Understanding water uptake in sugar beet

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Abstract

Sugar beet is grown worldwide for sugar production. In the UK the main growing area is East Anglia, which has an annual rainfall of c.600 mm and the crop is predominantly growth on sandy loam soils. Previous work has reported that sugar beet shows an average annual yield loss of 10% due to water deficits and during dry years this loss can increase up to 25%. It was also found that sugar beet can grow roots up to 1.5m deep but there is hardly any water uptake from depth which might contribute to yield loss due to drought stress.

Drought stress is common among many crops and studies into rooting patterns and water uptake have shown that within species. Not much work had been done on recent sugar beet varieties in terms of rooting patterns and root responses to variable water availabilities. During this PhD several experiments were designed to investigate possible limitations to water uptake and ways to mitigate potential issues.

The first experiment, conducted in the glasshouse, established what sugar beet roots were capable of in terms of growth and water uptake, in unlimited conditions. It was found that deep roots were formed, especially under drought stress, however there was a delay between roots arriving at a certain depth and water actually being taken up from that depth. A follow up experiment focussing on root anatomy showed that this delay could be explained by a lack of secondary xylem immediately after root formation. Once the xylem has matured and enough secondary xylem was formed, water could be taken up efficiently. Another glasshouse experiment looking at the effect of compaction below 50 cm depth showed that root growth and water uptake are limited when the bulk density is higher than 1.6 g cm^{-3}.

A box experiment, held over two years, allowed for closer monitoring of sugar beet root growth using minirhizotrons. Again, a delay between roots reaching a particular depth and there being water uptake from that depth was seen. It was also found that under prolonged drought, large yield losses would arise while shorter periods of drought followed by periods of rewatering did not result in strong yield penalties. Stomatal conductance measurements did show differences between the treatments, indicating that there were short term drought effects, regardless of the length of the drought period. Stomatal conductance dropped before water was taken up from depth, despite roots having been found in soil layers with sufficient water.

Lastly, two years of field experiments gave an indication of sugar beet growth under ‘normal’ conditions. Multiple varieties were grown alongside each other and during dry periods half of the plots could be irrigated. In both years only very mild drought was recorded, and no strong yield losses were observed. Soil cores were taken to look at the root growth at two moments during each year and enabled us to identify differences in rooting patterns between the varieties, some varieties had a greater root length density at depth. Under mild drought conditions these differences were more pronounced than under optimum conditions.

In summary, when soil conditions were non-limiting, sugar beet did produce deep roots and take up water from depth to mitigate drought. Delays in roots reaching deeper layers and there actually being water uptake from that depth was associated with a lack of mature xylem shortly after roots were formed. The sugar beet varieties grown in the field showed different rooting patterns, which could mean that it may be possible to breed for varieties that are able to extract more water from depth in periods of water shortage.

1. General introduction

Sugar beet and its growing conditions

Worldwide, sugar beet (*Beta vulgaris* L.) provides 20% of the total sugar production, the other 80% is derived from sugar cane. Whereas sugar cane is grown in tropical regions, sugar beet is grown in temperate regions. Since it can be grown in nearly all soil types, it is found on almost all continents (Draycott 2006a). Besides sugar production, sugar beet are used for animal feed and ethanol production, making it a potential biofuel (Demirbas 2008; Gerbens-Leenes et al. 2009; Dohm et al. 2013). Its versatility makes it an important crop worldwide and therefore it is essential to optimize growth and yield.

Sugar beet is a biennial halophyte which originates from the Mediterranean area (Boudry et al. 2002). The phylogenetic origin is described in Figure 1 (Kadereit et al. 2006). Before reproductive growth occurs plants have to be vernalised, seeds are sensitive to vernalisation from the moment they germinate (Boudry et al. 2002). From the middle ages onward sugar beet has been used as a natural sweetener, but it took until the eighteenth century before people tried to refine sugar from the beet. However, the extraction of pure sugar from the beet on a proper industrial scale did not start until the nineteenth century (Francis 2006). Breeding efforts in the last 200 years have resulted in an increase in sugar content from 8% to 18% in our current sugar beet varieties (Dohm et al. 2013). Besides breeding for yield improvements, the breeding programmes also focus on pest and disease tolerance and resistance, improvements to nutrient uptake and improvements to water uptake and drought tolerance.

