Project title:	Improving cane crop resilience by overcoming the legacy effects on photosynthesis during the recovery from short-term combined stresses.
Project number:	
Project leader:	Ece Moustafa, NIAB EMR
Report:	Annual report, October 2021
Previous report:	NA
Key staff:	Ece Moustafa, Mark Else, Andrew Simkin, Tracy Lawson, Amanda Cavanagh.
Location of project:	NIAB EMR, Kent, ME19 6BJ
Industry Representative:	Harriet Duncalfe, Berry Gardens
Date project commenced:	05 October 2020

DISCLAIMER

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

AUTHENTICATION

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

[Name]

[Position]

[Organisation]

Signature .... Date ....

[Name]

[Position]

[Organisation]

Signature .... Date ....

Report authorised by:

Mark Else

Head of Department, Crop Science and Production System

NIAB EMR

Signature ....Date ... 31 October 2021.....

[Name]

[Position]

[Organisation]

Signature .... Date ....

CONTENTS

GROWER SUMMARY

Headline

- Malling™ Bella recovers quickly from mild and transient coir water deficits but a more severe stress event reduces photosynthesis for several weeks

Background

The purpose of the work is to determine whether legacy effects of transient rootzone water deficits limit berry yields and quality in raspberry due to the suppression of photosynthesis. Upon exposure to limiting rootzone water availability, a series of plant adaptive responses are often triggered, and these include a slowing of leaf expansion and partial or complete stomatal closure, the aim of which is to limit water loss due to transpiration until the stress passes. However, these responses can also limit photosynthesis and overall carbon fixation capacity. The suppression of photosynthesis is thought to be due to either stomatal or metabolic limitations or perhaps a combination of the two. A longer-term suppression of photosynthesis after the stress has ended would likely limit Class 1 yield and berry quality.

Summary

Transient coir water deficits were imposed on potted Malling™ Bella primocane raspberries growing at the Water Efficient Technologies (WET) Centre at NIAB EMR to better understand: 1) the physiological responses to coir drying episodes; 2) how long these responses persist following rewetting; 3) how such legacy effects might affect crop productivity, berry quality, resource use efficiency.

Irrigation was scheduled automatically to well-watered plants to maintain coir volumetric moisture contents within a pre-determined narrow range and to achieve a target average daily run-off volume. In plants that are subjected to coir drying, one of the two drippers in each pot was removed so that a rootzone water deficit stress was imposed gradually. Three deficit irrigation treatments were imposed, each of a different severity and duration.

Physiological responses to each of the three deficit treatments were detected. Changes in shoot water balance were the first detectable responses to the coir drying treatments, followed by lowered stomatal conductance and photosynthetic rates. Upon re-watering to restore coir volumetric moisture contents to pre-stress levels, shoot water balance and leaf gas exchange recovered within a few days in plants that were subjected to the short and

medium-term stress episodes. However, in plants subjected to a more severe water deficit stress, legacy effects on rates of photosynthesis persisted for 15 days after plants were re-watered.

The outputs from this research will help to inform BGG raspberry growers' decisions on the optimum rooting volume and pot sizes for commercial raspberry crops, how to better manage tunnel phytoclimatic conditions during hot weather and how to manage irrigation scheduling strategies to ensure that transient or sustained coir water deficits do not limit commercial yields and berry quality via undetected stress legacy effects.

Financial Benefits

Sub-optimal irrigation and resulting transient stress events will reduce raspberry Class 1 yields and berry quality, but the likely financial impact of these effects is not yet known.

Action Points

Growers should ensure that irrigation to raspberry crops is scheduled effectively so that coir volumetric water contents are optimised throughout the day (and night). Growers choosing to implement a water deficit stress to control cane vigour, should be aware that unintended legacy effects may limit commercial yields and berry quality.

