	0.194
06/11/2023	0.148	0.164	0.675
09/11/2023	0.134	0.454	0.933

Table 5-10. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on average berry weight of Malling™ Bella. Significance is denoted with asterisks.*

Measurements of berry SSC (%BRIX) were made between 13 September and 09 November 2023. Soluble solid content values of berries from DD 4.7 were significantly higher than the other three treatments on three harvest dates ($p < 0.05$; Figure 5-15), with pot size generally causing the significant differences on each of the days (Table 5-11). Berry SSC values averaged over the season were slightly but significantly higher under the WW 4.7 treatment, 11.6 compared to 11.0 in the WW 7.5 treatment ($p < 0.05$). Soluble solid content values under the DD 4.7 and DD 7.5 treatments were slightly higher at 11.9 and 11.2, respectively (Figure 5-16), with pot size having a greater effect than the drying down treatment, but there was no interaction (Table 5-12).

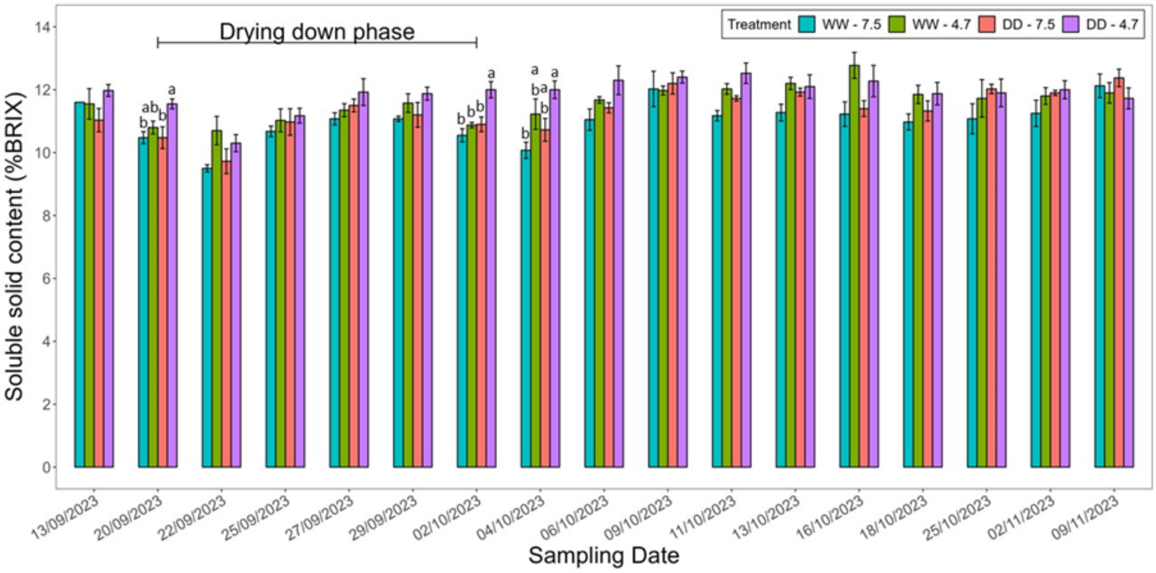

Figure 5-15. *Berry soluble solid content (%BRIX) of Malling™ Bella between 13 September to 09 November 2023. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
13/09/2023	0.979	0.203	0.336
20/09/2023	0.138	0.012*	0.138
22/09/2023	0.798	0.021*	0.369
25/09/2023	0.489	0.400	0.816
27/09/2023	0.095	0.228	0.790
29/09/2023	0.447	0.050	0.752
02/10/2023	0.004**	0.005**	0.087
04/10/2023	0.069	0.005**	0.864
06/10/2023	0.122	0.028*	0.685
09/10/2023	0.407	0.834	0.727
11/10/2023	0.025*	0.002**	0.905
13/10/2023	0.307	0.054	0.172
16/10/2023	0.691	0.010*	0.414
18/10/2023	0.555	0.040*	0.608
25/10/2023	0.234	0.569	0.405
02/11/2023	0.167	0.283	0.451
09/11/2023	0.911	0.209	0.532

Table 5-11. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on berry soluble solid content. Significance is denoted with asterisks.*

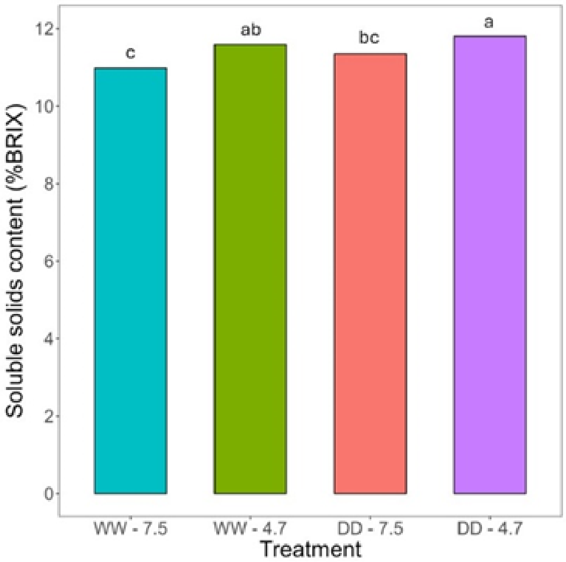

Figure 5-16. *The effect of drying down on overall berry soluble solids content (%BRIX) of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

	Overall berry soluble solids content
Drying down p-value	0.018*
Pot size p-value	0.006***
Interaction p-value	0.739

Table 5-12. *The tabulated P-values of the effects of the drying down treatment and different pot sizes on overall berry soluble solids content of Malling™ Bella. Significance is denoted with asterisks.*

5.3.4 The effect of substrate drying and rooting volume from the previous year on subsequent cropping potential

The floricane Malling™ Bella plants used as primocanes in experiments in 2023 cropped from 07 June to 30 August 2024, and the effects of the drying down treatment and rooting volume from the previous year on the following years cropping was investigated. The mean Class 1 yield for WW 7.5 and DD 7.5 was 3.3 kg/pot, whilst WW 4.7 averaged at 3.1 kg/pot compared to 2.5 kg/pot for DD 4.7 treatment, even though this was not statistically different (Figure 5-17A), although pot size had an effect on the differences in Class 1 yield (Table 5-13). Total berry numbers were significantly lower in DD 4.7 compared to WW 4.7, with 548 and 700 berries per pot (two plants per pot), compared to 671 and 717 berries per pot for the WW 4.7 and WW 7.5, respectively (Figure 5-17B).

A reduction in Class 1 yield from the DD 4.7 treatment was evident from the early harvest, with the first significant decrease detected on 17 June 2024 ($p < 0.05$; Figure 5-18), with pot size having the great effect throughout the experiment (Table 5-14). Class 1 yield remained lower between DD 4.7 and other treatments until 19 July 2024; thereafter, values were similar between treatments. Average berry fresh weight did not statistically differ between treatments (Figure 5-19; Table 5-15) and therefore not statistically different over the season (Figure 5-17C).

Measurements of berry SSC were made between 12 June and 27 August 2024. Apart from berry SSC measurements on 31 July 2024, mean berry SSC values did not differ between treatments, with the previous seasons coir drying treatment having no effect on berry SSC the following year (Figure 5-20; Table 5-16). Berry SSC values averaged at 11.7 and 11.5 for WW 4.7 and DD 4.7 compared to 11.4 for both DD 7.5 and WW 7.5.

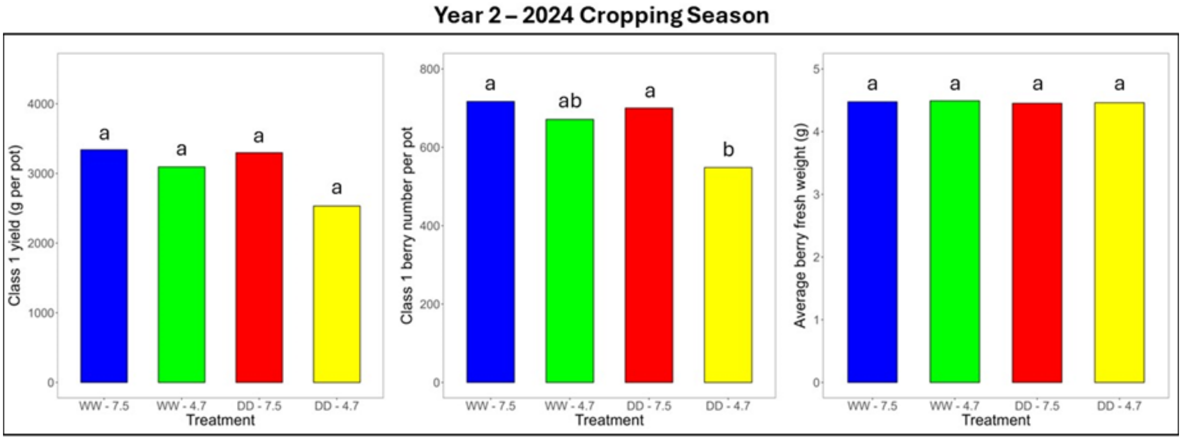
Year 2 – 2024 Cropping Season

Figure 5-17. *Effect of coir drying from the previous cropping season on A) overall Class 1 yield per pot, B) total Class 1 berry number per pot, and C) mean berry weight in the second year cropping of Malling™ Bella. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

	Class 1 yield per pot	Total Class 1 berry number per pot	Mean individual berry fresh weight
Drying down p-value	0.204	0.070	0.859
Pot size p-value	0.045*	0.016*	0.934
Interaction p-value	0.270	0.156	0.985

Table 5-13. *The tabulated P-values of the effects of the drying down treatment and different pot sizes from the previous cropping season on Class 1 yield per pot, total Class 1 berry number per pot and mean individual berry fresh weight on the second year cropping of Malling™ Bella. Significance is denoted with asterisks.*