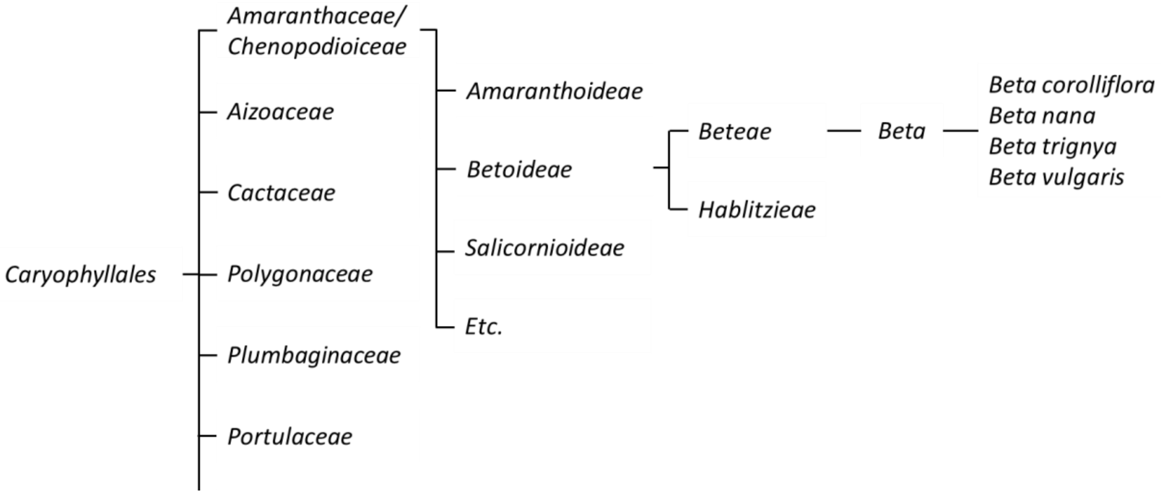

Figure 1 Phylogenetic background of *Beta vulgaris*, Phylogenbetic tree adjusted from Kadereit et al. (2006).

In the UK, sugar beet is generally sown in early spring and after emergence the leaf area expands rapidly. After 10-15 weeks the maximum leaf area is reached and the accumulation of sugar in the storage roots starts around June (Jaggard and Qi 2006). It is important for the leaf area to reach its maximum by the time the sugar accumulation starts since it enhances radiation interception which is directly related to yield (Clover et al. 2001). From August onward, the storage root will continue to grow and accumulate sugar and most beet are harvested between September and March.

After harvesting the beet are transported to one of the four UK factories, which are located in the East of England (Figure 2). Beet transport involves heavy loads and therefore sugar beet are only grown within reasonable proximity of the sugar producing factories. During processing of the beet several products will be produced and almost all products are used (British Sugar 2019). Firstly, sugar is being refined from the beet (British Sugar 2019). Next is the tough skin of the beet, which is reduced to a pulp that can be used as a high energy food source (British Sugar 2019). Then there is the excess heat that the factories produce which has in some cases been redirected to provide heating in glasshouses and electricity produced is sold to electricity supply companies (British Sugar 2019). Lastly there is the soil that came with the beet that has been washed off and is being mixed into different soil types for selling off as topsoil for gardens and sports fields (British Sugar 2019).