SCIENCE SECTION

Introduction

Raspberries (*Rubus idaeus*) are grown mainly in northern Europe and are cropped during the summer and autumn. Like all plants, raspberry morphology and physiology can be affected by abiotic and biotic stress events. Under stress events like mild water deficiency, the primary cause for the reduction in photosynthetic rate is stomatal closure (Flexas and Medrano, 2002). Reddy *et al.* (2004) summarised a range of responses displayed by higher plants exposed to water deficit stress. The three main categories were physiological, biochemical and molecular responses (Reddy *et al*., 2004). Some of the main physiological responses include loss of turgor and osmotic adjustment, a decrease in stomatal conductance (g_s), reduced internal CO_2 concentration, a decline in net photosynthesis (P_n) as well as reduced growth rates.

Under changing environmental conditions, stomata respond rapidly to optimise water use and CO_2 gain. Under a moderate water deficit, the short-term restrictions in net P_n are often caused by stomatal limitation (Flexas and Medrano, 2002, Pena-Rojas *et al*., 2004), where stomatal closure is thought to be triggered by a signalling process (hydraulic and/or chemical signalling) within the plant. It has also been noted that in the first weeks of drought, the relative decline in photosynthesis rate was less than in stomatal conductance (Socias *et al*., 1997). Under field conditions, photosynthesis is resilient to slowly developing soil water deficits, where stomatal closure is the main limiting factor (Chaves, 1991).

Significant drying of the soil or substrate will likely affect plant growth, development and functioning (Davies *et al*., 2002). Rootzone water deficits can have adverse effects on crop yield as they can limit plant physiological processes (Tombesi *et al*., 2015), for example, reduced water availability limits carbon gain via decrease in stomatal conductance which imposes a CO_2 diffusional constraint on photosynthesis (Medrano *et al*., 1997). A range of adaptive responses can be detected under stress, however, when water was reapplied after a period of water deficit stress, gas exchange recovered in all plants, however, rates of recovery were variable (Brodribb *et al*., 2010). Therefore, the variation in recovery rates depends on a range of factors including the type, magnitude and duration of stress, and the prevailing environmental conditions.

The aim of the experiment outlined in this report was to quantify the initial physiological responses in raspberry plants under a transient water deficit stress and understand the timing of recovery of these physiological responses following re-wetting the coir to well-watered levels.

Materials and methods

2.1 Plant material and growing conditions

The experiment was carried out at the Water Efficient Technologies (WET) Centre at NIAB EMR, Kent. Twenty-four 3-year-old plants of the raspberry variety Malling™ Bella growing in a Haygrove Pioneer polytunnel in 7.5 L pots of Cocogreen™ coir substrate were used for the experiment (Figure 1.1). Plants were located at the south end of a 40-m-long row, with four cropping canes per pot and two pots per linear metre.

Figure 1.1 – Malling™ Bella plants growing in a Haygrove Pioneer polytunnel in 7.5 L pots of Cocogreen™ coir. Photos were taken on 28 July 2021.

2.2 Fertigation System

Plants were watered and fed using a drip irrigation system where each plant-and-pot had two dripper stakes connected to Netafim CNL emitters (1.2 L/h). Irrigation was supplied automatically once coir moisture contents reached pre-determined values using a sensor-based closed loop irrigation scheduling system (Delta-T Devices Ltd, Cambridge, UK). Fertiliser was added at each irrigation event (fertigation) using a Netafim Octa NetaJet™4G nutrigation rig; fertiliser recipes were adjusted for vegetative and fruiting stages by following advice from the Berry Gardens Agronomy Team.

2.3 Environmental conditions

In the WET Centre, GP2 sensors were placed at different positions taking measurements of environmental conditions every 10 minutes. These measurements included relative humidity (RH), vapour-pressure deficit (VPD), air temperature, soil temperature and photosynthetically active radiation (PAR). The information from these sensors was used to interpret the plant physiological data sets.