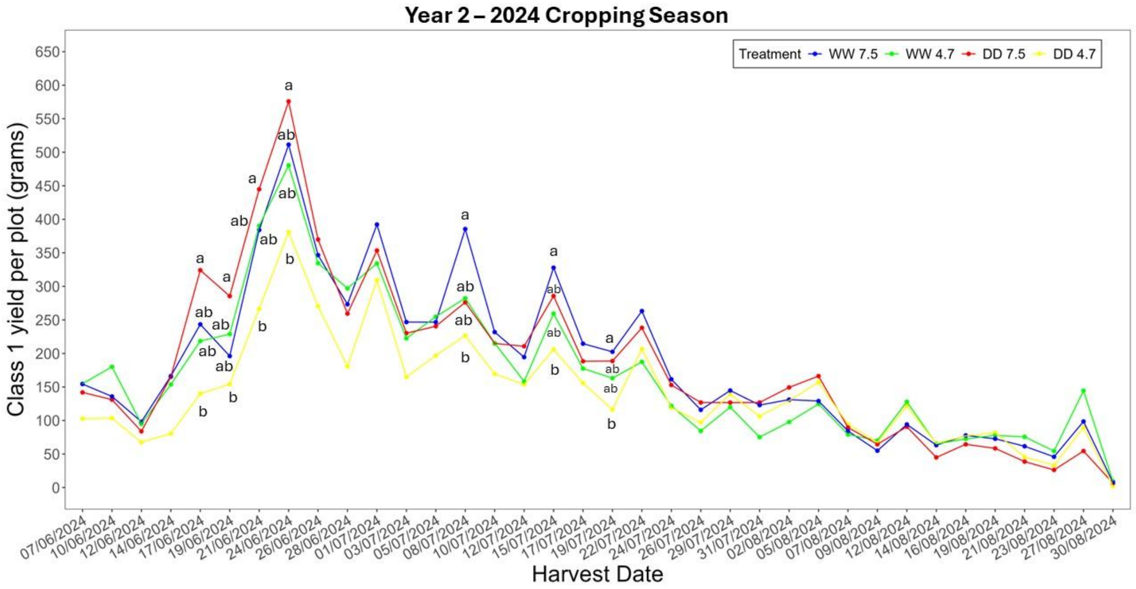

Figure 5-18. *Effects of the four treatments from the previous year on Class 1 yield harvested per pot from Malling™ Bella on each pick from 07 June to 30 August 2024. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
07/06/2024	0.265	0.502	0.491
10/06/2024	0.203	0.790	0.260
12/06/2024	0.207	0.548	0.689
14/06/2024	0.169	0.076	0.182
17/06/2024	0.980	0.030*	0.092
19/06/2024	0.817	0.130	0.015*
21/06/2024	0.453	0.047*	0.034*
24/06/2024	0.717	0.025*	0.097
26/06/2024	0.575	0.134	0.236
28/06/2024	0.041*	0.373	0.103
01/07/2024	0.379	0.162	0.846

03/07/2024	0.134	0.073	0.402
05/07/2024	0.260	0.527	0.360
08/07/2024	0.030*	0.043*	0.461
10/07/2024	0.215	0.221	0.562
12/07/2024	0.794	0.049*	0.642
15/07/2024	0.102	0.014	0.845
17/07/2024	0.232	0.087	0.907
19/07/2024	0.208	0.017*	0.456
22/07/2024	0.919	0.067	0.439
24/07/2024	0.769	0.061	0.878
26/07/2024	0.488	0.078	0.962
29/07/2024	0.970	0.749	0.327
31/07/2024	0.347	0.073	0.463
02/08/2024	0.245	0.219	0.748
05/08/2024	0.243	0.820	0.936
07/08/2024	0.563	0.991	0.778
09/08/2024	0.832	0.573	0.690
12/08/2024	0.846	0.218	0.954
14/08/2024	0.531	0.418	0.495
16/08/2024	0.842	0.862	0.681
19/08/2024	0.762	0.411	0.594
21/08/2024	0.027*	0.361	0.754
23/08/2024	0.084	0.498	0.950
27/08/2024	0.061	0.122	0.831
30/08/2024	0.096	0.555	0.235

Table 5-14. *The tabulated P-values of the effects of the drying down treatment from the previous year and different pot sizes on Class 1 yield per pot of the second cropping season. Significance is denoted with asterisks.*

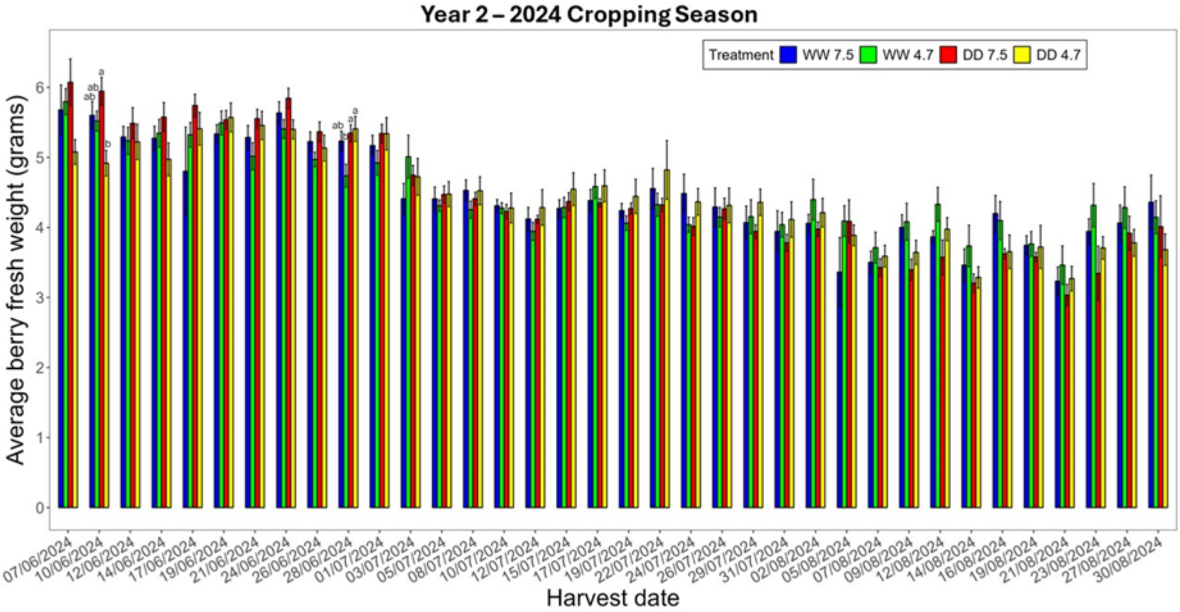

Figure 5-19. *Effects of the four treatments from the previous cropping season on average berry weight of Malling™ Bella on this year's cropping season from 07 June to 30 August 2024. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
07/06/2024	0.552	0.115	0.049*
10/06/2024	0.490	0.006**	0.016*
12/06/2024	0.663	0.440	0.611
14/06/2024	0.863	0.205	0.110
17/06/2024	0.281	0.278	0.524
19/06/2024	0.386	0.559	0.707
21/06/2024	0.056	0.312	0.629
24/06/2024	0.483	0.026*	0.456
26/06/2024	0.300	0.103	0.935
28/06/2024	0.009**	0.132	0.057
01/07/2024	0.088	0.457	0.471
03/07/2024	0.918	0.240	0.206

05/07/2024	0.413	0.736	0.709
08/07/2024	0.609	0.580	0.190
10/07/2024	0.760	0.951	0.745
12/07/2024	0.304	0.973	0.297
15/07/2024	0.276	0.557	0.620
17/07/2024	0.944	0.197	0.897
19/07/2024	0.166	0.953	0.208
22/07/2024	0.630	0.624	0.191
24/07/2024	0.669	0.776	0.023*
26/07/2024	0.705	0.797	0.599
29/07/2024	0.840	0.213	0.404
31/07/2024	0.844	0.340	0.598
02/08/2024	0.482	0.141	0.784
05/08/2024	0.426	0.420	0.162
07/08/2024	0.573	0.289	0.888
09/08/2024	0.013*	0.420	0.694
12/08/2024	0.116	0.038*	0.879
14/08/2024	0.112	0.420	0.662
16/08/2024	0.021*	0.858	0.786
19/08/2024	0.619	0.691	0.796
21/08/2024	0.347	0.283	0.986
23/08/2024	0.039*	0.191	0.972
27/08/2024	0.203	0.855	0.478
30/08/2024	0.431	0.491	0.834

Table 5-15. *The tabulated P-values of the effects of the drying down treatment from the previous year and different pot sizes on average berry weight of Malling™ Bella in the second cropping season. Significance is denoted with asterisks.*

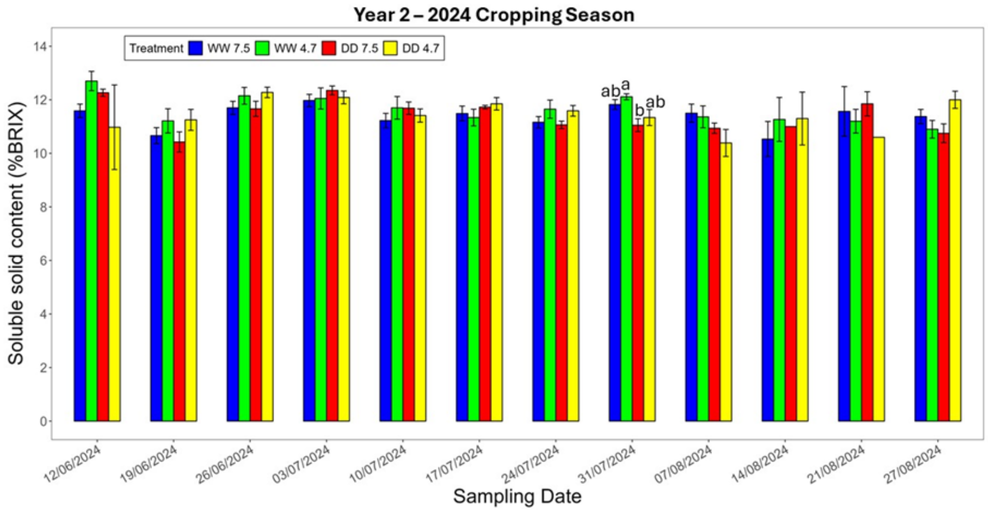

Figure 5-20. *Effects of the four treatments from the previous year on berry soluble solid content (%BRIX) of Malling™ Bella between 12 June to 27 August 2024. Different letters indicate a significant difference (F.prob < 0.05) in responses between treatments.*