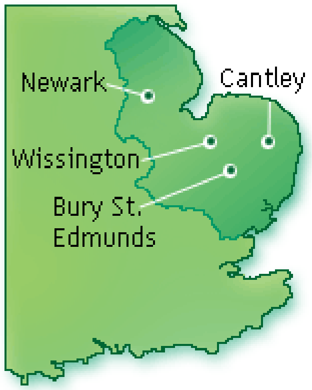

Figure 2 Sugar beet factories. In dark green it shows the sugar beet growing area.

To predict sugar beet yield a model, called the BEETGRO model, was developed at Broom's Barn. The model uses environmental factors to determine the potential yield. These factors include temperature, rainfall, soil available water capacity, evapotranspiration and radiation (Qi et al. 2005). The model can predict canopy growth, and this in combination with information on the radiation interception and evapotranspiration information predicts biomass accumulation (Werker and Jaggard 1997; Werker and Jaggard 1998). The model also considers that throughout the growing season there is leaf decay. The predicted biomass is then used to estimate the sugar content based on an equation by Werker et al. (1999).

Sugar beet growth and development

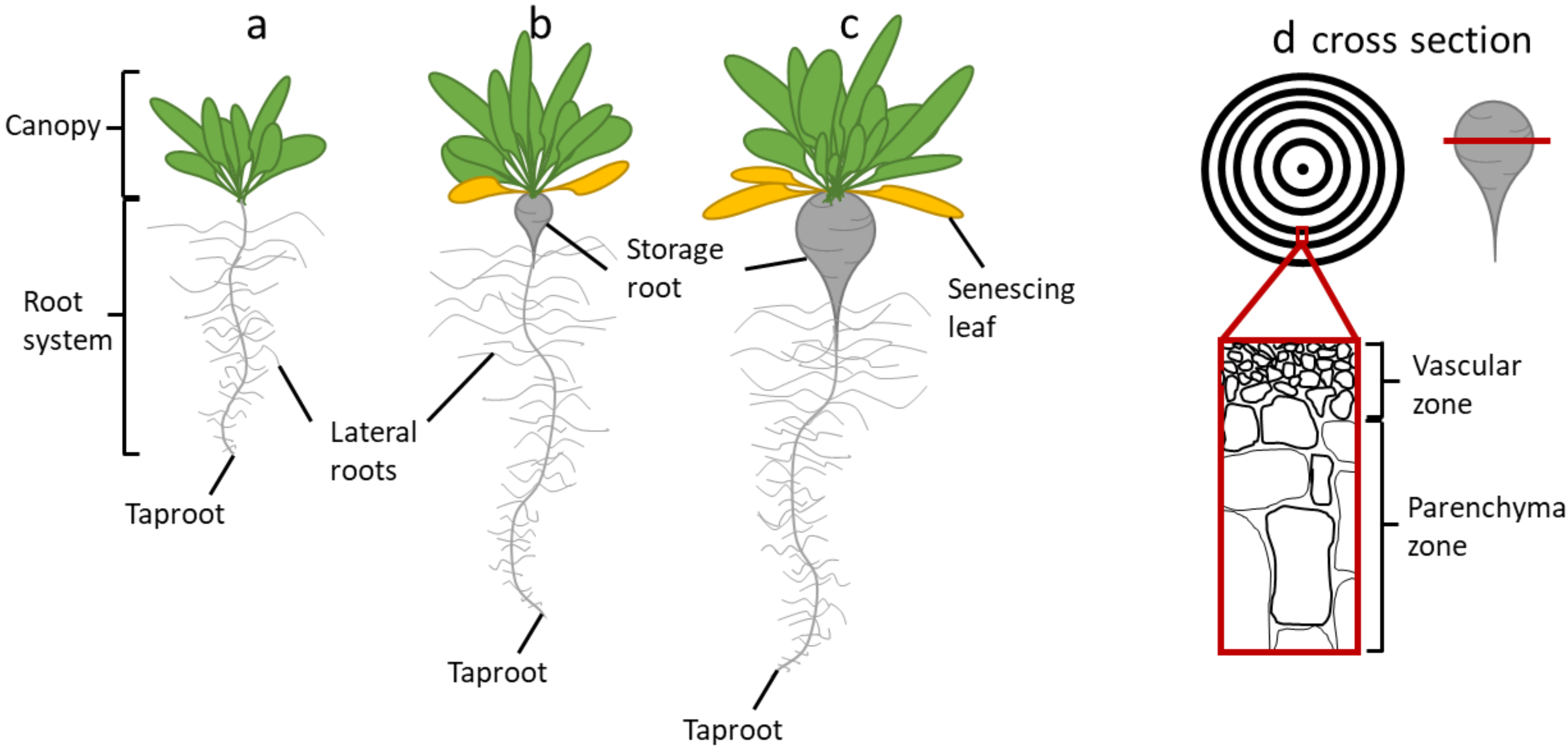

Figure 3 Different stages of sugar beet growth: a) early growth, b) maximum canopy expansion, c) mature storage root. And d) a cross section of the structure of the storage root at the end of the growing season.

Once sowing has commenced and the weather is favourable, the taproot will start growing at a steady pace, see Figure 3a. Once the cotyledons appear photosynthesis can take place and the first leaves will start to develop. Most of the growth in the first few months will be at the leaf level (Milford et al. 1985a; Milford et al. 1985b). At the same time the building blocks for the storage root development are prepared Figure 3b. The storage root consists of rings and each ring consists of parenchyma tissue and vascular elements and cambium (see Figure 3d)(Milford 2006). Once the leaf canopy is established, the storage root will start accumulating sugar which results in expansion of the storage root, Figure 3c. The sugar concentration is high in the vascular zone and reduces in concentration in the parenchyma zone (Milford 2006). Throughout the lifespan of the sugar beet new leaves are produced while the older leaves senescence.

