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Title: The effects of short-term water stress on raspberry

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1. Industry Summary

Abiotic stresses are increasingly prevalent in the changing climate, leading to significant implications for crop potential and product quality. In cane crops, such as raspberries, the occurrence of transient root water deficit stresses, resulting from elevated vapour pressure deficits and/or inefficient irrigation practices, are becoming more likely. These stresses can elicit immediate physiological responses, including stomatal closure, as well as long-term effects, such as impaired leaf gas exchange, which may adversely affect berry yield and quality. However, if these legacy effects could be predicted and overcome more rapidly, the risks to productivity could be better managed.

Responses to abiotic stresses differ between plant species, with physiological responses in strawberry plants' recovering more quickly following transient water deficit stress than in raspberry plants. Therefore, the aim of the research was to improve knowledge of the impact of transient rootzone water deficit stress on red raspberries and to better understand the signalling mechanisms that regulate physiological responses. During the experiments, effects on crop yield and berry quality were also quantified.

The key findings were:

- The duration of the rootzone water deficit stress affected the recovery rate of leaf gas exchange.
- Even a brief rootzone water deficit stress of four days resulted in legacy effects on photosynthesis that persisted for days after rewetting. Xylem-borne concentrations of the plant hormone abscisic acid, which regulates stomatal apertures, increased 50-fold during the drying down phase but returned to pre-stress values promptly following rootzone rewetting.
- When smaller pots were used, which meant that rooting volume was more limited, the stress legacy effect on photosynthesis persisted for longer than when larger pots were used.
- Rootzone water deficits lowered Class 1 yield and berry quality, along with a reduction in berry fresh weight that persisted for several weeks.

The findings suggest that chemical signals (e.g. abscisic acid) regulate the physiological responses to transient rootzone water deficit stress since no hydraulic signals were detected. UK soft fruit growers should choose pot sizes and irrigation methods carefully to help ensure that ineffective or inefficient irrigation scheduling does not lead to transient rootzone deficits, which could then impact productivity and berry quality.

2. Introduction

Plants are routinely subjected to a range of abiotic stresses, with droughts becoming more frequent and impactful due to climate change. These stresses affect plant growth, development, and overall functioning (Davies et al., 2002; Medyouni et al., 2021). Therefore, understanding how plants respond to and recover from water deficits is important when trying mitigate against yield losses, especially in commercial crops. This knowledge is particularly important for crops such as raspberry where water availability directly influences berry yield and quality.

Red raspberries (Rubus idaeus) are widely cultivated in Europe and North and South America and are economically significant due to their use both as fresh fruit and in processed products (Sargent et al., 2007; Ward et al., 2013). However, unpredictable weather conditions, such as the heatwave in the UK during early August 2020, resulted in a 15% decrease in yield and a 14% decrease in value for commercial raspberry growers (DEFRA, 2021). These climate-driven fluctuations underscore the need for a deeper understanding of how raspberries respond to such challenges, particularly those grown under rootzone water deficit stress conditions.

In this context, the study of stomatal behaviour becomes critical. Stomatal apertures, which regulate gas exchange, adjust in response to environmental cues such as soil moisture levels (Belko et al., 2012). While much has been studied on stomatal responses to water deficit in model species and other crops like grapevine (Beis & Patakas, 2010; Chaves et al., 2010; Khonghintaisong et al., 2017; Miyashita et al., 2005), there is still limited understanding regarding the specific response and recovery mechanisms in soft fruits like raspberries.

Rootzone water deficit stress has been shown to negatively impact leaf gas exchange and photosynthesis in various crops, with longer stress durations leading to prolonged recovery periods (Vassileva et al., 2011; Romero et al., 2017; Torres-Ruiz et al., 2015). Similar stress in woody perennials like grapevines has been linked to decreased photosynthetic rates (Maroco et al., 2002). Since photosynthesis is a key determinant of yield potential (Parry et al., 2011), any stress-induced reductions in photosynthetic rates are likely to reduce both marketable yield and berry quality (Wenter et al., 2018). Stomatal closure, primarily driven by the plant hormone abscisic acid (ABA), is a key mechanism that plants use to limit water loss during drought, but it also restricts CO2 uptake, further limiting photosynthesis (Flexas & Medrano, 2002; Comstock, 2002).

Given the potential for significant yield loss under drought conditions, it is essential for growers to better understand how raspberries respond to rootzone water deficits and how they recover. Insights into leaf physiological parameters, particularly those related to gas exchange, are vital for developing effective strategies for irrigation and water management. This is especially critical as more growers shift towards growing raspberries in pots or containers, rather than in soil, to manage root diseases and extend the growing season (Dolan et al., 2018). The use of smaller pots, in particular, can exacerbate water stress, as they hold less water and may lead to faster depletion of soil moisture, requiring more frequent irrigation (Targino et al., 2019).

