Title: Assessing water uptake in sugar beet (*Beta vulgaris*) under different watering regimes

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Abstract

Sugar beet yield worldwide is substantially reduced as a result of drought stress. Water uptake may be limited by the plant (e.g. low root density) or by soil physical constraints. An experiment was conducted to assess the ability of sugar beet to produce roots and take up water throughout the soil profile under contrasting water regimes. Sugar beet was grown in columns, 15 cm in diameter and 1 m height in a glasshouse. *In situ* soil moisture was monitored hourly, and stomatal conductance was measured weekly. Root length and diameter at different depths were assessed destructively at 78 and 94 DAS. Greater water availability resulted in a higher root length and lower water use efficiency. Water uptake was initially from the upper soil layers but, as demand for water increased, there was a strong increase in root length density at depth. However, it was a further 16 days, after roots reached the deep layers, before significant water was taken up. A possible reason for the delay, between presence of roots and water uptake by roots, was the absence of secondary xylem early on, which was supported by a second root anatomy study. Sugar beet can grow roots up to 1 m deep and take up water from depth, however this did not happen until the late stages of drought stress and thus storage root dry weight had already been

severely reduced, indicating that prevention of drought is necessary, early on, to minimise yield losses.

Key words: Drought, Roots, Soil physical limitations, Stomatal conductance

1. Introduction

Sugar beet (*Beta vulgaris*) is grown in many areas of the world but tends to thrive in the temperate climates found in large parts of Europe, North America and Asia. It is mainly grown as a sugar source, and more recently, for biofuel (Draycott 2006). In the UK, sugar beet production is centred in East Anglia, where the average annual rainfall is less than 700 mm, and is grown predominantly on sandy loam soils with a low water holding capacity (Brown and Biscoe 1985). Jaggard et al. (1998) reported that, on average in the UK, there is a 10% yield loss due to water limitation which can exceed 25% in dry years.

While sugar beet roots have been shown to exceed 1 metre depth (Brereton et al. 1986; Morillo-Velarde and Ober 2006), Brown and Biscoe (1985) found that 80% of the crop's water requirement was taken from the top 30 cm, with <12% coming from below 50 cm. Compaction of the soil is considered a major limiting factor to water uptake at depth because it restricts root growth in deeper soil layers and thus water availability (Kirkegaard and Lilley 2007). Brown and Biscoe (1985) hypothesised that; while sugar beet roots were found at depth, they may be confined to existing root channels and hence not be able to explore the soil effectively. Alternatively, roots may not be capable of taking up water from depth due to

physiological restrictions, such as immature root tissue (Varney and Canny 1993; Chimungu et al. 2014). When xylem develops it needs to mature from primary to secondary xylem. During this maturation stage, water uptake is suboptimal (Mapfumo et al. 1993).

Root plasticity allows roots to respond to changes in soil water availability by increasing root length or by forming root hairs (York et al. 2016). Previous studies, in maize, wheat and beans, as well as several species common in temperate grasslands, have shown rooting patterns change with contrasting water availability, and that generally a low water availability leads to increased root proliferation at depth (Li et al. 2002; Ho et al. 2005; Manschadi et al. 2008; Padilla et al. 2013). In sugar beet, roots are known to reach a depth of 1.5 m, if there are no soil restrictions, regardless of water availability (Brown and Biscoe 1985; Camposeo and Rubino 2003). However, sugar beet roots do not necessarily proliferate at depth under drought stress and the response has been shown to be variable between genotypes and seasons (Ober et al. 2004; Romano et al. 2005).

This study sought to address the following questions: a) how are sugar beet roots distributed under unrestricted conditions? ; b) does this change with water availability? ; and c) what are the key mechanisms responsible for extracting water from depth? To address these questions two experiments were conducted where plants were grown in a glasshouse in 1 m columns. One study considered the plant physiological responses to differing water regimes and the other experiment focused on root anatomy from 60 to 100 cm depth at different time points.

2. Material and methods

2.1 Experimental design: Plant physiology experiment

To measure physiological responses of *Beta vulgaris*, plants were grown in glasshouse conditions, heated to 18°C during the day and 8°C at night (min: 7°C, max: 38°C). Two sodium lights with a maximum output of 750 µmol m^{-2} s^{-1} (Master GreenPower CG T 400W E40 1SL, Turnhout, Belgium) supplemented the incident light for 8 hours per day. Sugar beet were grown in 1 m tall columns with a diameter of 15 cm, in a randomized block design with ten replicates. The soil medium was a sandy loam texture (12% clay, 19% silt and 77% sand) mixed with sharp washed sand and Kettering loam in a 1:1 ratio. The columns were partially filled and then watered to reduce slumping. This was done several times until the columns were completely filled. In six of the columns (two per treatment) volumetric soil moisture sensors, EC-5 (Decagon Devices, Labcell Ltd., Alton Hants, United Kingdom) were placed at four depths: 30, 50, 70 and 90 cm. Five Em5b data loggers (Decagon Devices, Labcell Ltd., Alton, Hants, United Kingdom) were used to log the hourly readings from the soil moisture sensors. Solid fertilizer (HortiMix Standard; NPK ratio 15:0.8:6, Hortifeed, Lincoln, United Kingdom) equivalent to 120 kg ha^{-1} was applied in the top 5 cm of the soil.

Two sugar beet seeds (cv. Master) were planted at 3 cm depth in each column. At 12 days after sowing (DAS) the first seedlings emerged and at 19 DAS columns were thinned to one seedling per column. Prior to the start of the experiment, field capacity (25% volumetric soil moisture content) was determined by watering the columns to saturation and then letting them drain for two days. The soil moisture sensors were calibrated to the soil used to get volumetric moisture contents representative to

the soil. From 1 to 39 DAS the columns were watered daily, by hand, to maintain field capacity. At 40 DAS, the three different watering regimes were imposed: FC (control): sufficiently watered, AR: average rainfall between 2010 and 2014 was simulated, DR: drought, no irrigation at all.