From the moment germination has happened the plant will develop its root system to optimize water uptake. Under non-restricted conditions sugar beet roots have been found to grow up to 2 m in depth. However, most of the water uptake takes place in the top 50 cm of the soil (Brown and Biscoe 1985). Brown and Biscoe (1985) even state that 80% of all water uptake happens in the top 30 cm of the soil. Several studies showed there was hardly any water uptake below 1 m and little water uptake from 30-100 cm (Brereton et al. 1986; Scott and Jaggard 2000). Deeper soil layers are only used for water uptake when water has become unavailable in the top soil. As soon as the top soil is replenished during a precipitation event, water uptake occurs from the top soil section again and not so much from deeper layers (Brown and Biscoe 1985). It is only when drought occurs that the plant will need to take up water from below 30 cm. It has been found in several crops that water uptake mostly occurs in shallow soil layers until this is depleted. Once this happens water uptake from deeper soil layers happens until the shallower layers are replenished (Brown and Biscoe 1985; Dara et al. 2015).

It was found that one hectare of sugar beet consumes 25 mm of water per tonne of sugar produced, during the critical period which is during storage root formation from June-August (Scott and Jaggard 2000). In the UK, the average yield over the past five years has been 12.76 tonnes sugar ha^{-1}. This means that one hectare of sugar beet needed 319 mm of water between June and August. When at field capacity, sandy loam soils, the most common soil type for sugar beet growth in the UK, have a water availability of 130 mm in the top 100 cm of the soil (Scott and Jaggard 2000). In addition, the average amount of rainfall in East Anglia (UK's sugar beet growing area) between June-August is 116 mm, so in total there could be 246 mm water available in the soil during this critical period (Ministry of Defence 2018). However, the soil is rarely at field capacity at the beginning of June, indicating that this is an overestimation of the available water during the storage root formation. This all suggests that, in an average year, sugar beet are limited in their water availability (Jaggard et al. 2007).