The research presented here investigates the impact of transient rootzone water deficit stress on leaf gas exchange in raspberry plants for different durations and in those grown in different pot sizes. This research also highlights the roles of chemical and hydraulic signalling that control the response to and the recovery from rootzone water deficits. Understanding these dynamics is essential for informing irrigation strategies and mitigating the effects of water deficit stress on photosynthesis, yield, and berry quality. The findings will help growers make more informed decisions about their cultivation practices, ultimately improving crop management and ensuring more stable yields despite the increasing unpredictability of climate conditions.

A full scientific record is available in the thesis.

3. Materials and methods

Detailed experiments are presented in the thesis and will be submitted as scientific papers to international journals. The materials and methods described here are a general overview of the most frequently used methods to analyse the effects of rootzone water deficits.

3.1. Plant material and growing conditions

3.1.1. Different drying-down durations

Malling™ Bella plants were grown in 7.5 L pots of Cocogreen™ coir substrate in a glasshouse compartment (NIAB East Malling, Kent, UK). Plants were arranged in two rows, orientated north to south, with four cropping canes per pot and approximately two pots per linear metre. The glasshouse compartment was fitted with eight LED lights (Lumatek Ltd., UK), which provided supplementary light between 06:00 and 20:00. Also, the relative humidity in the glasshouse compartment was set to 60%, while the temperature was set to 22°C during the 14-hour photoperiod and 16°C during the night.

3.1.2. Pot size experiment

Malling™ Bella plug plants supplied by Holland were potted into two different rooting volume pots, 4.7 L and 7.5 L (Figure 1). Two plug plants were potted in opposite corners in the pot in Cocogreen™

coir substrate. The plants were positioned on the west side of a polytunnel at NIAB East Malling, Kent, with approximately two pots per linear meter.

Figure 1. *The two different pot sizes were used for the two irrigation treatments (7.5 L on the left and 4.7 L on the right). Photo taken on 08 August 2023.*

Throughout both the different drying-down durations and pot size experiments, an agronomist from Berry Garden Growers provided guidance on crop husbandry, fertigation programs, and strategies for pest and disease management, and all recommendations were implemented promptly.

3.2. Experimental designs

3.2.1. Different drying-down durations

Experiments were set up as a complete randomised block design with two treatments and an equal number of plants per treatment. All plants were well-watered before commencing the drying treatments. The two irrigation treatments that were applied were: (1) a well-watered (WW) control with a target daily run-off water volume of 15%, and (2) a drying-down (DD) treatment, where the irrigation inputs were lowered so that coir volumetric moisture content (CVMC) values fell gradually. At the end of the drying-down phases, pots were re-wetted by raising the irrigation set point also to achieve the target run-off of 15%, as in WW controls.

3.2.2. Pot size experiment

A complete randomised design with four treatments was used in this experiment. There were 20 plants per treatment arranged in four replicate blocks. Two irrigation treatments were also applied for each pot size: (1) WW and (2) DD. Therefore, the notations used for the four treatments were: (i) WW - 7.5 L, (ii) DD - 7.5 L, (iii) WW – 4.7 L, and (iv) DD – 4.7 L.

3.3. Irrigation application

Each pot had two dripper stakes connected to Netafim CNL emitters (1.2 L h^{-1}) to provide a drip fertigation system to the plant. A sensor-based closed-loop system automatically supplied and scheduled irrigation once CVMC values reached pre-determined set points. Each treatment contained five Delta-T SM150T (Delta-T Devices Ltd., Cambridge, UK) sensors connected to a Delta-T GP2 Advanced Datalogger and Controller unit. A preloaded script on the GP2 allowed the average CVMC value to be calculated, and once the average CVMC value was equal to or less than the irrigation set point, the GP2 opened the solenoid valve. This precision irrigation system allowed the adjustment of the irrigation set point to deliver a target average daily run-off volume of 15% of the input volume. The GP2 units were connected to a Delta-T GPRS modem, allowing remote access for daily monitoring. The same irrigation application and scheduling technique were used for all experiments.

3.4. Measuring coir volumetric moisture content and physiology parameters

Measurements of CVMC values were also carried out using a hand-held WET-2 sensor connected to an HH2 meter (Delta-T Devices Ltd., Cambridge, UK). Measurements were carried out on all pots twice at two different heights once a day. A mean value per treatment was reported as the ratio of water volume in the coir to the total volume of the coir (m^3 m^{-3}).

Physiological measurements consisted of stomatal conductance (g_s), photosynthesis (Pn), and midday stem water potential (SWP) and were carried out every day during the drying down and recovery phase. Midday SWP measurements were carried out following the method described by McCutchan & Shackel (1992) using a pressure chamber (Skye Instruments, UK). A terminal leaf from the eastern side of each pot was covered in foil for 90 minutes before excision. A single sharp cut was made, and the leaf was quickly placed into the pressure chamber, which was then gradually pressurised. A hand lens was used to observe the protruding petiole, and the endpoint was recorded once the xylem sap darkened. All measurements were taken around 11:00.