2.2 Experimental design: Root anatomy experiment

A second experiment was conducted to study the root anatomy. Sugar beet were grown in 1 m tall columns in a non-heated glasshouse. The columns were cut in half and plexiglass was used to cover the open sides and to monitor root growth as the plants were growing. The variety Master cv. was grown in a sandy loam with solid fertilizer (HortiMix Standard; NPK ratio 15:0.8:6, Hortifeed, Lincoln, United Kingdom) equivalent to 120 kg ha^{-1} applied in the top 5 cm of the soil. Water was applied in sufficient amounts in all three columns. The seeds were sown at different times to ensure differential developmental stages at the time of harvest. The interval between sowing dates was 10-11 days. Since development can be fast the plants were sown in relatively quick succession to really look at the early root development. All plants were harvested when the plants that were sown last reached the bottom of the column.

To compare both experiments the thermal time was calculated for both experiments with a base temperature for sugar beet of 3°C.

2.3 Measurements

In the physiology experiment the fully expanded fifth leaf was used for gas exchange measurements using a Li-6400XT (Li-cor, Lincoln, Nebraska, USA). All measurements were taken

between 9.00h and 13.30h, but within a two hour timeframe on each sampling date. The settings were as follows: photosynthetically active radiation (PAR) 1200 µmol m^{-2} s^{-1}, Flow rate 500 µmol s^{-1}, CO_2 concentration 400 µmol and block temperature 18 °C. Von Caemmerer and Farquhar (1981) formulated the algorithms that the instrument uses to calculate the transpiration rate (mol m^{-2} s^{-1}), the stomatal conductance (mol_{H2O}^{-2} s^{-1}), and the net assimilation rate of CO_2 by the leaf (μmol_{CO2} m^{-2} s^{-1}).

2.4 Harvest

At 78 and 94 DAS destructive measurements were executed for the first experiment. These points were chosen based on roots reaching 1m depth at 78 DAS and towards 94 DAS the DR treated plants were stressed severely and the experiment was terminated. To monitor root growth more closely rhizotron columns were set up alongside the experiment to look at root growth over time under the different water regimes. Relative leaf water content (RWC) was calculated from the fresh weight, turgid weight and dry weight of leaf discs. The leaf area was measured (Li-3100C Area meter, Lincoln, Nebraska, USA), and the leaf dry weight was determined after drying at 85 °C for at least 48h. Specific leaf area (SLA) was calculated (Tsialtas and Maslaris 2008).

After the harvest, the columns were divided into four soil layers; 0-15 cm, 15-30 cm, 30-60 cm, and 60-100 cm. Previous studies showed that most water uptake is from the top 30 cm (Brown and Biscoe 1985), therefore the top 30 cm was divided in two sections of 15 cm. There is substantially less water uptake from below 30 cm, and thus those sections were kept larger. The roots from each layer were washed out and stored at 4 °C. Roots

were scanned on a flatbed scanner (EPSON expression, 11000XL Pro, Japan) and analysed with WinRHIZO software (Regent instruments Inc., Québec, Canada) to determine the total root length (cm), and the average root diameter (mm). The root length density (RLD) was calculated (Camposeo and Rubino 2003). Following WinRHIZO analysis, the roots were dried at 80 °C for at least 48h after which the dry weight was determined. Soil samples were taken at the different depths to measure bulk density. Total plant water use efficiency (WUE) was determined from the total plant dry weight and the total water uptake during the whole experiment.

Roots of the second experiment were harvested at 48, 59, and 69 days after sowing. The taproot of each plant was sectioned at the following depths: 60, 70, 80, 90, and 100 cm. Sections were embedded in resin (Technovit 3040, TAAB Laboratory equipment Ltd., Reading, United Kingdom) for further analysis. A microtome was used to create 2.5 µm sections which were kept on microscope slides. The sections were then stained with calcofluor white (Sigma-Aldrich, Poole, Dorset, United Kingdom), toluidine blue, and an anti-rat – FITC marker (LM11) (McCartney et al. 2005). An Olympus BX61 microscope (Olympus, Southend-on-Sea, United Kingdom) was used to take bright field and fluorescence images.

2.5 Statistical analysis

The main factor in the drought experiment was the water treatment. There were five blocks and two destructive measurements. A general ANOVA was performed on plant biomass data, leaf area data, root data and bulk density data. For stomatal conductance data, a repeated measures ANOVA was performed. GenStat 15th edition (VSN International Ltd.,

Hemel Hempstead, United Kingdom) was used for the statistical analyses.

3. Results

3.1 Soil moisture and water uptake

The watering regimes had a significant effect on the pattern of water uptake from different depths within the columns (Fig. 1a-c). The FC treatment did not show depletion of moisture from all layers (50, 70 and 90 cm) for the first 70 days. Towards 70 DAS, there was a decline in water content throughout the whole column, which coincided with a marked increase in air temperature (data not shown). The AR treatment started taking up more water than it received after 55 DAS, this was first observed at 30 cm depth after which water was also extracted from deeper layers. Towards 75 DAS the soil moisture in the top 60 cm of the soil had decreased below 17% and subsequently water was taken up more rapidly from 90 cm depth. At the end of the experiment, the upper 30 cm of the AR treatment had been depleted of soil moisture and there was limited water uptake from below 30 cm. The DR treatment, however, had a different pattern. There was water uptake from all layers, although water was not taken up from below 50 cm before the layers above it had been depleted to less than 15%. Water was taken up from 90 cm depth but only after the shallower layers had been depleted to 17% moisture.