Date	Drying down p-value	Pot Size p-value	Interaction p-value
12/06/2024	0.509	0.912	0.138
19/06/2024	0.799	0.086	0.727
26/06/2024	0.863	0.044*	0.748
03/07/2024	0.459	0.736	0.544
10/07/2024	0.776	0.745	0.228
17/07/2024	0.132	0.959	0.574
24/08/2024	0.736	0.043*	0.938
31/07/2024	0.001**	0.192	1.000
07/08/2024	0.057	0.381	0.598
14/09/2024	0.744	0.610	0.644
21/08/2024	0.932	0.478	0.619
27/08/2024	0/235	0.671	0.038*

Table 5-16. *The tabulated P-values of the effects of the drying down treatment from the previous year and different pot sizes on berry soluble solid content of Malling™ Bella in the second cropping season. Significance is denoted with asterisks.*

5.4 Discussion

5.4.1 Drying down and rooting volume effects on leaf physiological parameter

Water is essential for plant growth and survival, and reduced availability can affect various plant physiological responses (Bradford & Hsiao, 1982), including stomatal conductance and photosynthetic rate (Medrano *et al*., 1997; Beis & Patakas, 2010; Miyashita *et al*., 2005). Therefore, reducing the likelihood or preventing water deficit stress is important in ensuring optimal rates of photosynthesis. However, the use of smaller pot sizes in commercial raspberry production is becoming more frequent. Here, the effects of (1) using smaller 4.7 L pots compared to larger 7.5 L pots and (2) a rootzone water deficit treatment on leaf physiological parameters as well as the effects on berry yield and quality were assessed.

Midday SWP is considered to be a sensitive and reliable measure of plant-based water stress (McCutchan & Shackel, 1992; Choné *et al*., 2001). However, in this experiment, no differences in midday SWP between treatments were noted except from the last day of the coir drying treatment (Figure 5-5), and there were no significant changes in the environmental conditions on those days when midday SWP changes were evident that could have influenced these changes (Figures A-7 to A-10). On the last day of the drying down treatment, whilst differences in midday SWP between the 4.7 L WW and DD were apparent, a difference was not found between the 7.5 L WW and DD. Water potential gradients in each section of the plants can be controlled by changes in hydraulic resistance in those parts (Scharwies & Dinneny, 2019), suggesting that when a plant experiences a rootzone water deficit stress, the changes in hydraulic resistance may have controlled water potential, hence no significant differences were measured.

Research has reported that leaf growth is restricted when plants experience a water deficit stress (Zhang & Davies, 1989; Medyouni *et al*., 2021). In tomato, a partial rootzone drying treatment resulted in a slower LER five days after the water deficit stress commenced (Sobeih *et al*., 2004). In this experiment, the mild rootzone water deficit stress imposed did not affect LER, and neither did the different rooting volumes (Figure 5-6A). However, as the experiment continued, leaf expansion rate slowed in all four treatments as leaves matured. Air temperature (Figure-A7) and PAR (Figure-A10) continued to fall as the experiment progressed, resulting in the leaves not expanding quickly enough in the WW plants to detect a difference. With the experiment starting in late September and running into October, plants experienced fewer daylight hours compared to earlier months. Low light also causes stress to plants, and long-term low light exposure can lead to the destruction of membrane integrity (Zhu *et al*., 2017), preventing the leaves from growing. A similar trend was observed in SER values, with no significant differences noted between the pot sizes or due to the drying down treatment on any of the measurement dates (Figure 5-6B). Similarly, research has shown that water deficits did not cause a reduction in plant height growth compared to other treatment methods, like plant growth retardants (Alem *et al*., 2015). Aside from all the factors mentioned, as this experiment took place in the autumn, it was likely that plant development had slowed significantly compared to the summer months, and therefore as the plants matured, differences in LER and SER were not measured.

Measurements of Pn were low on 29 September 2023 when the saturating light was set to 600 µmol m^{-2} s^{-1}, which is below the light saturation point of 700 µmol m^{-2} s^{-1} for Malling™ Bella (Figure-A3), hence the measured lower Pn values of WW plants on that day. With the experiment taking place late September into October, weather conditions, including VPD, RH and air temperature (Figures A-7 to A-9), were less demanding than those usually prevalent over the summer months. The maximum air temperature recorded during the experiment was 26°C, and the highest VPD value was below 2.1 kPa. These minimal variabilities in environmental conditions are important in ensuring that other stresses did not contribute to the effects of different rooting volumes on response to, and recovery from, rootzone water deficit stress. In red raspberry, high VPD (1.75-2.25 kPa in the afternoon) and low leaf temperatures did not affect g_s or Pn (Qiu *et al*., 2017); these values of VPD were similar on some days during the experiment. Therefore, the differences in g_s and Pn on those days were unlikely to be caused by environmental conditions and more likely to be caused by the plants response to the rootzone water deficit stress and different rooting volumes. Values of higher VPD than that reported by Qui *et al*. (2017) may effect g_s or Pn, hence when carrying out leaf gas exchange measurements, environmental factors should be taken in consideration, to analyse the effects of the independent variable on the dependent variable. Research on lettuce has also shown stable environments can ensure higher stomatal conductance and photosynthesis (Inoue *et al*., 2021).

Plants under non-stressed conditions have a higher and more constant net photosynthesis rate (Brunetti *et al*., 2019), and other research has shown that drought-stressed plants can have a 70% lower photosynthetic rate compared to well-watered plants (Maroco *et al*., 2002). The drying down treatment caused a reduction in Pn and g_s (Figure 5-8), with responses to the drought stress in the different pot sizes occurring on the same day. Research has looked into the adverse effects on plant physiological processes caused by rootzone water deficits on grapevine (Maroco *et al*., 2002; Tombesi *et al*., 2015), tomato (Medyouni *et al*., 2021) and poplar trees (Brunetti *et al*., 2019). These studies all highlighted decreased stomatal conductance and photosynthesis as volumetric water content decreased, regardless of the rooting volume.

Responses to water deficit stress and the time taken to recover are thought to be mediated through signalling responses. There is a substantial amount of literature that indicates that increased xylem ABA concentrations cause stomatal closure when a plant experiences a water deficit stress (reviewed in Dodd, 2003). Research carried out on olives plants grown in 3 L pots (Torres-Ruiz *et al*., 2015) and tomatoes grown in 1 L pots (Sobeih *et al*., 2004) highlights the important role of both hydraulic and chemical signalling during stress events and during recovery, indicating that despite the difference in the rooting volume in the different species, hydraulic and chemical signalling were important in stress response and recovery. Torres-Ruiz *et al*. (2015) found no relationship between stem or root ABA and stomatal behaviour. Measuring ABA concentrations in different compartments is important, as ABA can redistribute depending on factors like pH, as increases in apoplastic pH, when a plant experiences stress, can increase ABA concentration by 2.5 fold in the xylem (Daeter *et al.*, 1993), resulting in altered concentrations in different compartments of the plant. However, leaf ABA and hydraulic signals can explain the regulation of stomatal conductance and Torres-Ruiz *et al.* (2015) mentioned that hydraulic signalling was the main effector in the stomatal response by distal organs of the plant (like leaves) imposing a hydraulic limitation. As hydraulic capacity of a root system to deliver water to the shoot can increase with root surface area (Gambetta *et al*., 2012), the increase in rooting volume in the experiment described in this chapter could have affected the hydraulic signalling once rewetting of the coir commenced, with a quicker hydraulic recovery compared to the smaller rooting volume.

An increase in xylem sap pH was constantly observed when explaining reasons for stomatal closure; increased xylem sap pH can act as a signal to close stomata without xylem ABA concentrations increasing (Sobeih *et al*., 2004). With the uptake of water potentially greater in the larger rooting volume, the recovery of xylem sap pH back to pre-stress values could have occurred quicker, hence the quicker recovery of g_s values in the larger rooting volume pots. Stomatal conductance is primarily regulated by hydraulic mechanisms in grapevine, and foliar ABA limits leaf gas exchange over a longer period of time, which is thought to prevent the recovery of stomatal aperture once rewetting commences

(Tombesi *et al*., 2015). The rate of removal of ABA from leaves by the phloem can affect the ABA signal; if less ABA exits the leaf, then there is a higher chance that the remaining ABA will penetrate the guard cells (Wilkinson & Davies, 2002). There is more ABA transported out of detached maize leaves in the phloem when the buffer is at a well-watered pH (5.5) rather than a droughted pH (7.5) in the xylem (Jia & Zhang, 1997), and a water deficit stress can affect the xylem pH (Gloser *et al*., 2016). A plant continuously adapts its root system architecture to optimise water needs (Hong *et al*., 2013) and when under drought stress, root hair density is enhanced as the plant tries to increase water uptake (Schnall & Quatrano, 1992). Larger pots can result in a greater volume and longer roots (Obede da Silva Aragão *et al.*, 2020). All these factors would have increased water uptake rates in the larger pot size, potentially affecting the signalling mechanism and the recovery of xylem pH in plants in the larger rooting volume pots, enabling a quicker recovery from the water deficit stress.