Measurements of g_s and Pn were carried out using either a LI-6400XT (7-day experiment) or a LI-6800 (4-day and pot size experiment) Portable Photosynthesis system (LICOR Biosciences Inc., Lincoln, Nebraska, USA). 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). All measurements were carried out around midday using a fully expanded leaf that was exposed to sunlight on the eastern side of the canopy.

3.5. Xylem and leaf collections and ABA analyses

3.5.1. Different drying-down durations

Xylem sap was collected using the vacuum extrusion method described by Bollard (1953). Two leaf samples between node 20 and the apex of the cane were collected. *For more details, please see the thesis*. Both xylem sap and leaf samples were frozen in liquid N_2 immediately before storage at -80°C until hormone analysis.

Liquid chromatography methods were used for the solid phase extraction to prepare the sap for hormone analysis. Once samples were ready, they were injected into a GC-MS (Agilent GC/MS 6890N – 5973N MSD, Agilent Technologies, Santa Clara, CA, USA).

For more details, please see the thesis.

3.6. Fruit yield and quality

3.6.1. Pot size experiment

Ripe berries were harvested three times a week, and all berries were graded into Class 1 and waste, while the number of berries and the fresh weight in each category was recorded.

3.7. Statistically analyses

Graphs were plotted, and statistical analyses were carried out using RStudio (version 2023.06.0).

3.7.1. Different drying-down durations

One-way ANOVA tests were carried out to determine whether differences between irrigation treatments, and Tukey HSD values for $p < 0.05$ were calculated.

3.7.2. Pot size experiment

Two-way ANOVA tests were carried out to determine whether differences between irrigation treatments and pot size and Tukey HSD values for $p < 0.05$ were calculated.

4. Results

4.1. Different drying-down durations

Frequent measurements of CVMC during the experiments were carried out to quantify the rate and severity of the coir across the different drying durations. Day 0 refers to the day the drying-down treatment was imposed (Figures 2 and 3). The WW plants had an average CVMC value above 0.5 m^3 m^{-3} throughout the 7- and 4-day drying-down treatment experiments. The imposition of the drying down treatment resulted in lower CVMC values in the DD plants, resulting in statistically significant differences in CVMC values between the WW and DD plants.

During the 7-day DD treatment, values were significantly lower ($p < 0.05$) after Day 2 (Figure 2), while during the 4-day DD treatment, values were significantly lower ($p < 0.05$) soon after the DD treatment had commenced, on Day 0. Following rewetting, CVMC values recovered within three and four days for the 7-day and 10-day DD treatment (respectively).

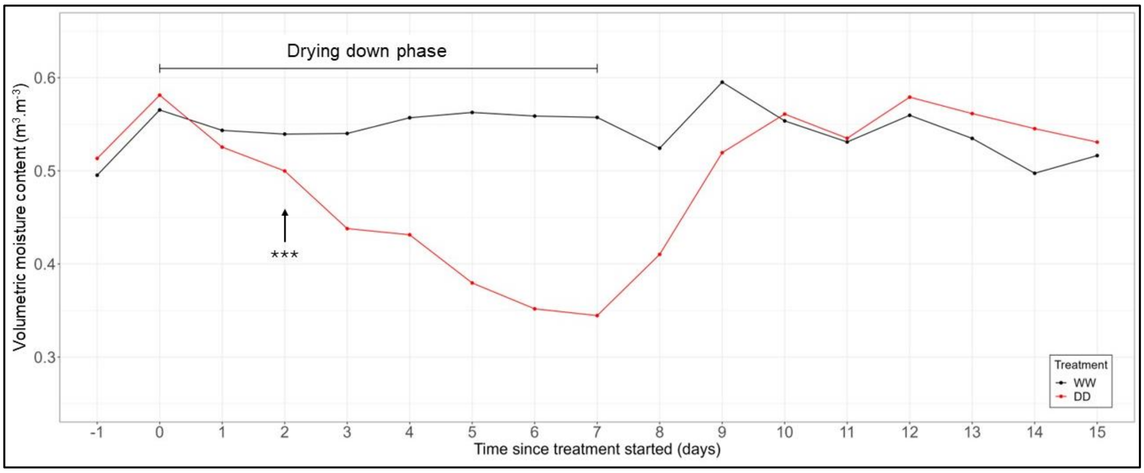

Figure 2. *The effects of the 7-day DD treatment on coir volumetric moisture content (CVMC) of Malling™ Bella, made by carrying out "spot" measurements using a Delta-T WET sensor. Each point represents an average CVMC value during the phases of drying down (n = 12) and recovery (n = 6). Asterisks indicate when statistically significant differences (p < 0.05) between treatments were first measured. 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.*