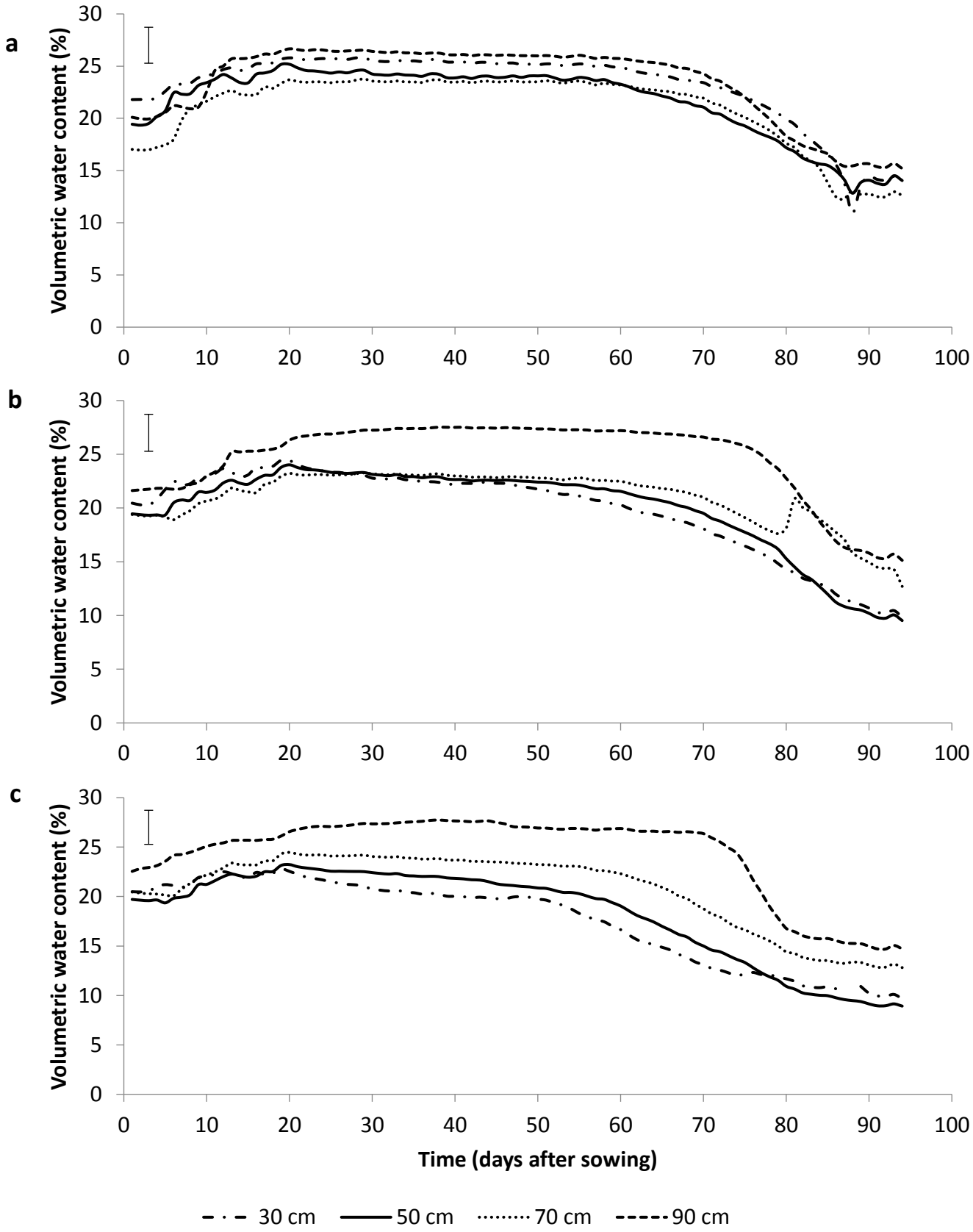

Figure 1 Volumetric water content (%) of a) control treated plants, b) average rainfall (AR) treated plants, and c) drought (DR) treated plants at different depths; 30cm, 50 cm, 70 cm and 90 cm. The bar shows the least significant difference (time*treatment*depth)

242 Water use efficiency appeared to decrease with increased

water availability. The control plants received 7686 ml of water during the experiment and had a water use efficiency (WUE) of 3.2 g L^{-1}, while the DR treated plants received only 3887 ml of water but had a WUE of 4.1 g L^{-1}. The AR treated plants took up 5237 ml of water and had a WUE of 4.3 g L^{-1}, which was the highest of the three treatments.

3.2 Stomatal conductance and photosynthesis

Most changes were seen later in the experiment, particularly at 80 DAS. Changes in water uptake influenced stomatal conductance after 72 DAS, control plants showed a reduction in stomatal conductance from 0.36 to 0.15 mol_{H2O} m^{-2} s^{-1} when the water uptake was reduced at 88 DAS (Fig. 2). The AR treated plants had a lower stomatal conductance at 80 DAS compared to the control plants and showed a strong decline in stomatal