2.4 Coir water deficit treatments

A rhizosphere drying-down treatment was imposed on x24 Malling^{TM} Bella plants from 02 to 11 August 2021. Four different irrigation treatments were imposed: a well-watered (WW) control; and a deficit irrigation treatment that resulted in the coir drying for 4 days (DD4), for 7 days (DD7) or for 10 days (DD10). To impose gradual coir drying, one dripper from each pot was removed and the remaining dripper was repositioned in the centre of the pot to ensure a more even distribution of irrigation water across the root system. In this way, plants in the drying down treatments received 50% of the daily water (and fertiliser) volume that WW plants received. The dripper that had been removed from the pots were bent and taped until the imposition of coir drying was ended. Drippers were then returned to their original positions and the previously dried plants were irrigated in the same way as the WW controls.

2.5 Coir volumetric moisture content

The coir water status was measured as volumetric moisture content (VMC) using a hand-held WET-2 sensor connected to an HH2 meter (Delta-T Devices, Cambridge, UK). Measurements of coir VMC were made three times a day at the top and bottom of the pots, and an average was taken 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 electrical conductivity (EC) and coir temperature.

2.6 Midday stem water potential

Midday stem water potential (SWP) measurements were made following the method described by Scholander *et al.* (1965) using a pressure chamber (Skye Instruments, UK). A terminal leaf from one of the canes in each pot was covered in foil for 90 minutes prior to excision (Figure 1.2). A single sharp cut was then made while the leaf was still covered in foil and then it was sealed quickly around the petiole into the pressure chamber that contained damp tissue at the bottom. The chamber was filled with pressure and using a hand lens the protruding petiole was observed. The end point was recorded once the xylem sap darkened from the xylem vessels at the cut surface; any minor bubbles emitted before the end point were dabbed with a tissue.

Figure 1.2 *– The terminal leaf covered in foil for 90 minutes prior to excision ready to make midday SEP measurements. Photo taken on 15 June 2021.*

2.7 Leaf gas exchange parameters

Leaf gas exchange parameters were assessed using the LI-6400XT and LI-6800 Portable Photosynthesis systems (LICOR Biosciences, Inc. Lincoln, Nebraska, USA). An automatic leaf chamber was used with the same conditions in each of the systems (6cm^2 leaf area, flow rate of 500 µmol s^{-1}, 65% reference humidity, CO_2 at 400 µmol mol^{-1} and a 1,000 µmol saturation point m^{-2} s^{-1} PAR). Photosynthetic rate (A) and stomatal conductance (g_s) were measured every 3 to 4 days at midday using the 2nd fully expanded leaf that was exposed to sunlight. Leaf gas exchange parameters were also collected using a portable, open-flow gas exchange system (ADC Bioscientific, UK) using the same conditions as those described above. Data sets collected from all three systems were analysed and compared.

2.8 Experimental design and statistical analysis

A randomised block experimental design generated using GenStat was used. Each block consisted of four pots where each pot received one of the four treatments. Graphs were produced using RStudio and data were analysed using GenStat and statistical differences ($p<0.05$) among treatments were determined by analysis of variance (ANOVA) and least significant differences (LSDs).

Results

3.1 Coir volumetric moisture content

Coir volumetric moisture content (CVMC) in the WW treatment was maintained between 0.6 and 0.75 m^3 m^{-3} throughout the experiment (Figure 2.1). Before the onset of coir drying, measurements were made on Day 0 (shown by an arrow in Figure 2.1). Measurements made before the onset of the coir drying were referred to as negative (-) days. Substrate drying in the DD treatments began on 2 August 2021 (Figure 2.1). During the DD treatment, the lowest average CVMC value recorded was 0.32 m^3 m^{-3}, a decrease of more than 50%. The pots were re-wetted as soon as each DD treatment was ended and then all DD pots received the same daily irrigation volume as the WW controls; CVMC values were then maintained at WW values until the end of the experiment.