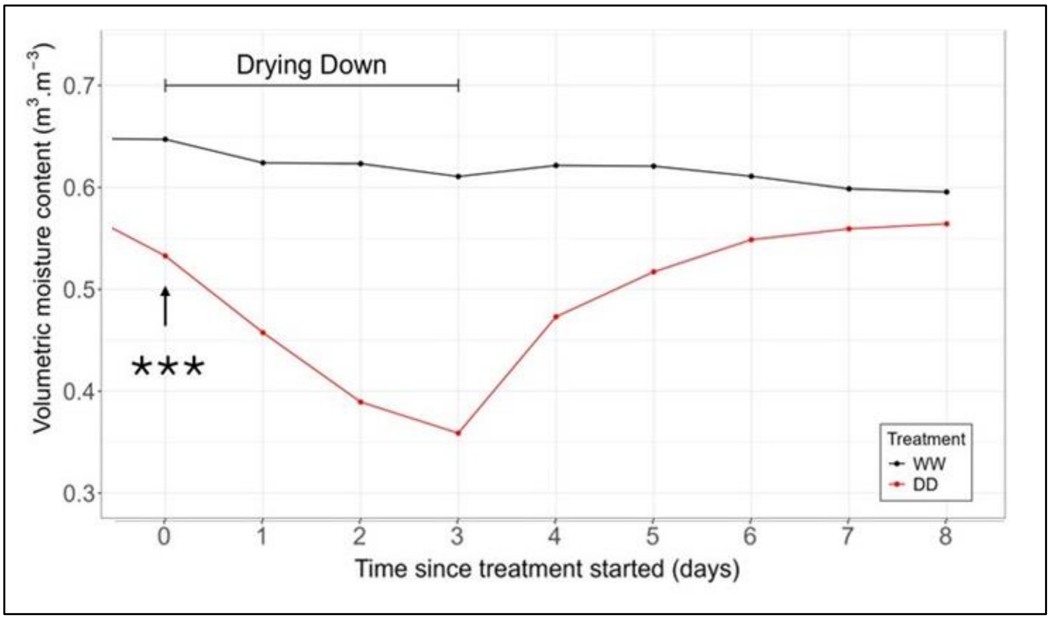

Figure 3. *The effects of the DD treatment on coir volumetric moisture content of the twelve Malling™ Bella, made by carrying out "spot" measurements using a Delta-T WET sensor. Each point represents the mean CVMC value from the six pots in each block. The duration of the drying-down treatment is shown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

Midday SWP values were among the first detectable changes when a DD treatment was imposed. In both the 7- and 4-day DD treatment, the mean midday SWP value was above -0.5 MPa throughout the experiment. The first statistically significant difference between the two experiments, with changes first detected on days 3 and 2, for the 7-day and 4-day DD treatment, respectively (Tables 1 and 2).

Once rewetting commenced, midday SWP values recovered quickly in both experiments. Recovery to values similar to WW values occurred within 5 hours after the 7-day DD treatment (Table 1) and within two days after the 4-day DD treatment (Table 2).

Time since treatment started (days)	7-day DD treatment effects on Midday SWP (MPa)		Statistical significance
	WW	DD	
Day 0	-0.230	-0.243	NS
Day 2	-0.416	-0.479	NS
Day 4	-0.374	-0.529	**
Day 6	-0.423	-0.711	**
Day 7	-0.449	-1.049	**
Day 8	-0.495	-0.531	NS

Table 1. *The effects of the 7-day DD treatment on midday stem water potential. The results are an average of the values from all the blocks. The asterisks indicate the significance of the difference, while NS denotes Not Significant.*

Time since treatment started (days)	4-day DD treatment effects on Midday SWP (MPa)		Statistical significance
	WW	DD	
Day 0	-0.342	-0.398	NS
Day 2	-0.404	-0.716	**
Day 4	-0.410	-1.206	**
Day 5	-0.359	-0.624	**
Day 6	-0.411	-0.401	NS

Table 2. *The effects of the 4-day DD treatment on midday stem water potential. The results are an average of the values from all the blocks. The asterisks indicate the significance of the difference, while NS denotes Not Significant.*

Following the changes in midday SWP, further adaptive responses to the coir drying were evident in midday g_s and Pn values, which decreased over time. Significant differences between the WW and DD plants were measured on Day 4 for both g_s and Pn during the 7-day DD treatment (Table 3), however, during the 4-day DD treatment differences were measured on Day 2 (Table 4).

Once coir rewetting commenced, midday g_s and Pn values remained significantly lower in the DD plants compared to the WW plants for different durations. Values of g_s recovered the day after rewetting commenced in the 7-day DD treatment, however, the recovery of g_s values took four days to recover after the 4-day DD treatment. On the other hand, values of Pn recovered five days and four days after rewetting commenced, for the 7-day and 4-day DD treatment (Table 3 and 4).