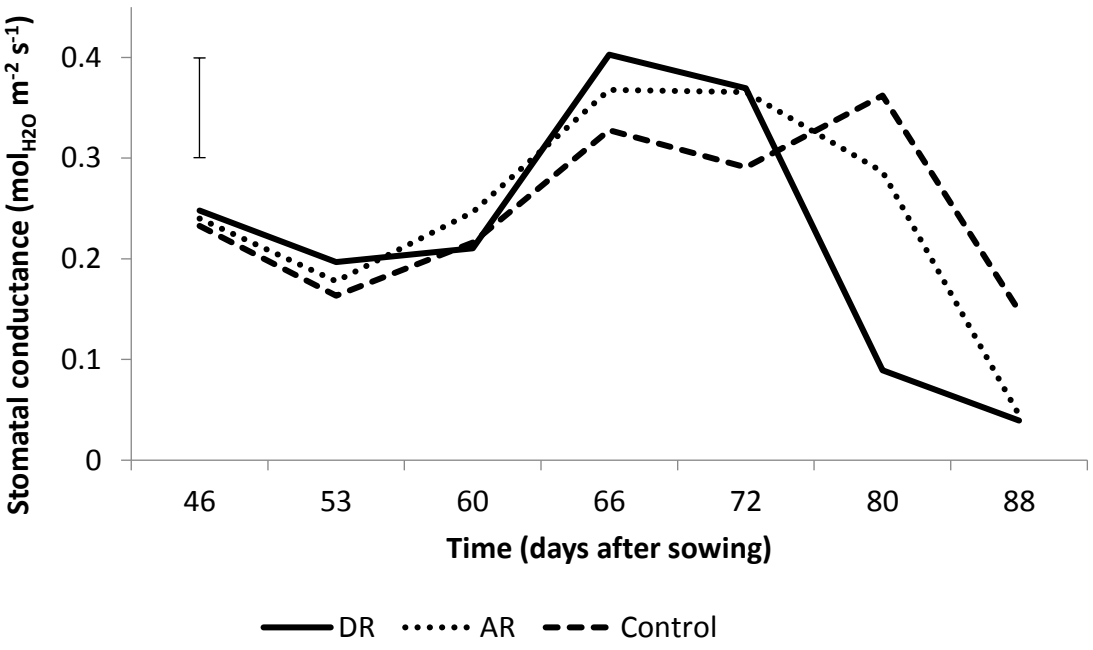

Figure 2 Mean stomatal conductance (mol_{H2O} m^{-2} s^{-1}) over time for each treatment; drought (DR), average rainfall patterns (AR), and control. The bar shows the least significant difference (time*treatment).

conductance when the water uptake was reduced from 0.29 to

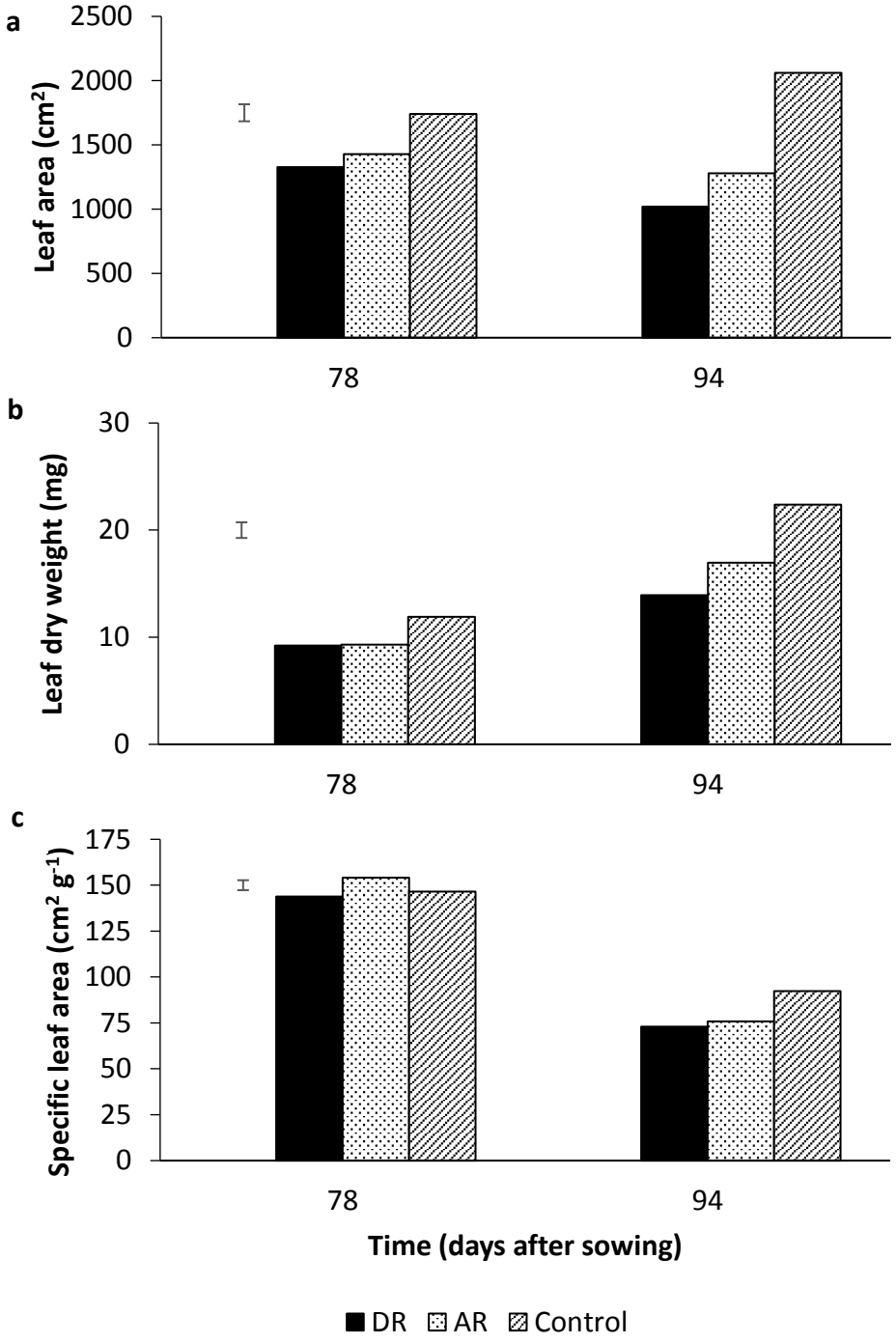

Figure 3 a) Leaf area (cm^2), b) Leaf dry weight (mg), and c) specific leaf area ($cm^2\ g^{-1}$) at 78 DAS and 94 DAS. The bar shows the least significant difference (treatment).