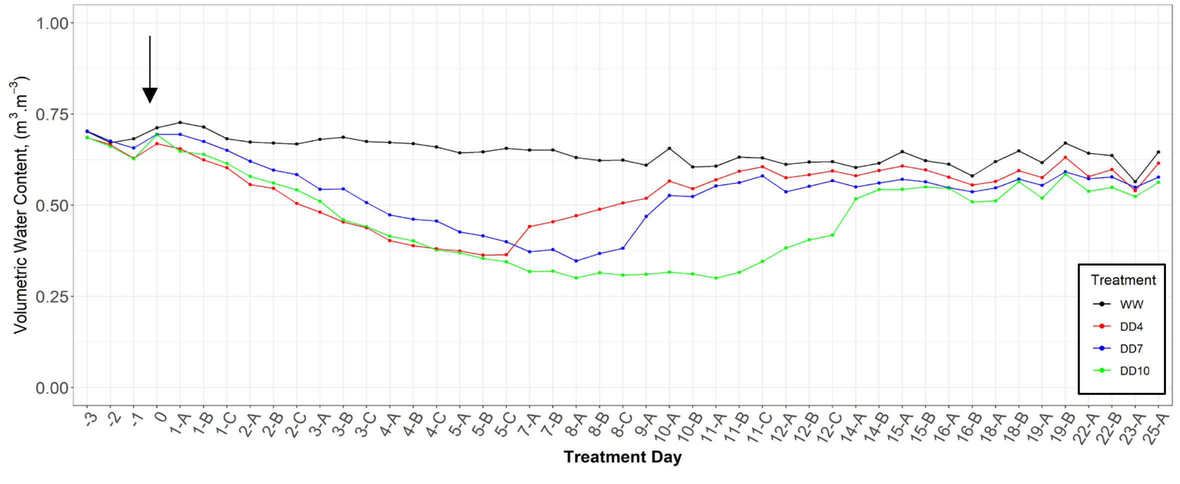

Figure 2.1 *– The effects of the DD treatment on coir volumetric moisture content of the twenty-four Malling™ Bella. Each point represents an average CVMC of the 6 pots in each of the different blocks. Each colour represents a treatment that was applied; black represents WW, red represents DD4, blue represents DD7, and green represents DD10 plants. Arrow represents day 0 measurements. On x-axis, numbers refer to measurements made since the onset of water deficit stress in days and readings were made either in the morning (A), midday (B) or afternoon (C).*

A one-way ANOVA showed that the effect of the DD treatment on the CVMC was statistically significant [$p(<2e^{-16})$]. Minor differences in CVMC between WW plants and DD plants were apparent on Day 1 (1-A), 2 August 2021, and differences became significant [*p (0.006)*] on Day 2 (2-A), but only in DD4 plants and DD10 plants. A day later, on Day 3 (3-A), differences in CVMC between WW and all DD plants were significant [*p (<.001)*].

Once DD plants were rewatered CVMC values were eventually restored to those in WW plants, albeit over different lengths of time. Coir volumetric moisture contents were returned to pre-stress levels on 11 (11-A) in DD4 plants (7 days), on Day 16 (16-B) in DD7 plants (9 days), and on Day 23-A in DD10 plants (13 days).

3.2 Midday stem water potential

On Day 0, midday SWP values were all around -0.478 MPa (+/-0.065). The first responses to drying down of the coir were observed on Day 3 when significant differences between WW, DD7 and DD4, DD10 [*p (<0.05)*] where noted (indicated by a black asterisk in Figure 2.2). These results suggest that a CVMC value of less than 0.5 m^3m^{-3} is needed to trigger a fall in midday SWP and so on Day 3, when the average CVMC was 0.54 m^3m^{-3} in DD7 plants, there was no detectable change in midday SWP. However, when CVMC fell below 0.5 m^3m^{-3} on Day 4, a change in midday SWP in DD7 was observed (indicated with a red asterisk in Figure 2.2).