Time since treatment started (days)	7-day DD treatment effects on g_s (mol m^{-2} s^{-1})		Statistical significance in g_s values	7-day DD treatment effects on Pn (μmol m^{-2} s^{-1})		Statistical significance in Pn values
	WW	DD		WW	DD	
Day 0	0.152	0.123	NS	11.7	10.4	NS
Day 2	0.143	0.128	NS	12.1	11.4	NS
Day 4	0.248	0.176	*	12.0	10.2	*
Day 7	0.211	0.044	***	12.7	5.3	***
Day 9	0.141	0.059	NS	9.8	5.1	*
Day 11	0.240	0.098	*	11.7	7.8	*
Day 13	0.251	0.200	NS	11.9	12.4	NS

Table 3. *The effects of the 7-day DD treatment on stomatal conductance (g_s) and photosynthetic rate (Pn). The results are an average of the values from all the blocks. The asterisks indicate the significance of the difference, while NS denotes Not Significant.*

Time since treatment started (days)	4-day DD treatment effects on g_s (mol m^{-2} s^{-1})		Statistical significance in g_s values	4-day DD treatment effects on Pn (μmol m^{-2} s^{-1})		Statistical significance in Pn values
	WW	DD		WW	DD	
Day 0	0.132	0.088	NS	9.6	7.4	NS
Day 2	0.114	0.027	*	9.6	2.8	*
Day 4	0.122	0.018	**	10.1	2.6	***
Day 5	0.158	0.027	**	10.5	3.5	**
Day 7	0.132	0.054	*	11.0	5.4	**
Day 9	0.219	0.189	NS	11.1	9.2	NS

Table 4. *The effects of the 4-day DD treatment on stomatal conductance (g_s) and photosynthetic rate (Pn). The results are an average of the values from all the blocks. The asterisks indicate the significance of the difference, while NS denotes Not Significant.*

4.2. Chemical signalling

In the collected xylem sap and leaf samples, ABA was detected only on all the sampled days during the 4-day DD treatment.

Xylem ABA concentration ([ABA]) in WW plants was similar across all the sample days, and similar consistency in WW plants in foliar [ABA] was measured. This ensured that comparisons between the WW and DD plants accounted for. On the last day of the drying down treatment, Day 4, there was a 50-fold increase in xylem-borne [ABA], while the foliar [ABA] was just over 1.5 times greater in the DD plants than in the WW plants (Table 5). Following the rewetting of the coir on Day 5, both xylem [ABA] and foliar [ABA] returned to pre-stress values by Day 7 and remained at pre-stress values for the duration of the experiment (Table 5).

Time since treatment started (days)	4-day DD treatment effects on xylem [ABA] (nM)		Statistical significance in xylem [ABA]	4-day DD treatment effects on foliar [ABA] (nM)		Statistical significance in foliar [ABA]
	WW	DD		WW	DD	
Day 1	4.22	17.43	***	336.77	418.13	***
Day 4	2.22	151.09	***	309.78	533.85	***
Day 7	3.23	2.35	NS	385.22	421.95	NS
Day 10	1.10	1.14	NS	382.21	388.30	NS

Table 5. *The effects of the 4-day DD treatment on xylem ABA concentration and foliar ABA concentration. The results are a mean value from four sampling canes for the xylem sap samples and three samples for each treatment for the foliar samples. The asterisks indicate the significance of the difference, while NS denotes Not Significant.*

4.3. Pot size experiment

Plants respond to mild substrate water deficits by adjusting the physiology of the shoots to limit transpiration (water loss) and conserve leaf water balance. The drying-down treatment caused a drop in CVMC; subsequently, values recovered during the rewetting of the coir (Figure 4). Well-watered values for both pot sizes were kept within a narrow range between 0.5 m^3 m^{-3} and 0.6 m^3 m^{-3}. The lowest recorded average CVMC values were 0.42 m^3 m^{-3} for DD 4.7 and 0.36 m^3 m^{-3} for DD 7.5 (Figure 4).