0.04 $mol_{H2O}\ m^{-2}\ s^{-1}$. The DR treated plants ended with the lowest stomatal conductance; at 72 DAS it was 0.37 $mol_{H2O}\ m^{-2}\ s^{-1}$ and at 80 DAS it had dropped to 0.09 $mol_{H2O}\ m^{-2}\ s^{-1}$. After a warm

period in the glasshouse (with temperatures up to 30 °C), at 88 DAS, the stomatal conductance of the DR treated plants dropped to below 0.04 mol_{H2O} m^{-2} s^{-1}. There was a significant time x treatment interaction of stomatal conductance (p<0.001, DF=78.59, l.s.d.=0.099) caused first by the drop in DR treated plants, and the later drop in AR treated plants. The net photosynthetic assimilation followed a similar pattern to the stomatal conductance with severe reductions in net photosynthetic assimilation in all treatments between 80 and 88 DAS. The control plants had a net photosynthetic assimilation of 24 μmol_{CO2} m^{-2} s^{-1} at 80 DAS which halved to 12 μmol_{CO2} m^{-2} s^{-1} at 88 DAS. The AR treated plants decreased to 7 μmol_{CO2} m^{-2} s^{-1}, and the DR treated plants decreased to 6 μmol_{CO2} m^{-2} s^{-1} at 88 DAS.

3.3 Canopy measurements

Higher water uptake resulted in greater leaf area and dry weight in the FC treatment (Fig 3a-b). However, there was a decrease in specific leaf area (SLA) between 78 and 94 DAS in all treatments (Fig. 3c). DR treated plants had the lowest SLA at both time points (p=0.003, DF=19, l.s.d.= 5.44), and even though the AR treated plants had the highest SLA at 78 DAS their SLA was severely reduced after a warm period. The SLA of the control plants reduced slightly, due to the warm period. Between 78 and 94 DAS there was a significant drop in relative leaf water content (RWC) (p<0.001, DF=19, l.s.d.=0.025). At 78 days after sowing the RWC of all treatments was around 80% with the DR treated plants being 3% lower (p<0.001, DF=19, l.s.d.=0.031) than the other treatments (Fig. 4). At 94 DAS the relative leaf water content in the DR treated plants decreased to 46%, while the control plants still had a RWC of 75%. The AR treated plant's RWC dropped 30% between 78 and 94 DAS. The

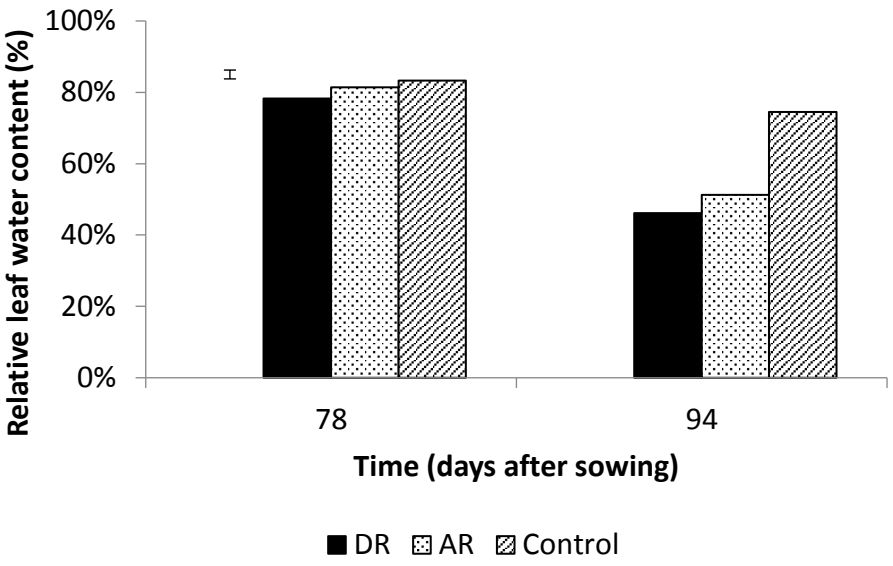

Figure 4 Relative leaf water content (%) at 78 DAS and 94 DAS. The bar shows the least significant difference (0.025) (treatment).

RWC of control plants dropped 9% between 78 and 94 DAS. All reductions in RWC corresponded with reduced stomatal conductance and water uptake (Fig. 2 and Fig. 4). The changes in water use efficiency between the two harvests showed that the DR treated plants had increased less (3.4 g l^{-1} to 4.1 g l^{-1}) than the FC (2.4 g l^{-1} and 2.7 g l^{-1}) and AR (3.2 g l^{-1} and 4.3 g l^{-1}) treated plants.

3.4 Root length and diameter

Total root length density (RLD) significantly increased over time, however at both points in time the DR treated plants had a lower RLD than the FC plants. The DR plants had an overall RLD of 0.43 cm cm^{-3} at 78 DAS which increased to 0.93 cm^3 cm^{-3} at 94 DAS, while the FC plants went from an overall RLD of 0.82 cm^3 cm^{-3} at 78 DAS to 1.79 cm^3 cm^{-3} at 94 DAS (p<0.001, DF=13, l.s.d.=0.181). The water availability strongly influenced the RLD distribution (Fig 5). The DR plants had a significantly lower RLD in the 0-15 cm soil section compared to the control plants (p=0.026, DF=67, l.s.d.=0.564), this difference increased