The reported relationship between rooting volume and photosynthesis rates appears to vary across plants species. With decreasing pot size, photosynthetic rate increases in vegetative bean (Carmi *et al*., 1983), decreases in tobacco during flowering and fruiting (Herold & McNeil, 1979) and did not change in vegetative soybean (Krizek *et al*., 1985). While the developmental stage may have effected the results in these experiments, decreasing pot size can have different effects on photosynthetic rate in different species. While xylem ABA increases during drought stress, rooting volume did not cause changes in xylem ABA concentration in *Vigna unguiculata* (Ismail *et al*., 1994). In pepper subjected to root growth restrictions by reducing the rooting volume, increases in xylem sap ABA concentration were somewhat the factor for the reduction in g_s values (Ismail & Davies, 1998), hence the indication that signalling mechanisms play a role in the regulation of g_s and Pn values in different rooting volumes. Rooting volume did not affect Pn or g_s values in WW plants under the reported conditions as plants were supplied with fertigation throughout the day to ensure an overall 15% run-off at the end of the day, which is thought to be optimal (M. Davies, 2021, pers. comm.). However, recovery rates of stomatal conductance and photosynthesis values following a rootzone water deficit stress were affected, with plants in smaller pots taking longer to recover. As the drying-down treatment occurred simultaneously for both rooting volume pots and reached similar CVMC values at the end of each drying-down phase, neither environmental conditions nor the coir moisture content *per se* could have resulted in the legacy effect on photosynthesis.

Water deficit stress raised the leaf temperature in all genotypes of common bean, and it was stated that drought-tolerant genotypes had warmer leaves associated with lower stomatal conductance (Ghanbari *et al*., 2013). However, the measurements in this chapter did not indicate that water deficit stress caused changes in leaf temperature in raspberry. When g_s was lowered in DD plants, an anticipated rise in leaf surface temperature was expected. However, not obtaining significant differences in those results suggests a need for greater sensitivity to these measurements, particularly in detecting minor changes in leaf surface temperature. This was similar to research in grapevine, which also indicated no difference in leaf temperature when under drought conditions (Maroco *et al*., 2002), suggesting partial stomatal closure (and not full closure) was enough to keep leaf temperatures within the range of control plants.

5.4.2 Coir drying reduced Class 1 yield in the same cropping season

Ripe raspberries were harvested for 10 weeks in the first cropping season, and continued until two weeks before the first frosts, whereby yields per pot had significantly reduced. Flowering occurs over a longer period of the growing season when temperatures are cooler (Hatfield & Prueger, 2015), allowing the cropping to continue into early November in the first cropping season.

Class 1 yields in WW plants were similar to those of other raspberry cultivars (Morales *et al*., 2013). Pot size did not affect yield in blueberry production (Pinto *et al*., 2017). However, a rootzone water deficit stress did affect Class 1 yield and number of berries in Malling™ Bella and similar results were reported in other raspberry cultivars (Ortega-Farias *et al*., 2022; Morales *et a*l., 2013); in tomato, fresh weight, fruit size and production dry matter were all reduced following a water deficit stress (Medyouni *et al*., 2021).

The effect of coir drying on individual berry weight may not be apparent at the time of drying, but have a legacy effect on fruit that is harvested some weeks after the event; presumably, the effect was exerted on unripe berries that were present on the plant at the time of limited water availability, ripening some weeks later and displaying this prolonged legacy effect on berry size. Water deficit stress during the cell expansion of the fruit has the largest impact on final berry size and, therefore, affects yield (Molitor & Junk, 2019). Under a water deficit stress, plant growth is reduced due to inhibition of cell expansion (Hsiao *et al*., 1997) and reductions in fruit growth are common (Ebel *et al*., 1993). With less water available, fewer berries, as well as smaller berries, were formed under the coir drying treatments. When plants are under water deficit stress, this can affect the ripening process and accelerate the ripening of berries (Castellarin *et al*., 2007). Hence, berries ripen and are smaller under water deficit stress. Smaller berries were also more prevalent in 4.7 L compared to 7.5 L WW plants, which could be due to the water and nutrient availability in the different rooting volume pots. Berry weight in grapes also decreased as a result of water deficit stress, as water deficit stress modified cell structural properties and limited subsequent enlargement of pericarp cells (Ojeda *et al*., 2001). Increases in SSC in berries were measured in those that were imposed to a water deficit stress and as water availability decreases, the skin-to-pulp weight ratio increases as berry size is reduced, causing an increase in phenolic compounds (Ojeda *et al*., 2002).

In the second cropping season, raspberries were harvested for a total of 12 weeks to see whether legacy effects on berry production from the treatments imposed in the previous year impacted on cropping potential in the following year. Apart from significant reductions in the mean Class 1 berry number per pot in the smaller rooting volume pots that were exposed to a rootzone water deficit stress the cropping season before, legacy effects from the previous cropping season were not apparent. On larger scale production systems, the reduction of Class 1 berry numbers per pot in smaller rooting volume pots could affect total Class 1 yield and, therefore, affect total revenue.

5.5 Conclusions

The impact of rooting volume on plant physiological parameters following rootzone water deficit stress and during the recovery phase were investigated. Primocane Malling™ Bella grown in smaller 4.7 L pots are expected to exhibit more pronounced legacy effects on leaf gas exchange than those potted into 7.5 L pots. Following the rewetting of the coir, leaf physiology took longer to recover in the DD 4.7 treatment, taking 8 days to recover, compared to the recovery of 6 days in plants in the DD 7.5 treatment. This legacy effect on leaf physiology then eventually affected berry yield and quality parameters, as limited coir water availability in the DD 4.7 treatment reduced total Class 1 yield and total berry number significantly compared to WW 4.7 values. Furthermore, individual berry fresh weight in DD 4.7 and DD 7.5 treatments was significantly reduced after the coir had been rewetted, and this legacy effect persisted for 3 weeks. Also, the imposed rootzone water deficit stress increased berry SSC in both DD 4.7 and DD 7.5 treatments, presumably *via*. effects on individual fruit fresh weight.

The risks of using 4.7 L pots in commercial production can be detrimental even if a mild transient rootzone water deficit stress was to occur. This work demonstrated the importance of ensuring that plants receive effective and efficient irrigation to crops and that using larger pots for raspberry cultivation is beneficial for increased yield and berry quality.

6 General Discussion

The frequency and severity of drought is expected to increase globally with climate change (Cook *et al*., 2014). As temperatures have been increasing, so have the frequency of summer droughts in England and Wales (Cole *et al*., 2006). Drought events limit the water availability in the rootzone, causing plants water deficit stress. The uptake of water for transpiration coupled with limited water availability causes the substrate to dry, which can affect plant growth, development and function (Davies *et al*., 2002). Depending on the duration and intensity of a rootzone water deficit stress, plant physiological processes can be affected (McDowell *et al*., 2008; Tombesi *et al*., 2015). Plant responses to a rootzone water deficit stress are controlled by chemical and hydraulic signalling, although different species can and do respond to rootzone water deficit stresses differently, and so some species are better able to tolerate and recover from drought events than others. These transient stresses are particularly important in crop plants, whereby even minor transient stresses can impact marketable yields and fresh produce quality. These negative impacts can occur at relatively high, but still sub-optimal, substrate water contents.

The research in this PhD programme focused on: (i) understanding the leaf physiological responses to rootzone water deficit stress in red raspberry and (ii) investigating the causal signalling that regulates the stress response and recovery to a rootzone water deficit stress. The research will quantify the likely effects of this stress on marketable yields and berry quality; this information could help UK raspberry growers to make informed decisions about how to minimise the risk of transient root water deficits in commercial production.

6.1 Transient rootzone water deficit stresses and signalling mechanisms

Research has been undertaken on red raspberry responses to a range of different abiotic stresses, including low-temperature (Chang *et al*., 2023), high temperature (Stafne *et al*., 2001), low and high VPD (Qiu *et al*., 2017) and drought stress (Morales *et al*., 2013; Ortega-Farias *et al*., 2022). However, published drought-related research on substrate-grown red raspberry focused on understanding the impact of prolonged drought episodes, *e.g.* several weeks (Morales *et al*., 2013) and do not focus on the recovery phase. However, results from Chapter 2 highlighted that shorter durations of rootzone water deficit stress could also impact leaf physiological responses, and the persistence of decreased leaf gas exchange values was still evident even after rewetting. The research here provides a greater understanding of the response and recovery of Malling™ Bella to a temporary rootzone water deficit stress, the extent and duration of which is likely to be relevant to commercial substrate-grown raspberry production.

The use of potted raspberry plants is relevant to commercial UK production (Chapters 2-5), and was favoured in previous studies on red raspberry (Morales *et al*., 2013; Stafne *et al*., 2001). This approach ensured that measurements of CVMC within the rooting zone could be measured accurately at and around the time points when the effects of the transient rootzone water deficit stress were first perceived by the plant in terms of altered leaf physiology. In addition, the implementation of sensor-based automatic irrigation facilitated consistent drying rates across experiments (Chapters 3-5), thereby allowing for a more focused and meaningful comparative analysis.