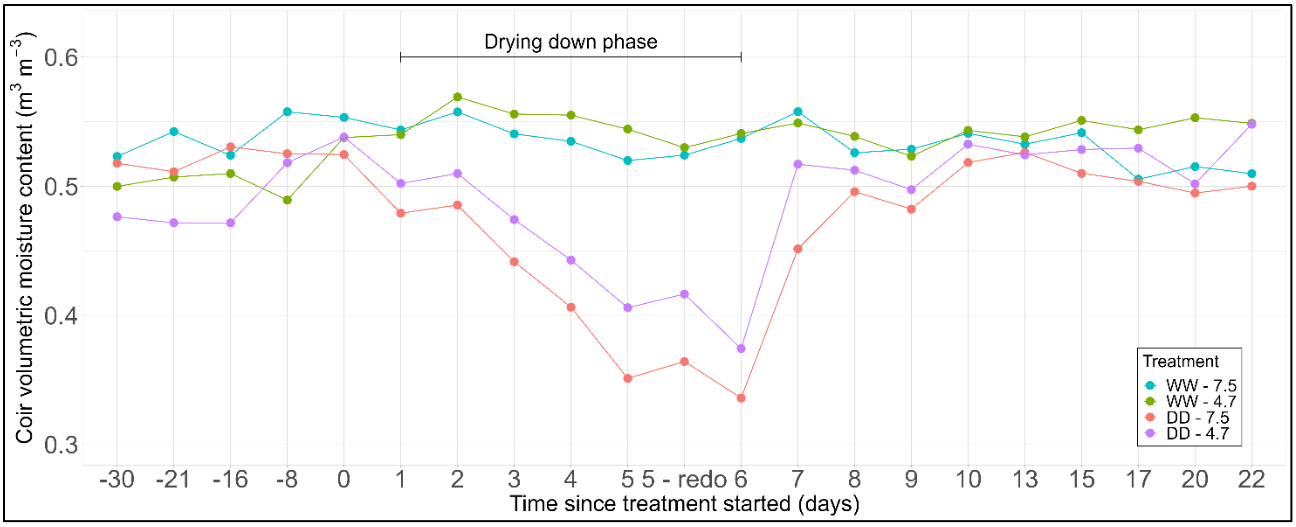

Figure 4. *The effects of the DD treatment on coir volumetric moisture content on the two different pot sizes of Malling™ Bella that were made by carrying out "spot" measurements using a Delta-T WET sensor. The duration of the drying-down treatment is shown for information.*

A reduction in the rate of Pn in response to coir drying was first seen on Day 5, when the average CVMC in DD 4.7 and DD 7.5 had reached 0.41 m^3 m^{-3} and 0.35 m^3 m^{-3}, respectively (Figure 5). All DD pots were re-wetted early on Day 7. Pn values remained significantly reduced in the previously DD-treated plants for different durations depending on pot size, with values in DD 7.5 returning to pre-stress values on Day 13, and for DD 4.7 full recovery returned four days later on Day 17 (Figure 5).

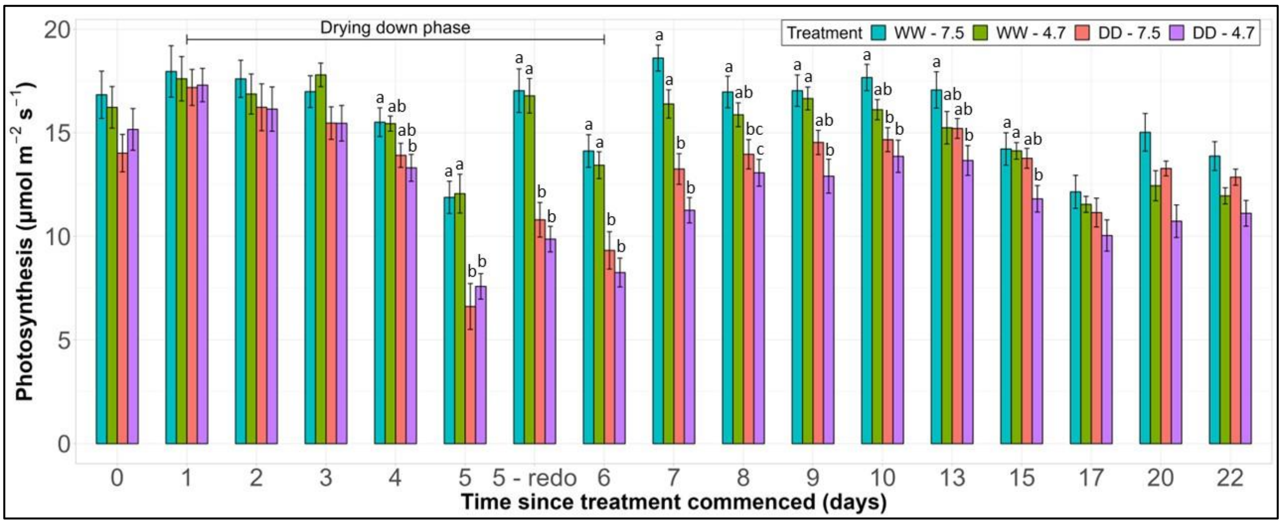

Figure 5. *The effects of the DD treatment on the photosynthetic rate of the two different pot sizes of Malling™ Bella that were made by carrying out "spot" measurements using a Delta-T WET sensor. The duration of the drying-down treatment is shown for information.*

The drying down treatment had an impact on both the yield and quality of the berries, with noticeable effects across all measurements, including total Class 1 yield, average berry fresh weight, and total berry number

For WW 7.5, the average Class 1 yield was 2.2 kg/pot, while DD 7.5 yielded only 1.5 kg/pot; for WW 4.7, the yield was 2.4 kg/pot, and DD 4.7 produced 1.3 kg/pot (Table 6). The drying down treatment led to a decrease in average Class 1 yield, but the difference in yield between the WW and DD pots was more pronounced in the smaller, 4.7 L, pots (1.1 kg/pot difference) as opposed to the larger, 7.5 L, pots (0.7 kg/pot difference).

In the WW 4.7 pots, the average weight of individual berries was lower compared to the WW 7.5 pots. The average berry weight for WW 4.7 was 4.6 g/berry, whereas for WW 7.5, it was 5.1 g/berry (Table 6), indicating a 10% reduction in berry size for plants cultivated in the smaller pots. Although this effect was marginally outside statistical significance [p = 0.082], the decrease in berry size is an essential factor for consideration for raspberry cultivation in smaller pots.