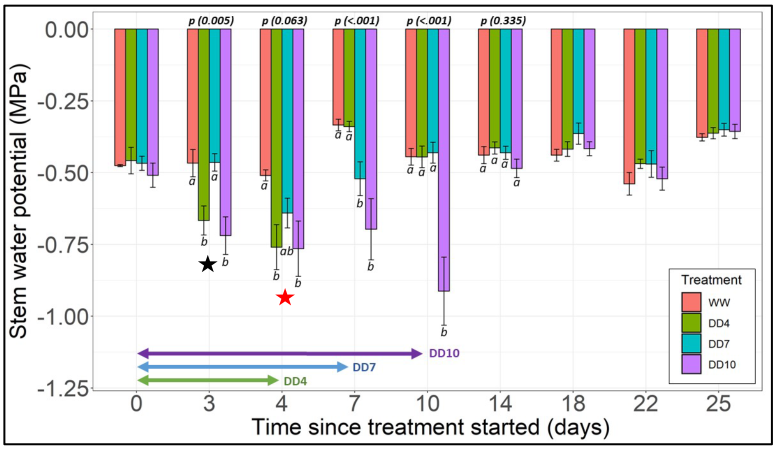

Figure 2.2 *– The effects of the DD treatment on midday stem water potential. Results are an average of the value from all the blocks. Error bars are ±SE, where n=24. Horizontal lines represent the duration of the DD treatment for each of the different conditions. Same letters indicate there is no difference and different letters represent significant difference.*

On day 10, the last day of the water deficit treatment in DD10 plants there was a significant difference [*p (<.001)*] between WW and DD10 plants. When a Tukey HSD was performed using the results from the ANOVA, significant differences between WW-DD10, DD4-DD10 and DD7-DD10 (where adjusted $p = <0.003$) were detected on Day 10. The Tukey HSD also showed that there was no difference between values of midday SWP in plants under the WW, DD4 and DD7. This is shown by the letters representing significant differences (Figure 2.2).

On Day 10, 11 August 2021, there were no significant differences in midday SWP between WW, DD4 and DD7 plants, but DD10 values remained significantly lower.

After rewetting of the coir in each of the different treatments, recovery of midday SWP took approximately 3-4 days. DD4 plants recovered by 8 August 2021 (Day 7, 3 days after rewetting), DD7 plants recovered by 11 August 2021 (Day 10, 3 days after rewetting) and DD10 plants recovered by 15 August 2021 (Day 14, 4 days after rewetting). On 15 August 2021 (Day 14), by which time all DD plants had been rewetted, midday SWP levels were restored in all previously DD-treated plants (Figure 2.2).

3.3 Leaf gas exchange

Responses to coir drying were also detected in leaf gas exchange parameters; with P_n and g_s decreasing over time (Figure 2.3 A&B). Due to technical issues, values on Day 0 were not recorded. However, 1 week prior to beginning the DD treatments, assimilation rate and stomatal conductance values were all within the same range with no significant difference between plant destined for each of the treatments. Due to changeable weather conditions and phytoclimate within the polytunnel a common trend for all DD treated plants was not evident and the duration of the drying down affected the recovery phase of leaf gas exchange parameters.