Midday SWP is a reliable and sensitive measurement that can be used to determine whether a plant is perceiving stress (McCutchan & Shackel, 1992), and so midday SWP measurements have been used as a reference for irrigation scheduling for some commercial crops like pecan, olive and apple trees (Othman *et al*., 2014; Moriana *et al*., 2012; Naor *et al*., 2005; Shackel *et al*., 1997). Here, differences in midday SWP values between WW and DD plants was the first detectable plant response to the imposed transient rootzone water deficit stress, with responses generally occurring before changes in g_s and Pn (Chapters 2 and 3) or occasionally on the same day (Chapter 4). This could have occurred due to the differences in environmental differences between experiments, notably higher VPD (Chapter 4 compared to experiments in Chapters 2 and 3). A correlation was notable between midday SWP and xylem-borne ABA; during the drying down phase, as midday SWP values decreased, xylem-borne ABA increased. During the rewetting phase, when xylem-borne ABA decreased, midday SWP values were restored to pre-stress values, suggesting that these two are causally linked. Similar trends have been measured in other crops like rice (Bano *et al*., 1993). Water potentials within a plant can be adjusted when water availability decreases, as stomata closure and hydraulic conductance are altered to reduce water loss and maintain cell turgor (Scharwies & Dinneny, 2019) and increases in xylem-borne ABA with decreased g_s values have been reported many times in the literature across plant species (Dodd *et al*., 2006; Socias *et al*., 1997; Shatil-Cohen *et al*., 2011), suggesting that ABA plays a vital role in promoting stomatal closure, to reduce transpirational water loss (Comstock, 2002; Ng *et al*., 2014). However, following the rewetting of the coir, midday SWP values recovered quickly and before the recovery of values of g_s and Pn (Chapters 2, 3 and 4). Therefore, midday SWP measurements are a reliable and accurate indicator of the onset of stress in red raspberry. However, following rewetting the coir, midday SWP values to recover quickly, but the legacy effects on g_s and Pn values were not evident unless taking measurements with a porometer or IRGA. Growers that use midday SWP values to estimate irrigation scheduling should be aware that following a transient rootzone water deficit stress, while midday SWP values recover quickly, legacy effects on g_s and Pn values may still be present. Hence, growers should not rely just on measurements of midday SWP to understand if a plant has recovered from the stress and should use other tools, such as porometers and leaf spectrometers, to inform their decision-making.

The values of g_s and Pn following a transient rootzone water deficit stress fell irrespective of the duration of the stress (Chapters 2, 3, 4 and 5). As the rate of drying was similar between experiments (Chapters 3, 4 and 5), the xylem-borne ABA changes following the four-day DD treatment (Chapter 4) could explain the responses in the longer durations of a rootzone water deficit stress (Chapter 3 and 5). As values of xylem-borne ABA increased following the drying-down treatment, reductions in g_s and Pn values promptly followed, with similar results previously reported (Dodd *et al*., 2006; Socias *et al*., 1997). As the coir dries, ABA accumulates in the plant roots (Zhang *et al*., 2021b), by promoting the expression of ABA biosynthesis genes, and this ABA synthesis plays a pivotal role in facilitating adaptive responses in plants under conditions of drought stress (Zareen *et al*., 2024). Drought stress can lead to an increase in ABA synthesis in the roots, resulting in a greater concentration of ABA to be transported via the xylem sap to the leaves (Davies & Zhang, 1991), and this increase in xylem-borne ABA is highly likely to be sourced from the drying roots. However, to be able to confirm that increases in xylem ABA resulted from increases in root ABA, future experiments should take into consideration the analyses of root ABA concentrations during a rootzone water deficit stress.

While the timing of the measured leaf physiological responses to the drying down treatments were similar, recovery following a transient rootzone water deficit varied with the extent and degree of coir drying. The longer the duration of the transient rootzone water deficit stress, the longer the recovery time of g_s and Pn values back to pre-stress values once rewetting commenced. This could be due to a protracted export of ABA that was synthesised and accumulated in the roots during the deficit stress (Dodd *et al*., 2006). However, with the rapid return of xylem and foliar ABA concentrations to low pre-stress values following rewetting (Chapter 4), the variable rates of recovery of g_s and Pn values were likely due to other signalling mechanisms. Altered root-to-shoot signalling of other plant hormones, *e.g.* auxins, ethylene and gibberellins, may alter the stomatal sensitivity to ABA (Dodd *et al*., 1996; Bradford & Hsiao, 1982), however, as ABA was the only phytohormone that was analysed in these experiments, the role of other phytohormones in the recovery of g_s and Pn values in red raspberry could not be determined. Other phytohormones should also be considered as research suggests that ethylene can inhibit stomatal opening (Dodd *et al*., 2015), while auxin is known to have a role in stomatal opening (Merritt *et al*., 2001), and these phytohormones concentrations may also affect the signal strength of the plant hormone ABA. While foliar material was collected, there was inadequate time available to perform experiments to measure the changes in other phytohormones. However, these may have provided further understanding of the roles of other chemical signalling processes that may control the legacy effects on photosynthesis following rewetting.

The legacy effects on photosynthesis have been shown throughout all experimental chapters (Chapters 2-5), however, the recovery times differed depending on drying durations. The hysteresis of substrate matric potential versus soil water content provided explanations for the longer recovery of stomatal conductance following the longer drying-down durations (Figure 3-8). Whereby in Chapter 3, following the rewetting after the longer drying down duration the recovery of the stomatal conductance values took longer although coir water content increased, suggesting that there was reduced water available to the plant leading to sustained stomatal closure or partial stomatal reopening (Carminati & Javaux, 2020). Although coir has a high rewetting capacity (Wever et al., 1997), coir can still have this hysteresis effect where soil moisture content increases but stomatal conductance values do not, suggesting that there is reduced water availability to the plant at that given moment. Future experiments should investigate the hysteresis effect caused by longer rootzone water deficit stress events to help explain and then prevent legacy effects on photosynthesis, to reduce effects on marketable berry yield and quality.

Leaf hydraulic conductance did not significantly differ during the drying down or subsequent rewetting phase. Since, leaf hydraulic conductance is only measured once on a signal leaf, as it is a destructive method, it makes it hard to track changes over time. Furthermore, measurements were carried out once per day, but leaf hydraulic conductance can be affected by environmental factors when using the evaporative flux method (Wang *et al*., 2022), causing high variability in data. Although the time of day of measurements were kept consistent throughout the experiment, changes in daily environmental conditions undoubtedly may have caused variability in the data set. Although differences were not measured in the experiments described here, other research has shown that exogenous ABA led to a decrease in leaf hydraulic conductance, highlighting that ABA may play a role in regulating leaf water status by affecting leaf hydraulic conductance (Coupel-Ledru *et al.*, 2017). Once again this highlights the significant role that ABA plays in response to a water deficit stress. The duration of the drying down treatment in Chapter 4 was shorter than that in Chapter 3, making it more difficult to pick up the subtle changes that may have occurred. Therefore, future research may benefit from trying to understand the effects of longer drying-down treatments, but should also be reminded of the hysteresis effect that can occur following longer drying durations.

The recovery of g_s and Pn values took longer in smaller rooting volume pots compared to the larger rooting volume pots (Chapter 5), suggesting that the signalling mechanisms for the recovery following rewetting depend on many factors and not only on the duration of the transient rootzone water deficit stress or the phytoclimate during the recovery period (Chapter 3). While research on *Vigna unguiculata* has shown that rooting volume did not cause changes in xylem ABA concentration (Ismail *et al*., 1994), root restriction did cause an increase in xylem sap ABA concentration in pepper (Ismail & Davies, 1998) and tomato (Hurley & Rowarth, 1999). The possibility of an extra supply of xylem ABA in the smaller rooting volume pots, due to the extra stress caused by root restrictions, may have affected the recovery period, as an increase in ABA concentration can lead to a loss of guard cell turgor leading to stomatal closure (Schroeder *et al*., 2001b). Rooting volume also may have affected the readily available water in the pots. With less water available in the smaller rooting volume, this may have resulted in greater synthesis of ABA, and once rewetting commenced, an extra short-lived ‘pulse’ of xylem ABA may have occurred, which has been previously measured in the literature (Dodd *et al*., 2006; Dodd *et al*., 2015). This augmented xylem-borne ABA signal may have contributed to the longer recovery time for the smaller rooting volume pots and the longer durations of rootzone water deficit stress (Chapter 3). Therefore, future experiments should take into account the measuring of concentration differences in xylem ABA in different rooting volume pots during the drying down and subsequent recovery phase.

The drying down treatments (Chapters 3 and 5) affected berry yield and quality, as did rooting volume (Chapter 5). Following the imposed root water deficit stress, Class 1 berry yield per plant was reduced due to a combination of effects on individual berry fresh weight and the number of berries in the Class 1 category in both experiments (Chapters 3 and 5). Similar effects of water deficit stress on berry marketable yield and quality have been previously reported in raspberry (Ortega-Farias *et al*., 2022; Morales *et al*., 2013) and tomato (Medyouni *et al*., 2021). Reductions in fruit growth are expected during a water deficit stress (Ebel *et al*., 1993), as plant growth is restricted due to inhibition of cell expansion (Hsiao *et al*., 1997). In grape berries, acceleration of ripening during water deficit stress (Castellarin *et al*., 2007) can also result in smaller berries. However, a rootzone water deficit stress and a smaller rooting volume raised berry SSC, likely due to the decreased dilution of sugars with less water available. While this may be favourable for growers intending to market sweeter berries, growers should be reminded that Class 1 yield is concurrently reduced. Other factors important to consumers, including texture and shelf life, may have also been impacted by the drying down treatment and the smaller rooting volume pots, which should be further investigated. However, growers should be reminded that the risks of using 4.7 L pots in commercial production can be detrimental even if a mild transient rootzone water deficit stress was to occur, since primocane Malling™ Bella grown in 4.7 L pots are likely to perceive a rootzone water deficit stress sooner than those in 7.5 L pots.