During the drying down phase of the study, there were no reductions in average berry size in the DD pots compared to the WW pots of the same size. The first indication of the drying down treatment's effect on average individual berry fresh weight emerged three days after coir rewetting began. Individual berry fresh weight remained lower in plants previously exposed to coir drying for up to 3 weeks after rewetting the DD pots.

Treatment	Average total Class 1 yield (kg/pot)	Average berry fresh weight (g/berry)	Average total Class 1 berry number (no./pot)
WW 7.5	2.2 a	5.1 a	426 ab
WW 4.7	2.4 a	4.6 ab	433 a
DD 7.5	1.5 b	4.5 b	379 bc
DD 4.7	1.3 b	4.5 b	364 c

Table 6. *Treatment effects on average total Class 1 yield per pot, average berry fresh weight and average Class 1 berry number of Malling™ Bella. Different letters indicate a significant difference (F.prob <0.05) in responses between treatments.*

5. Discussion

The experiments described in this report were designed to better understand the legacy effects on photosynthesis and stomatal conductance following a transient rootzone water deficit stress. The mechanisms that govern the response to stress and the subsequent recovery process were investigated to gain a deeper understanding of the causal signals so that impacts on berry yield and quality could be minimised if a transient rootzone water deficit stress in commercial crops could not be avoided.

To support these objectives, experiments were conducted in a glasshouse compartment, allowing for greater control over environmental variables compared to a polytunnel, to help understand the causal signals that regulate the responses. Conversely, the pot size experiment was conducted in a polytunnel to more accurately represent real-world growing challenges that growers might face.

5.1. Using sensor-based automatic irrigation

In the experiments outlined in this report, a sensor-based automated precision irrigation system was utilized to facilitate independent irrigation for each treatment. This methodology enabled rigorous control of CVMC values and ensured that a narrow, predetermined range was maintained for WW plants. Furthermore, this system allowed for confident comparisons between WW and DD plants, as any observed differences in plant performance were directly attributable to variations in CVMC values. Sensor-based automatic irrigation systems have also been used to support the growth of young *Cymbidium* where consistent volumetric water contents were achieved (An *et al.*, 2020). An *et al.* (2020) indicated that *Cymbidium* grown at lower volumetric water contents had significantly smaller leaves, biomass and lower photosynthetic rates compared to those grown at higher volumetric water contents.

5.2. Shoot water balance responses

Midday SWP values of WW plants remained consistent with minimal day-to-day fluctuations, enabling reliable comparisons with DD plants under rootzone water deficit stress. Midday SWP is a sensitive and reliable water stress indicator, capable of detecting changes as small as 0.05 MPa (McCutchan & Shackel, 1992). Seasonal patterns of midday SWP, with more negative values as the year progresses, have also been observed in peach trees (Marsal et al., 2015). The minimal variability in WW plants allowed for precise detection of the response and recovery phases during and after drying treatments. When water availability decreased, stomatal closure and hydraulic adjustments minimized water loss and maintained cell turgor, as supported by Scharwies and Dinneny (2019) and Tombesi et al. (2015). Midday SWP recovered rapidly upon rewetting, indicating quick restoration of water potential, consistent with findings from Li et al. (2015), who suggested that embolism repair in the xylem facilitates recovery after severe water stress.

5.3. Leaf gas exchange

The response time of g_s to coir drying was similar across treatments, with differences observed on Day 4 in the 7-day DD treatment and Day 2 in the 4-day DD treatment. A transient rootzone water deficit stress triggers the synthesis of ABA in the roots, which is transported to the leaves via xylem sap, promoting stomatal closure by regulating downstream signalling pathways (Davies & Zhang, 1991; Liu et al., 2022). Accumulation of foliar ABA limits gas exchange by reducing stomatal aperture (Tombesi et al., 2015). Hydraulic signals also play a role, as water deficit stress reduces turgor pressure, increases solute concentration, and enhances stomatal sensitivity to chemical signals from the roots (Christmann et al., 2013; Jia & Zhang, 2008).

Upon rewetting, g_s and Pn recovery differed based on the duration of the drying treatment. Foliar ABA accumulation during the drying phase may inhibit full recovery of stomatal aperture after rewetting, as sustained reductions in transpiration favour embolism repair (Tombesi et al., 2015). Drought stress also induces reactive oxygen species production, causing cellular damage and impairing photosystems, particularly photosystem II and the electron transport chain (Qiao et al., 2024). Metabolic limitations, such as reduced RuBP and ATP availability, can restrict Pn even when g_s remains high (Flexas & Medrano, 2002; Lawlor, 2002). Stomatal limitations further exacerbate this by reducing CO_2 availability (Pena-Rojas et al., 2004). The reduced Pn values observed after rewetting in both treatments suggest that either metabolic or stomatal constraints, or both, contributed to the incomplete recovery of photosynthetic activity.