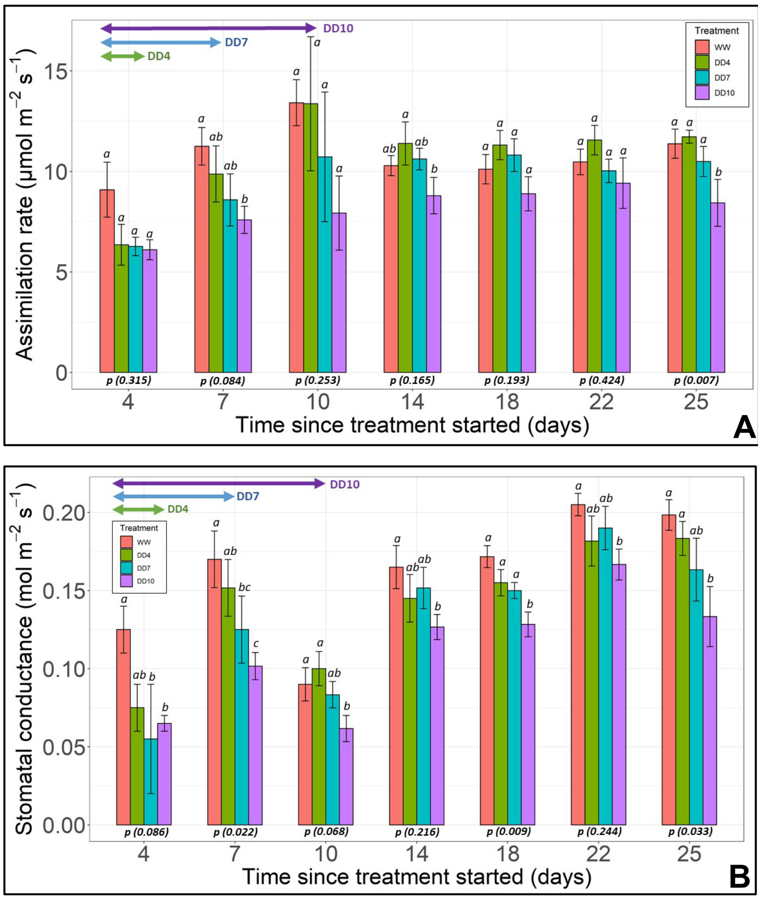

***Figure 2.3** – A) Assimilation rate and B) stomatal conductance of Malling™ Bella during and after the drying down phase. Results are an average of the value from all the blocks. Error bars are ±SE, where n=24. Horizontal lines at the top of the graphs represent the duration of the DD treatment for each of the different conditions. Same letters indicate there is no difference and different letters represent significant difference.*

Gradual coir drying triggered stomatal closure after 4 days and stomata remained partially closed for different lengths of time after rewatering depending on the duration of DD treatments. On Day 4, even though there was a fall in assimilation rate between WW and all DD treated plants, this difference was not significant [*p (0.315)*], at a CVMC value of 0.32 m^3m^{-3} (±0.15; Figure 2.1). There was no significant difference [*p (0.086)*] in stomatal conductance on Day 4 either.

There were no differences in measured assimilation rate between the treatments apart from on Day 25 (Figure 2.3A). However, stomatal conductance varied much more in response to treatments depending on the severity and duration of water deficit stress. In DD4 plants, there was no significant differences in assimilation rate or stomatal conductance throughout the treatment and during recovery.

Once rewetting of DD7 plants began, stomatal conductances returned to WW vlaues within 3 days (Figure 2.3B). DD10 plants responded differently to DD4 and DD7 plants; a significant [*p (0.007)*] fall in assimilation rate was first detected on Day 25. On the other hand, stomatal conductance in DD10 was significantly different to WW plants throughout the treatments and subsequent recovery phase.

Discussion

In this experiment, we compared the impact of a transient water deficit stress over different time frames on physiological parameters of Malling™ Bella. Plants responded by a lowering of midday SWP once a certain CVMC threshold was reached, and changes in leaf gas exchange parameters were detected a day after the fall in midday SWP. These results will help to inform irrigation treatments and growing practices to maintain berry yield and quality; these will be further explored in future experiments.