The changing climate is already impacting on crop yields and quality, and such effects are likely to become more widespread and more frequent. Prioritising research into how crops adapt to variable weather conditions is imperative if the resilience of cropping systems is to be raised so that UK targets for food and nutrient security can be achieved. The slow recovery of leaf gas exchange outlined in the experiments here highlights the importance of understanding stress responses in crops likely to experience abiotic stress with the changing climate. The Department for Environment, Food & Rural Affairs (DEFRA) in the UK collates information on production, trade, and valuation of horticulture crops in the UK annually. In 2020, DEFRA reported that due to the variable weather in August, including a heatwave in the first half of August, there was an overall 15% decrease in average raspberry yield per hectare (DEFRA, 2021). In contrast, in 2023, in the most recent report, due to the cold spring, picking commenced a week later than in 2022 for raspberry; however, early raspberry crops yielded better than expected, but the overall yield was 7.3% lower in 2023 than in 2022 (DEFRA, 2024). However, 2022 was the warmest year on record for the UK for maximum and mean temperatures since 1884 (Met Office, 2023). Many crops, including raspberry, are consistently challenged by fluctuating environmental conditions, varying not only between seasons but also daily. However, in raspberry at least, little is known about the signalling systems that regulate and integrate immediate-, short- and longer-term plant responses to these variable conditions. The research here enhances our understanding of the signalling mechanisms associated with the recovery process from a short-term rootzone water deficit. The work carried out here highlights the importance of understanding how plants respond to, and recover from, a rootzone water stress and how different signalling mechanisms are likely involved in the perception and adaptation to the stress, and in the subsequent recovery of leaf gas exchange once the stress has passed. This understanding is crucial to help to minimise the impacts of the short-term rootzone water deficit stresses that are likely experienced in commercial substrate-raspberry production.

6.2 Research limitations and future work

There are, of course, various ways the experiments outlined in this thesis could be further improved. The application of root zone water deficit stress facilitated a gradual and controlled reduction in coir volumetric moisture content, thereby contributing to an enhanced understanding of how leaf gas exchange responded to, and recovered from, the stress. It is important to note that this particular gradual drying of the substrate is a possible scenario for growers. A water deficit stress is more likely to materialise as an unexpected interruption of irrigation supply by accidental removal or blockage of a single dripper or inaccurate irrigation scheduling, which could lead to a rapid drying of the coir and consequently elicit a different response to the water deficit stress than that documented here. Also, water deficit stress is unlikely to occur on its own and more likely to occur as a combined stress, especially so given the changing climate. The combination of two different abiotic stresses is often distinct and cannot necessarily be inferred from the response of plants to each individual stressor (Mittler, 2006). Although these experiments have outlined foliar responses to stress and subsequent recovery in raspberry plants and the possible signalling mechanisms that control these responses, when in combination with other abiotic stresses, responses and signalling mechanisms may also vary.

Some differences in leaf physiological measurements were observed between experiments conducted in the more controlled environment of a glasshouse compartment (Chapters 3 and 4) than those in the polytunnel (Chapter 5), with the latter-more closely resembling large-scale commercial plantings. However, commercial production of raspberry in heated polytunnels and in glasshouses is becoming increasingly common as growers try to take advantage of the higher market prices at the shoulders of the typical grower season. While reports detailing experiments comparing leaf physiological measurements in a glasshouse and a polytunnel for raspberry canes are not available in the literature, experiments on other crops and plant aspects indicate that responses may vary depending on the experimental setting (Zhou *et al*., 2018) and depending on the cultivar; for example, strawberry cultivars responded to water deficit stress differently (Grant *et al*., 2010). Therefore, future experiments will need to consider other raspberry cultivars to determine if response and recovery times are cultivar-specific or are similar for all cane crops.

Furthermore, the collection of samples solely during the 4-day transient rootzone water deficit stress failed to provide insight into the extended recovery period associated with prolonged rootzone water deficit stress. Moreover, the practicality of obtaining samples from large potted raspberry plants on consecutive days to monitor daily variations in xylem-borne ABA was limited. Conversely, in other experiments where samples have been obtained from more tractable crop plants and more frequently, it was, presumably, easier to establish a correlation between xylem ABA and changes in leaf gas exchange (Dodd *et al*., 2006; Else *et al*., 2001).The possibility of using individual leaf xylem sap as measurements as a representative shoot xylem sap should also be considered and therefore compared to xylem ABA concentrations collected from the shoot, that way if there are not large differences, it could increase the practicality of collecting samples. In the glasshouse compartment, accommodating more than 24 plants at a commercially-relevant plant spacing was not possible. Consequently, it was necessary to consider both destructive measurements and leaf physiological measurements simultaneously, and so xylem sap collection on consecutive days was not possible. Therefore, future experiments should take into account the utilisation of a larger glasshouse compartment to accommodate a greater number of plants, thereby facilitating an expanded sampling programme, which would help in identifying the causal signals that regulate the recovery of leaf gas exchange parameters from a rootzone water deficit stress in raspberry canes.

The research findings in this thesis suggest several recommendations for growers to reduce the likelihood of legacy effects on photosynthesis after a transient rootzone water deficit stress. It is important to implement effective and efficient irrigation schedules that consider plant water needs at different growth stages and under varying environmental conditions. As such, measurements of volumetric moisture content of the substrate should be carried out as frequently and across as large a range of pots as the grower can economically afford. Growers need to be mindful that a transient rootzone water deficit stress of a few days can lead to a reduction in Class 1 yield and impact the berry fresh weight for several weeks after the coir is re-wetted, potentially leading to substantial revenue losses. Based on the work here, growers should be informed about the potential risks associated with using 4.7 L pots and should be encouraged to use pots with larger rooting volume to minimise the adverse effects of a transient rootzone water deficit stress, if it occurs. The work described here aims to assist growers in establishing the best growing conditions for raspberry crops, ensuring that global raspberry production remains unaffected by fluctuating weather conditions. In order to protect all crops globally from the detrimental impact of drought stress, more studies like the ones carried out here are essential to maintaining crop production stability with the changing climate.

7 References

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8 Appendices

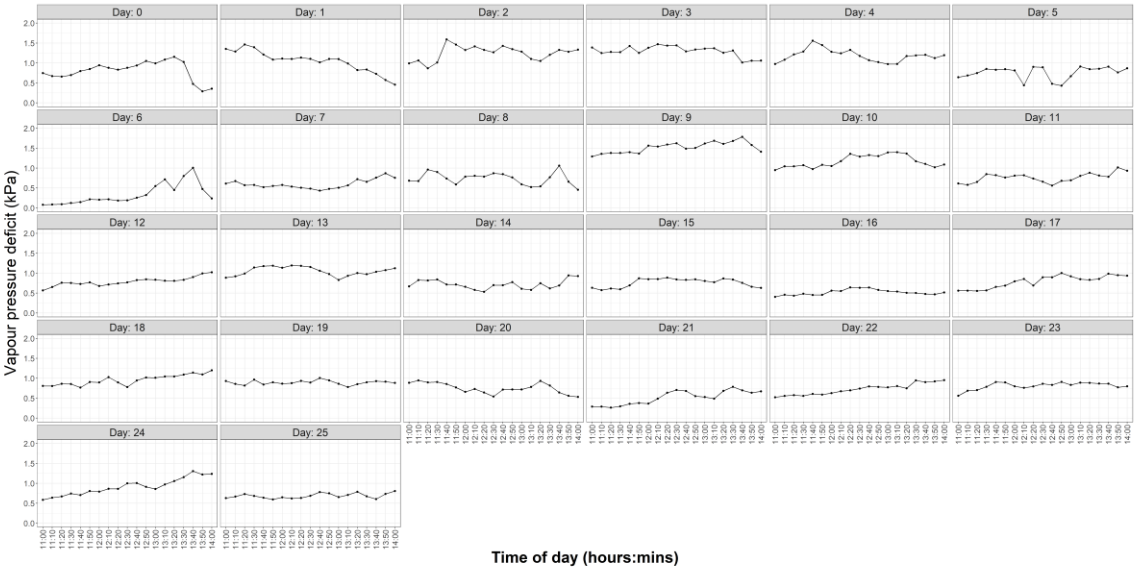

Figure-A1. *Vapour pressure deficit (VPD) values between 11:00 and 14:00 recorded by sensors in the tunnel throughout the duration of the experiment (Chapter 2).*

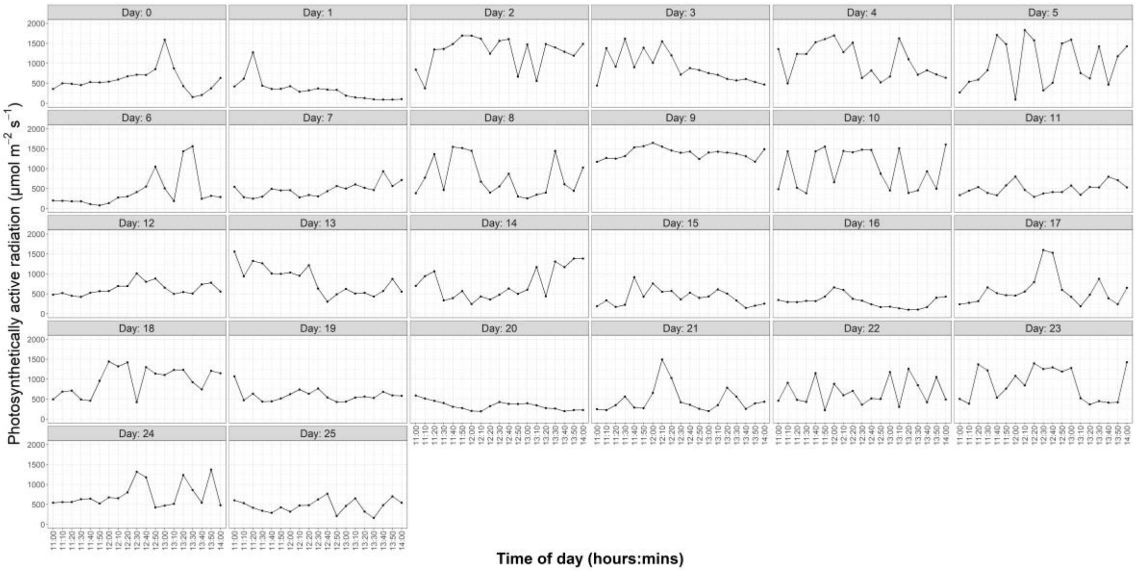

Figure-A2. *Photosynthetically active radiation (PAR) values between 11:00 and 14:00 recorded by sensors in the tunnel throughout the experiment (Chapter 2).*