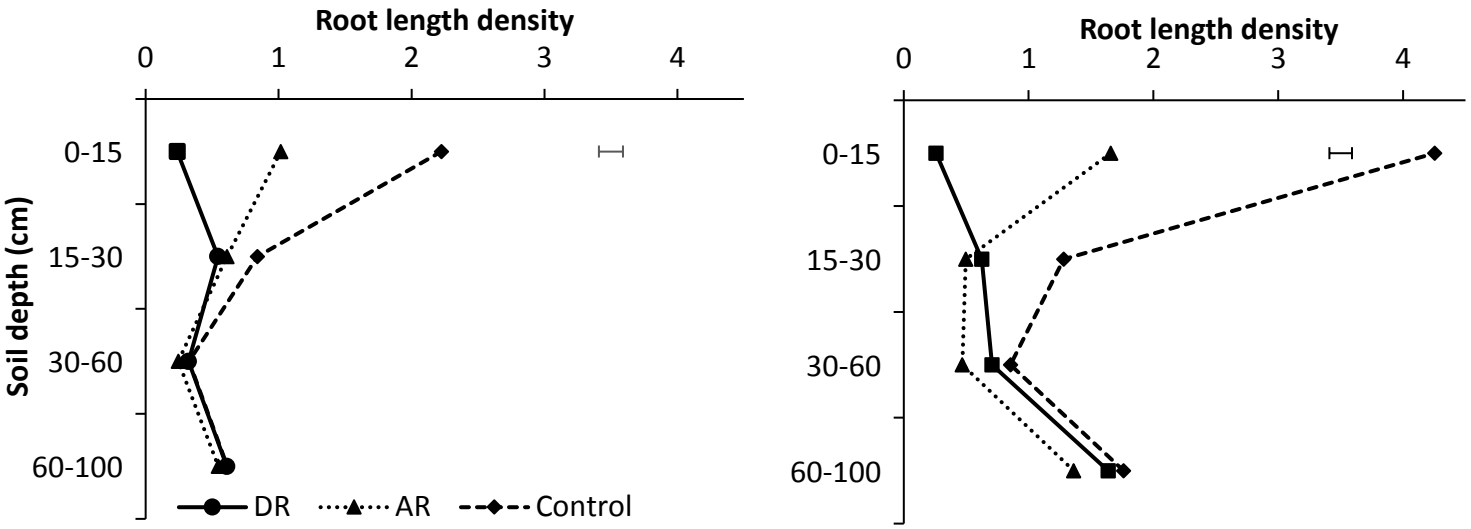

Figure 5 a) Root length density (cm cm^{-3}) at 78 DAS, and b) root length density (cm cm^{-3}) at 94 DAS. The three treatments have been divided into layers: 0-15 cm depth, 15-30 cm depth, 30-60 cm depth, and 60-100 cm depth. The bar shows the least significant difference (time*treatment*depth).

between 78 and 94 DAS. Both AR and control plants significantly increased their RLD in the 0-15 cm soil section between 78 and 94 DAS. The DR plants only showed a slight increase in RLD. In the 15-60 cm section of the column there were only minor

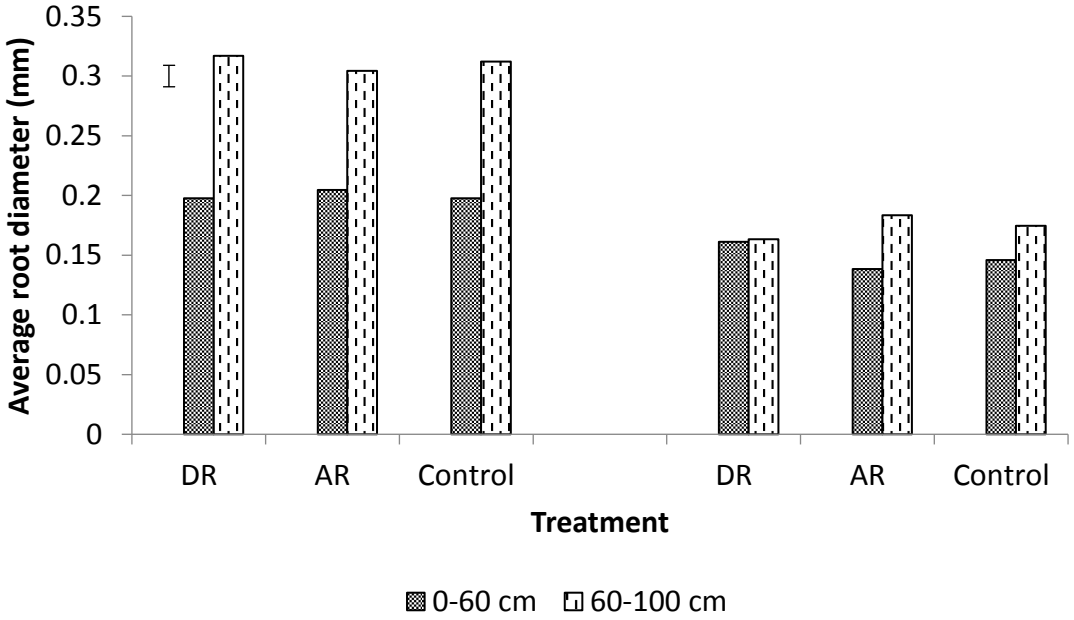

Figure 6 Average root diameter (mm) at 78 DAS (left) and 94 DAS (right). The three treatments have been divided into layers: 0-60 cm and 60-100 cm. The bar shows the least significant difference (time*treatment*depth).

changes in RLD distribution between the different treatments at 78 and 94 DAS. However, the 60-100 cm section showed that the RLD increased significantly between 78 and 94 DAS regardless of the treatment.

Figure 6 shows the average root diameter, which was overall significantly higher in the 60-100 cm layer at 78 and 94 DAS (p<0.001, DF=67, l.s.d.=0.018). At 94 DAS the difference in diameter had been severely reduced, with a general decrease in the overall average diameter of the roots. Bulk density measurements showed no changes between 78 and 94 DAS; there was only an increase in bulk density with increasing depth (p=0.031, DF=32, l.s.d.=0.092). The storage root weight was severely affected by drought. The control plants had an average storage root weight of 19.4 g (se +/- 1.1) at 94 DAS, the AR and DR treated plants had a much lower average storage root dry weight, 9.9 (se +/- 0.41) and 8.4 g (se +/- 0.36) respectively.