Ensuring that CVMC optimised is important for the plant growth as water is required to maintain turgor pressure, which drives cell expansion and stem elongation. Consequently, a deliberate imposition of a transient water deficit stress has been used by some to control plant height (Cameron *et al.*, 2006, Alem *et al.*, 2015). Rootzone water deficits can result in changes to cell wall properties, such as the extensibility of the cell wall as well as the minimum turgor required for cell expansion (van Volkenburgh, 1999). However, rootzone water deficits have also been noted to affect physiological parameters such as midday SWP as well as photosynthesis and stomatal conductance. The effects of a rootzone water deficit and subsequent recovery on these physiological parameters were explored in this experiment.

4.1 Midday stem water potential

One of the earliest detectable responses that a plant is perceiving a stress is a change in midday SWP values. Water is an important component in the light dependent reaction of photosynthesis as photosystem II is capable of water-splitting as it is an enzymatic complex (Caffarri *et al.*, 2009). Changes in SWP could be used to detect the onset of rootzone water deficits ahead of other stress responses such as stomatal closure (McCutchan and Shackel, 1992). Results from this experiment show changes in midday SWP to be the first physiological response to the transient water deficit stress. Midday SWP changes were first recorded on Day 3 for DD4 and DD10 plants and on Day 4 for DD7 plants. The time at which plants first perceived the declining coir water availability was estimated to be when midday SWP values began to change. DD4 and DD10 plants first responded to the transient, with a lowering in midday SWP, on Day 3, when the CVMC was less than $0.5m^3m^{-3}$ (Figure 2.1). On Day 3, when the CVMC for DD7 plants was more than $0.5m^3m^{-3}$, there was no change in midday SWP. A day later, on Day 4, when values fell below $0.5m^3m^{-3}$, DD7 plants responded to the water deficit stress with a lowering in midday SWP (Figures 2.1 and 2.2). Conclusively, this supports the statement: "when volumetric water content decreases, SWP is also expected to decrease" (McCutchan and Shackel, 1992). Midday SWP values recovered quickly once rewetting began, taking three-to-four days, but this was not the case with the leaf gas exchange parameters. For example, on Day 4, when there were significant differences noted in midday SWP, there were no significant differences in leaf gas exchange parameters.

4.2 Leaf gas exchange

On Day 0 of the experiment, technical issues arose that meant collecting Day 0 leaf exchange parameters was not possible. The weather conditions throughout August 2021 were variable with days that were quite warm and other days that were cloudy. The changing weather condition would have undoubtedly affected assimilation rate, as it is highly dependent on photosynthetically active radiation (PAR). It is therefore important to compare the values of assimilation rate on a single day rather the changes across the whole experiment. The average assimilation rate for any given time and day were measured in WW control plants and since diurnal changes can affect the values of assimilation rate and stomatal conductance, it was important to make measurements across replicate plants from each treatment in a given timeframe. Valentini *et al.* (1995) conducted in situ estimations of gas exchange parameters and noted diurnal changes over different years and different seasons due to different weather conditions. In that work, data collected from July 1992 and September 1992 were quite similar in terms of the trends in diurnal changes, but with different A_{max} values recorded over different timeframes (Valentini *et al.*, 1995).

There have been many studies on a wide range of plants that have reported that a water deficit stress limited photosynthesis due to the restriction of gas exchange resulting from stomatal closure, a so-called stomatal inhibition of photosynthesis (Zhou *et al*., 2013, Zhen *et al.*, 2014, Currey *et al.*, 2019, Nam *et al.*, 2020, An *et al.*, 2020). The results from this experiment also suggest a stomatal inhibition of photosynthesis, since A was reduced only after g_s was lowered in response to coir drying. Data shown in from Appendix 1 were collected on two separate days, 17 June and 1 July 2021, from leaves at different positions in the canopy. There was a positive correlation between stomatal conductance and assimilation rate when plants were not under stress conditions. However, the results collected in this experiment supports the notion that a decline in photosynthesis rate was less noticeable than in stomatal conductance during the first weeks of a water deficit stress (Socias *et al.*, 1997).