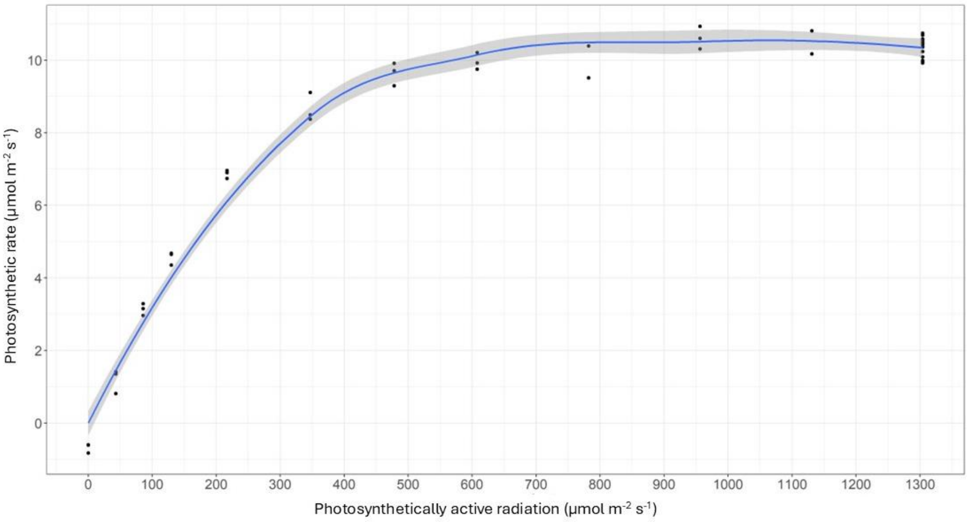

Figure-A3. *The light response curve of Malling™ Bella on a terminal leaf on node 20.*

Table A-1. *The mean RH and VPD values in the glasshouse compartment depending on the type of curve formed on the day of the measurement for WW plants during the 10-day DD experiment (Chapter 3).*

Type of Curve	**Mean RH (%)**	**Mean VPD (kPa)**
Single peak	64.6	2.75
Double peak	72.3	2.66
Constant drop	59.8	2.81

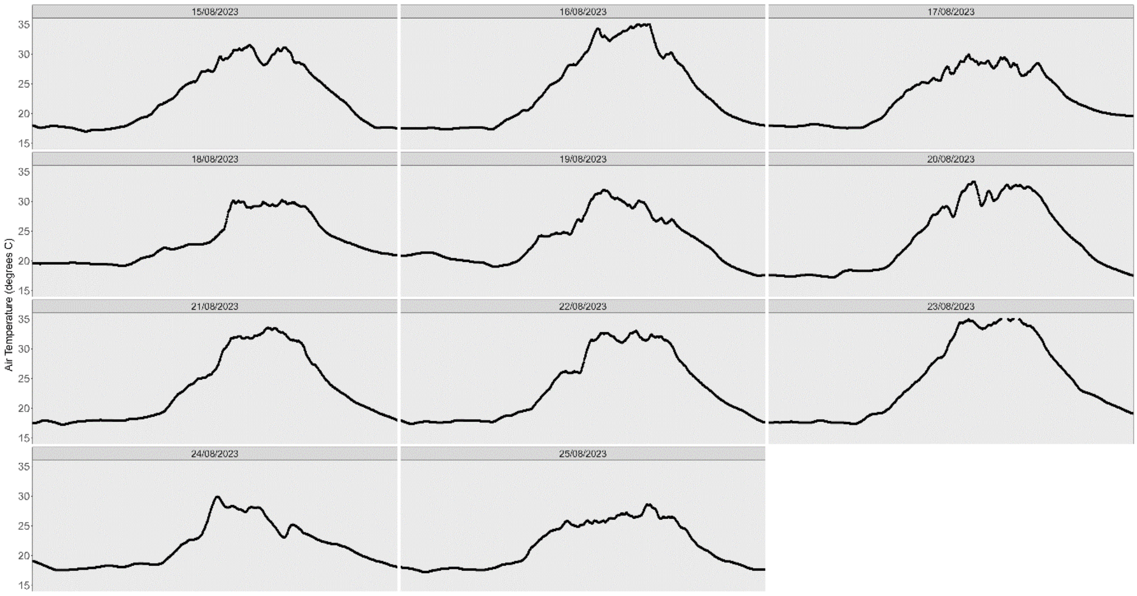

Figure-A4. *Air temperature from Day 0 (15/08/2023) till Day 10 (25/08/2023) was recorded by sensors in the glasshouse compartment throughout the duration of the experiment (Chapter 4).*

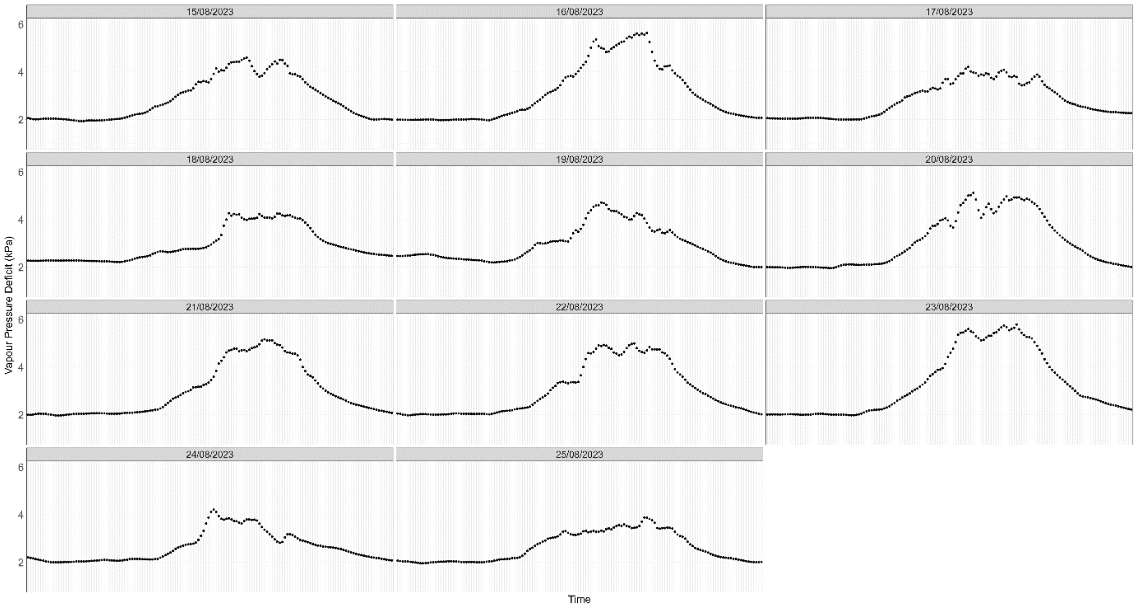

Figure-A5. *Vapour pressure deficit (VPD) from Day 0 (15/08/2023) till Day 10 (25/08/2023) was recorded by sensors in the glasshouse compartment throughout the duration of the experiment (Chapter 4).*

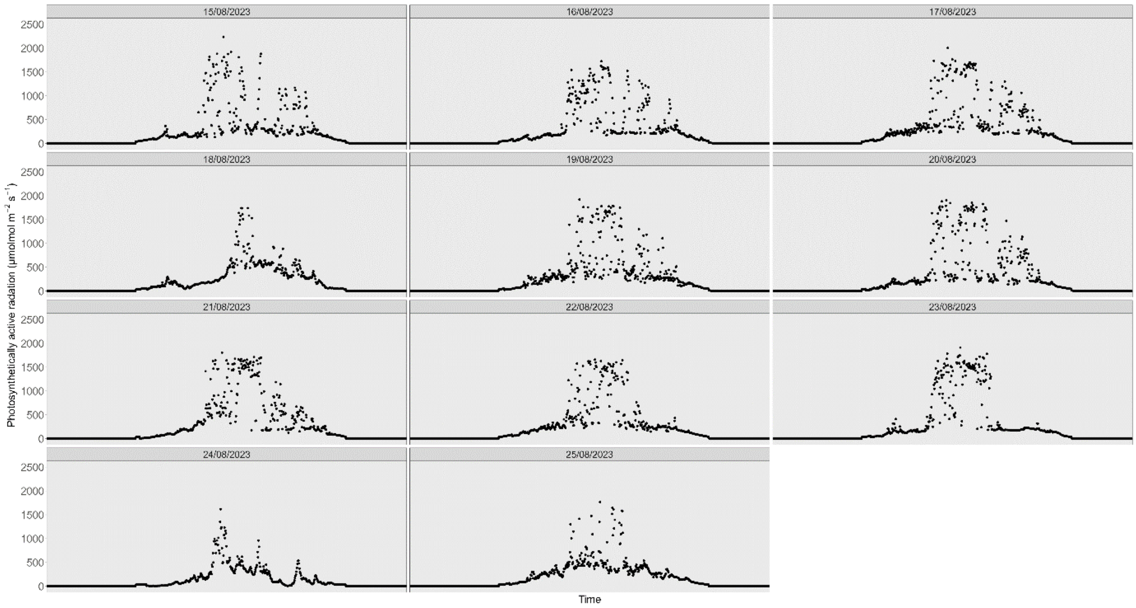

Figure-A6. *Photosynthetically active radiation (PAR) from Day 0 (15/08/2023) till Day 10 (25/08/2023) was recorded by sensors in the glasshouse compartment throughout the duration of the experiment (Chapter 4).*