To compare both experiments the thermal time was calculated. Table 1 shows how the two experiments compare developmentally.

Table 1 Comparison of days after sowing between both experiments with the help of the thermal time.

Thermal time (°C days)	First experiment	Second experiment
534	48 DAS	48 DAS
748	66 DAS	59 DAS
919	79 DAS	69 DAS

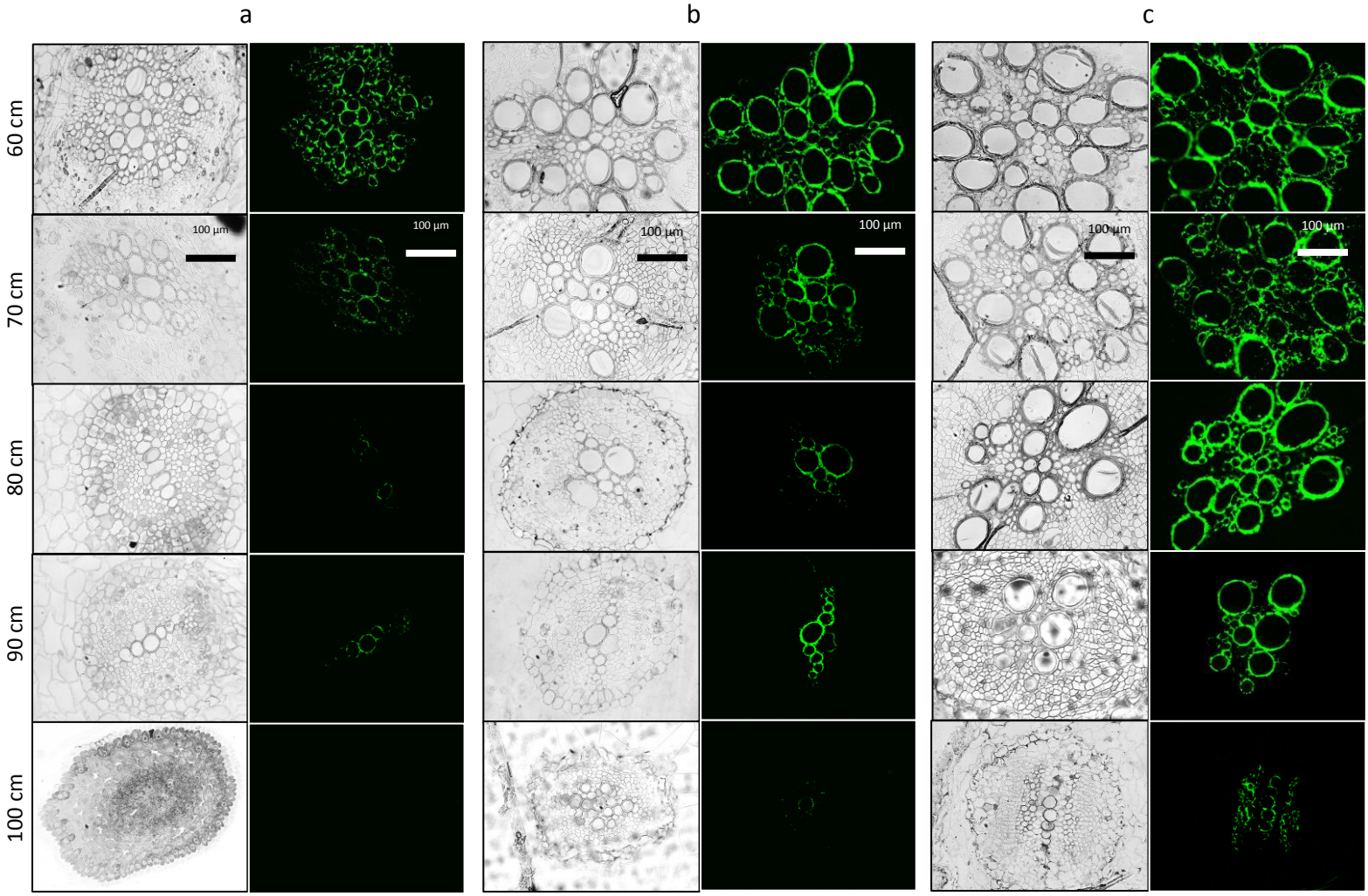

Figure 7 Cross section of the taproot in bright field and under the fluorescent microscope after staining with anti-rat FITC marker LM11 at 60, 70, 80, 90, and 100 cm depth. a) 48 DAS; b) 59 DAS; c) 69 DAS. The bar indicates 100 µm.

Each panel in Figure 7 shows a cross section of the root in bright field on the left, then using a fluorescent marker to highlight secondary xylem, in green, on the right. At 48 DAS roots had reached 100 cm depth but no xylem tissue was found at that depth. However at 60 cm there was a significant amount of secondary xylem. Samples taken at 59 DAS showed that the taproot at 100 cm had developed xylem, however the lack of green fluorescence indicates that the xylem had not yet matured, unlike the xylem at 60 and 70 cm depth. At 69 DAS secondary xylem was abundant at 60 to 80 cm depth with a good amount of secondary xylem at 90 and 100 cm.