Experiments planned for 2022 will investigate the effects of legacy effects on P_n from a water deficit stress on Class 1 yield and berry quality in Malling™ Bella and in other commercial raspberry varieties. The aim is to understand the nature of the chemical and/or hydraulic signals that regulate shoot and fruit physiological responses during recovery from rootzone water deficit.

Conclusions

Gradual coir drying triggered physiological responses in Malling™ Bella raspberry plants. Midday SWP responses in all DD plants were relatively the same, taking 3 days to respond to the rootzone water deficit stress and 3 days to recover, with longer periods of drying not affecting recovery. Assimilation rates in WW and DD plants subjected to shorter water deficit stress were similar, however, longer periods of drying down (DD10) caused a significant reduction in assimilation rate. On the other hand, stomatal conductance was lower four days after drying down began and stomata remained partially closed for different durations after rewatering depending on the length and severity of the DD treatments.

Knowledge and Technology Transfer

- Poster presentation at the Graduate Forum for the University of Essex.
- There are a few more opportunities to share results via a poster presentation in the near future; including the BCPC Congress.

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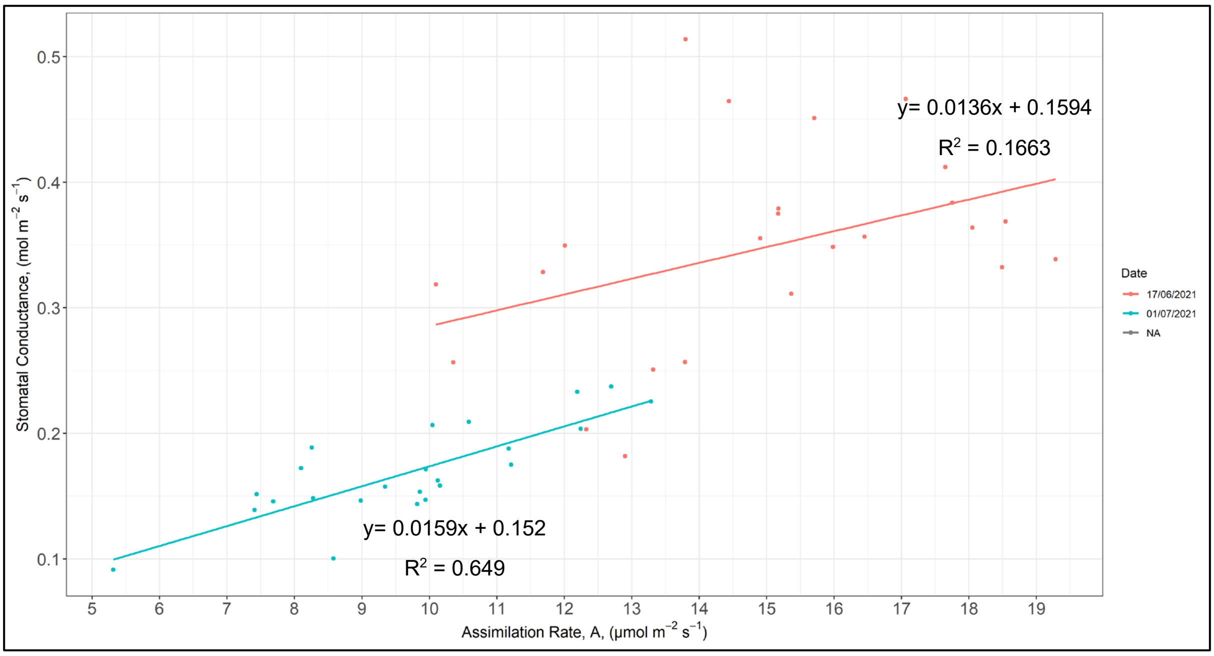
Appendices



***Appendix 1** – Relationship between stomatal conductance and assimilation rate on measurements made on Malling™ Bella prior to the drying down treatments on two different days with different weather conditions. Each point represents a survey measurement and the line representing a line of best fit for a single day.*