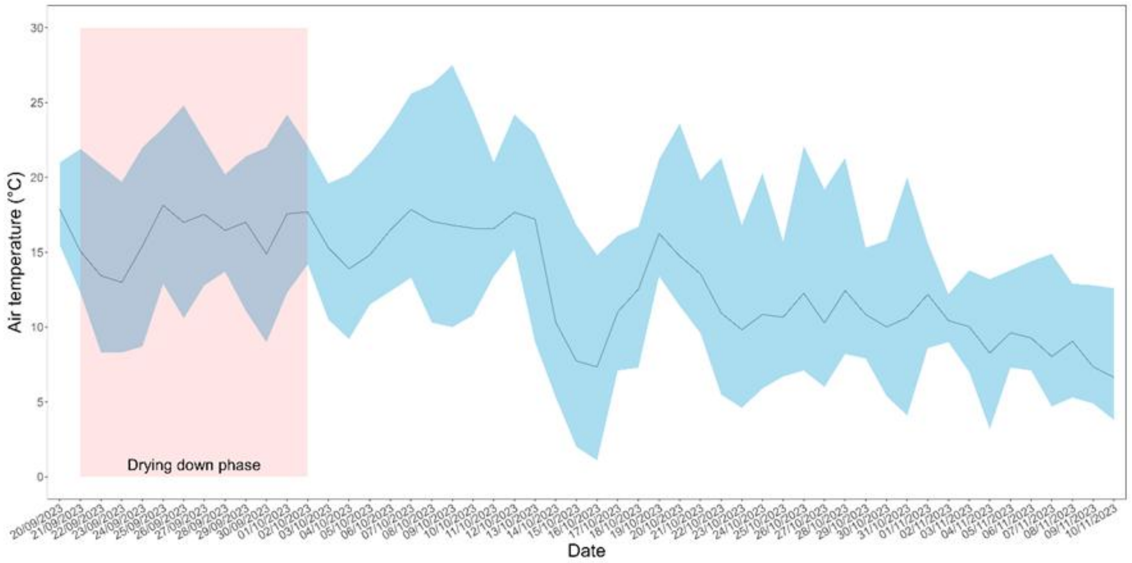

Figure-A7. *Average air temperature during the duration of the drying down phase (22/09/2023 – 02/10/2023) and the subsequent recovery phase (03/10/2023 – 10/11/2023) of the experiment. The dark blue line indicates the average air temperature recorded, while the shaded area represents the minimum and maximum temperatures recorded. The highlighted pink area indicates the drying-down phase of the experiment (Chapter 5).*

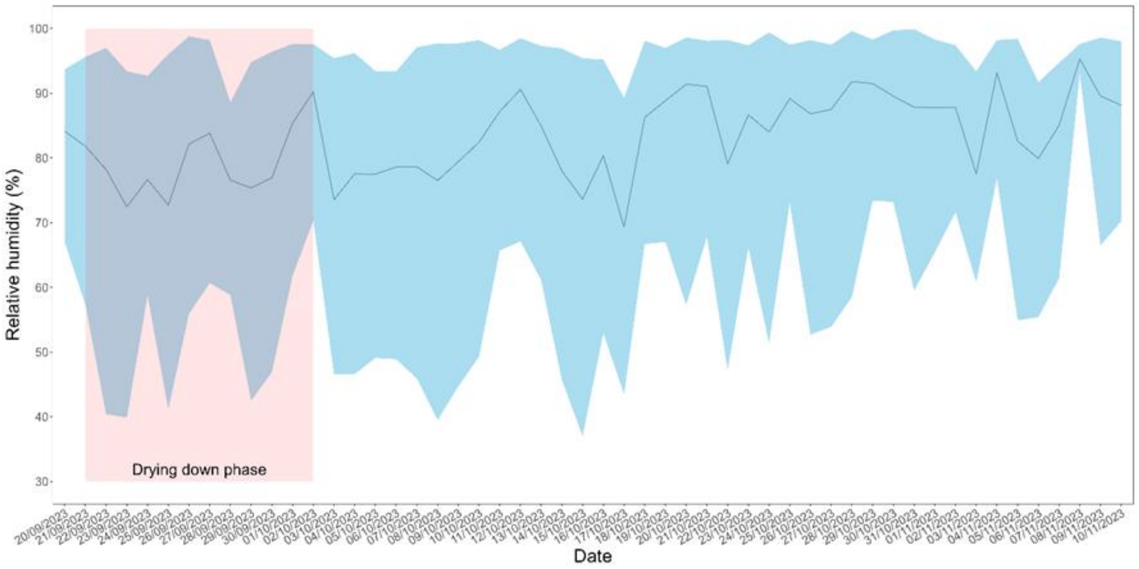

Figure-A8. *Average relative humidity (RH) during the duration of the drying down phase (22/09/2023 – 02/10/2023) and the subsequent recovery phase (03/10/2023 – 10/11/2023) of the experiment. The dark blue line indicates the average RH recorded, while the shaded area represents the minimum and maximum RH recorded. The highlighted pink area indicates the drying-down phase of the experiment (Chapter 5).*

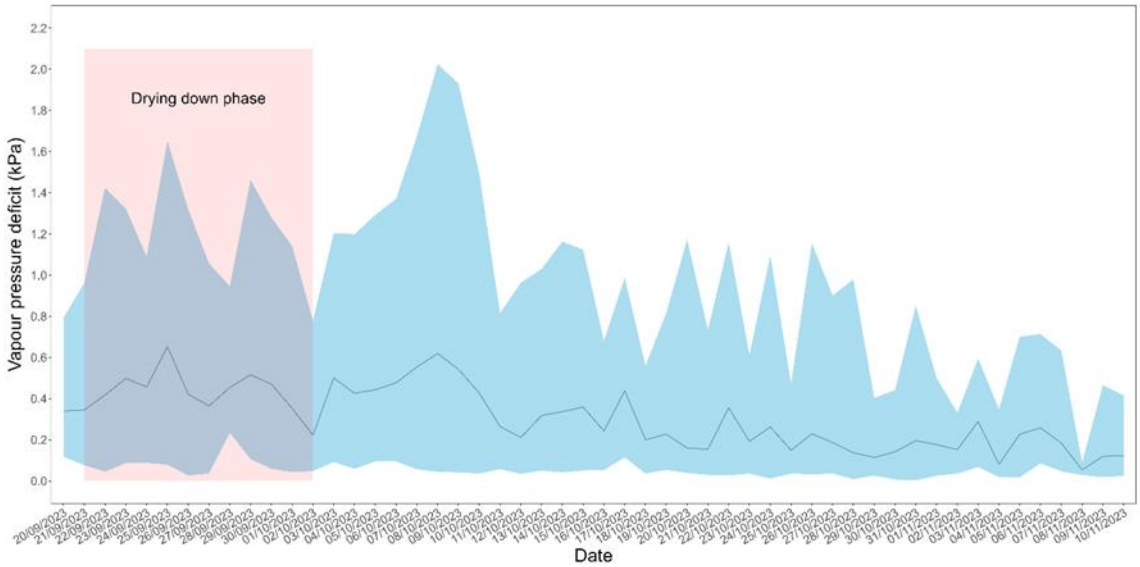

Figure-A9. *Average vapour pressure deficit (VPD) during the duration of the drying down phase (22/09/2023 – 02/10/2023) and the subsequent recovery phase (03/10/2023 – 10/11/2023) of the experiment. The dark blue line indicates the average VPD recorded, while the shaded area represents the minimum and maximum VPD recorded. The highlighted pink area indicates the drying-down phase of the experiment (Chapter 5).*

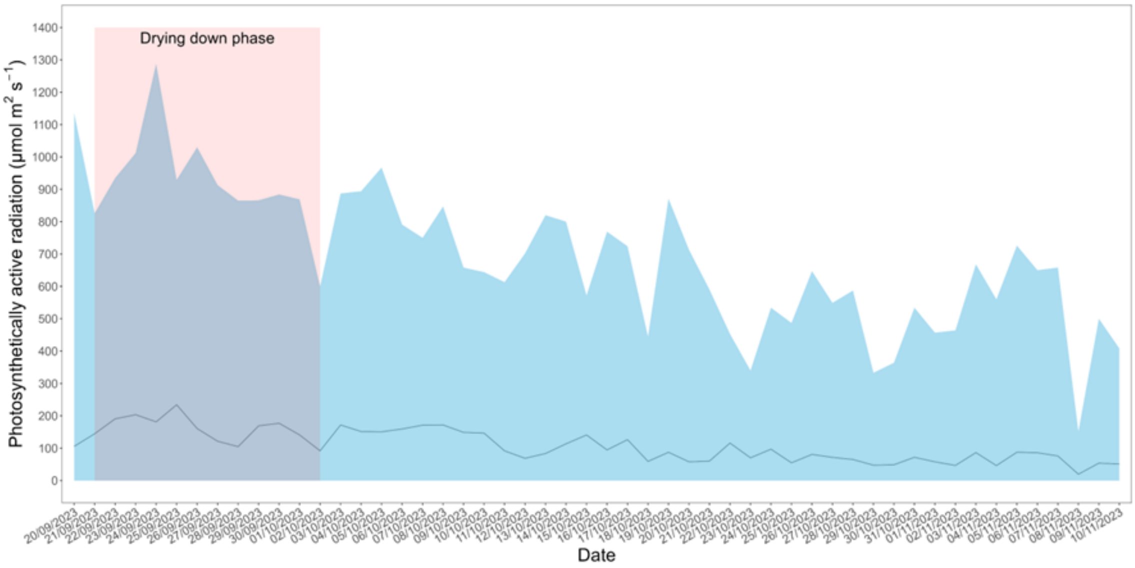

Figure-A10. *Average photosynthetically active radiation (PAR) during the duration of the drying down phase (22/09/2023 – 02/10/2023) and the subsequent recovery phase (03/10/2023 – 10/11/2023) of the experiment. The dark blue line indicates the average PAR recorded, while the shaded area represents the minimum and maximum PAR recorded. The highlighted pink area indicates the drying-down phase of the experiment (Chapter 5).*