4. Discussion

Water use efficiency was higher when water availability was reduced. This has been previously reported in C3 cereals (Araus et al. 2002) and in sugar beet with drought stress (Araus et al. 2002; Bloch et al. 2006; Rinaldi and Vonella 2006). However, at 94 DAS the WUE of the DR treated plants had increased less than the FC and AR treated plant, from 3.4 g l^{-1} to 4.1 g l^{-1}, indicating that severe drought stress can reduce WUE. Araus et al. (2002) suggested the improvement of WUE is in part caused by a decrease in stomatal conductance, and by an increase in photosynthetic capacity. The reduction in available soil moisture resulted in decreased stomatal conductance, however it was unclear whether this was the result of a plant protection mechanism or a water conservation strategy of the plant (Ober et al. 2005). The net photosynthetic assimilation was reduced following the same pattern as the stomatal conductance. This indicates that improved WUE was a result of stomatal closure rather than maintenance of photosynthetic assimilation. Previously Flexas and Medrano (2002) showed that a stomatal conductance of 0.10 mol m^{-2} s^{-1} is considered a threshold value between severe and moderate drought stress in C_3 species such as sugar beet. At 80 DAS the stomatal conductance of the DR treated plants dropped below 0.10 mol m^{-2} s^{-1}, and at 88 DAS both the DR and the AR treated plants had values below this threshold indicating severe drought stress and thus explaining the drop in photosynthetic assimilation. Gollan (1985) found that soil water content rather than relative leaf water content controlled leaf gas exchange. When approximately half of the extractable water in the soil had been taken up there was a decrease in leaf gas exchange. Relative leaf water content

383 reductions do not always seem to interfere with the
384 photosynthetic assimilation, but they are thought to limit plant
385 growth (Anderson and McNaughton 1973). This corresponds
386 with our findings of a lower leaf area and dry weight in DR and
387 AR treated plants compared to the control plants and also
388 translates into a lower specific leaf area for the DR treated
389 plants. Brereton et al. (1986) found that plants can adapt to
390 negative soil conditions by reducing the leaf area expansion
391 instead of reducing the stomatal conductance by reduced leaf
392 water potential. The reduction in SLA, over time, was most likely
393 a result of older, larger leaves dying and new, smaller leaves
394 forming (Milford et al. 1985).
395
396 Considering the total amount of water taken up (on average
397 5600 ml for all treatments), there was limited water uptake
398 from 90 cm depth (Figure 1) before the shallow soil layers were
399 depleted, as previously observed in the field (Brown et al.
400 1987). Our findings suggest this is, at least in part, due to a lack
401 of secondary xylem, as previously been seen by Mapfumo et al.
402 (1993). In the control plants there were no significant changes
403 in soil moisture content, since the extracted water was
404 replenished almost immediately. Towards the end of the
405 experiment, temperatures in the glasshouse had risen above a
406 daily average temperature of 18°C (min 7°C, max 35°C),
407 compared to an overall daily average of 14 °C, resulting in an
408 increase in water uptake. During this period, the DR and AR
409 treated plants took up more water from depth, where water
410 was still available, while the control plants appeared to extract
411 water throughout the whole column and not just from the
412 shallow layer where the water was replenished immediately.
413 There may be several reasons why water was not taken up from
414 depth until later in the experiment; a) roots did not reach the

deeper soil layer, b) the axial pressure was too low, because the root was too thin (Clark et al. 2003), and c) roots grew in the deep soil layer but the xylem had not matured, resulting in reduced water uptake (Steudle and Peterson 1998; Carminati and Vetterlein 2013). Our data showed that roots reached 90 cm at 51 DAS which discounts that roots were not physically in place (data not shown). The roots below 30 cm were thicker than the roots in the first 30 cm, so axial pressure is not expected to have caused limitations to water uptake. In the anatomy experiment, we observed that there was a 16 day delay between roots arriving at a given depth and water being taken up. This corresponded closely to the time of secondary xylem developing. We therefore suggest that the reason for the delay between roots reaching deep layers of the soil profile, and taking up water from that depth, was due to the time required for secondary xylem to develop.

The reason for the increased root thickness below 30 cm was most likely an increase in soil bulk density (Clark et al. 2003; Alameda et al. 2012; Tracy et al. 2012). The bulk density below 30 cm was 0.2 g cm^{-3} higher than the bulk density in the first 15 cm, which was 1.08 g cm^{-3}. A reason for the difference in bulk density could be soil slumping and the pressure of the top soil weighing down the soil below that, creating a gradient of increasing bulk density with depth. This has likely caused the difference in root diameter between the first 15 cm and below 60 cm seen at the first harvest (78 DAS). At 94 DAS this difference in root diameter with depth had disappeared in all treatments. The period in the glasshouse, where temperatures rose to 18°C on average, after 78 DAS resulted in drought stress and all plants had foraged for water, as seen in an increase in root length density (RLD) below 60 cm. Previous studies have

shown drought stress can result in an increase in root length, especially at depth (Shaw et al. 2002; Asch et al. 2005). The newly formed roots had a smaller diameter and therefore the overall root diameter was reduced. It is not clear whether these newly formed roots were capable of taking up water. Carvalho et al. (2014) and White et al. (2015) found that, in spring barley and bread wheat, a RLD of 1 cm cm^{-3} was necessary to take up 90% of the available water. In this study, 90% of the available water had been taken up in every treatment, even though the RLD of the DR and AR treatment was between 0.4 and 0.9 cm cm^{-3}. This indicates that the threshold for sugar beet to take up 90% of the available water is lower than barley, wheat and oilseed rape.

5. Conclusions

Sugar beet can grow roots to depths of 1 m under non-limiting conditions, and are able to extract water from this depth. However, there seems to be root physiological restrictions that limit water uptake when the roots have only just formed (Frensch and Hsiao 1994). The lack of secondary xylem in the first three weeks (from roots appearing until they mature) supports this hypothesis. Over time root constraints in the form of increased bulk density appeared to be overcome by the need to forage for water or by roots maturing. Considering the difference in root diameter, between 78 and 94 DAS, the new roots that formed indicated that the constraints were overcome by root foraging. Either way, at this point in time, there was already a 50% reduction in storage root dry weight. Further work is necessary to explore varieties that are capable of extracting water from depth before severe drought stress occurs, which could potentially increase sugar yield for growers in drought prone areas.

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