5.4. Chemical signalling in response to a rootzone water deficit stress

Xylem-borne and foliar ABA levels remained consistently low in all WW plants, indicating an absence of stress, which was maintained by the steady CVMC achieved through the PI irrigation system. In contrast, elevated xylem and foliar ABA levels were detected in DD plants from Day 1, reflecting increased root-synthesized ABA being transported via the xylem to the leaves as the coir dried (Davies & Zhang, 1991). Altered leaf gas exchange parameters following the increase in xylem and foliar ABA highlights the significant role of ABA in signalling in response to a water deficit stress.

5.5. The effects of different pot sizes of leaf gas exchange recovery and on berry yield and quality

Research carried out on different crops and pot sizes, olive plants in 3 L pots (Torres-Ruiz *et al*., 2015) and tomatoes grown in 30 L pots (Sobeih *et al*., 2004) highlighted the importance of both hydraulic and chemical signals in stress response and recovery, despite species-specific differences. While Torres-Ruiz et al. (2015) found no direct link between stem or root ABA and stomatal behaviour, they emphasized the role of hydraulic signals from the root system in regulating stomatal conductance. Larger rooting volumes, which increase root surface area and hydraulic capacity, may facilitate quicker recovery after rewetting due to improved water delivery to shoots (Gambetta et al., 2012), which could explain the quicker recovery of g_s and Pn values in the 7.5 L compared to the 4.7 L pots.

The relationship between rooting volume and photosynthetic rates varies across species. While photosynthesis decreases in some plants with smaller rooting volumes, such as tobacco (Herold & McNeil, 1979), it may increase in others like beans (Carmi et al., 1983) or remain unaffected as in soybean (Krizek et al., 1985). Although xylem ABA concentrations rise during drought stress, rooting volume did not directly alter xylem ABA levels in some species, such as cowpea (Ismail et al., 1994). However, restricted rooting volume in peppers was linked to increased xylem ABA and reduced stomatal conductance (Ismail & Davies, 1998). In the experiments explained in this report, rooting volume influenced recovery rates of photosynthesis and stomatal conductance following water deficit stress, with smaller pots showing slower recovery. Since the drying phase reached similar CVMC levels across pot sizes, environmental conditions or coir moisture content were unlikely to explain these differences, suggesting a lasting impact of rooting volume on recovery.

Class 1 yields in WW plants were comparable to those of other raspberry cultivars (Morales et al., 2013). However, rootzone water deficit stress reduced Class 1 yield and berry number in Malling™ Bella, consistent with findings in other raspberry cultivars (Ortega-Farias et al., 2022; Morales et al., 2013). Similarly, water deficit stress in tomatoes decreased fresh weight, fruit size, and production dry matter (Medyouni et al., 2021).

The legacy effect of coir drying on berry weight becomes evident weeks after the event, likely due to water deficit stress impacting unripe berries present during the stress period, leading to smaller berries at ripening. Water deficit stress during cell expansion has the greatest impact on final berry size, reducing yield (Molitor & Junk, 2019) as plant and fruit growth are inhibited due to reduced cell expansion (Hsiao et al., 1997; Ebel et al., 1993). Water deficit stress can also accelerate berry ripening (Castellarin et al., 2007), contributing to smaller berry size. Limited water availability in coir drying treatments resulted in fewer and smaller berries, with smaller berries more common in 4.7 L pots compared to 7.5 L WW pots due to differences in rooting volume.

For detailed information on the experiments conducted and their results, please refer to the thesis.

5.6. Research limitations and actions for growers

- **Gradual and Controlled Stress Application:** The experiments effectively facilitated a gradual reduction in coir volumetric moisture content, providing valuable insights into leaf gas exchange responses and recovery. However, real-world water deficit stresses are often sudden and caused by factors like irrigation blockages or scheduling errors, which may elicit different responses than those documented here.
- **Impact of Abiotic Stress Combinations:** Water deficit stress rarely occurs in isolation and often combines with other abiotic stresses, especially with the challenges posed by climate change. Future experiments should explore how combined stresses affect plant responses and signalling mechanisms, as these are often distinct from individual stress responses (Mittler, 2006).
- **Experimental Environment and Cultivar Specificity:** Physiological responses varied between experiments conducted in a controlled glasshouse environment and those in a polytunnel, which more closely resembles commercial conditions. Future studies should consider testing different raspberry cultivars to determine if stress response and recovery are cultivar-specific or similar across cane crops.
- **Sampling Limitations and Facility Constraints:** The limited sampling of xylem sap and leaf samples during the 4-day rootzone water deficit stress and the small area of the glasshouse compartment restricted the ability to monitor extended recovery periods and daily variations in xylem and foliar ABA. Future studies should include larger facilities to accommodate more plants and enable expanded sampling to better identify causal signals regulating recovery.
- **Recommendations for Growers:** Efficient irrigation schedules that account for plant water demand and environmental conditions are critical if legacy effects on photosynthesis after transient water deficit stress are to be minimised. Growers should consider using larger pots, such as 7.5 L, to mitigate the adverse effects of stress on yield and berry weight, since smaller pots (e.g., 4.7 L) were shown to exacerbate these issues. Regular monitoring of substrate moisture content across multiple pots is also advised to minimise revenue losses.

6. References

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