Climate change

Climate change is happening worldwide and different regions are affected in different ways. Over the past 100 years the global temperature increased 0.6 °C, however regional changes have far more impact on the environment than global averages. In the mid and high latitudes of the northern hemisphere temperature changes resulted in warmer winters and more frost-free periods (Walther et al. 2002; Luterbacher et al. 2004). At the same time summers have become warmer, and more periods of extreme heat have been registered (Coumou and Rahmstorf 2012). Along with temperature changes, changes to the precipitation patterns have been observed in western Europe, with an increase in precipitation in autumn and winter (Walther et al. 2002). Overall, weather patterns have been changing to include more drought and more extreme weather (Rosenzweig et al. 2001).

Climate change trends have resulted in intensification of drought limitations seen in crops, leading to an increase in yield losses (Ober et al. 2005). At the same time crop yield improves with rising CO_2

levels. However, the positive effects of elevated CO_2 will soon be overshadowed by the negative effects of rising temperatures and increasing weather extremes (Supit et al. 2012). There have already been severe yield losses because of drought. Especially in warm years, years with extreme summer heat and rainfall deficits (Ciais et al. 2005). To get a better understanding of the impact of climate change on crop growth and yield in the future looking at climate change scenarios will be crucial.

Modelling climate change is a great challenge since the models have to be able to accurately predict: site specific changes; a large number of climate variables; changes in mean climate variability; and to predict scenarios over many years (Semenov and Barrow 1997). Despite these difficulties scientists have managed to create models that can give us a reasonable estimate of possible climate change scenarios and most scenarios for Western Europe show increasing temperatures and more extreme weather events (Walther et al. 2002; Ciais et al. 2005; Coumou and Rahmstorf 2012; Supit et al. 2012). These climatic changes have already started happening and effects can be seen in crops worldwide (Moriondo et al. 2011; Lobell et al. 2014).

One major limitation that is occurring more often and in an increasingly wide area is drought. Since the start of this century several severe droughts have occurred worldwide. In 2003, the majority of Europe suffered from drought which resulted in significant losses to almost all crops grown that summer (Ciais et al. 2005). Then another severe drought happened in most parts of Europe with the exception of the UK and Scandinavia, in 2015, resulting in losses in crops such as sugar beet, potatoes and maize (Van Lanen et al. 2016). Europe is not the only region to suffer losses as a result of drought, large parts of Asia have seen multiple years where 20% of the rice growing area suffered yield losses as a result of drought (Pandey et al. 2004). Australia has suffered increasing yield losses as a result of drought with 2014 being an extremely dry year resulting in large losses of sorghum yield (Lobell et al. 2015).

Water stress

Water stress in crops is a worldwide problem where plants experience a state of stress associated with physiological consequences. These physiological consequences usually result in negative effects on plant growth, production and yield (Jaggard et al. 1998; Ober and Luterbacher 2002; Fotovat et al. 2007). Factors contributing to the water status of plants are: evapotranspiration, hydraulic conductivity of the plant and soil and water use efficiency (WUE). Getting a better understanding of these physiological processes in the plant and soil may result in mitigation of water stress in sugar beet.

As seen before, rainfall plays an important role in water provision for the plant. However, part of the rainfall will evaporate from the soil before the plant has a chance to take it up. Taking the sum of the soil evaporation and the plant transpiration is the evapotranspiration, a value that was found to have a strong positive relationship with yield (Werker and Jaggard 1998). Werker and Jaggard (1998) found that, on average, there is a 0.42% yield loss for every 1% loss in potential evapotranspiration. Although others have argued that it is better to measure rainfall and transpiration to determine the effect on yield (Wright et al. 1997; Clover et al. 2001; Kenter et al. 2006). With the current trends in climate change leading to higher temperatures there will also be an increase in evapotranspiration. On the other hand, increases in temperature might lead to shorter growing seasons reducing the negative effects of increased evapotranspiration (Raddatz and Shaykewich 1998).

Hydraulic conductivity (“*describes how readily water can move across a membrane*” (Taiz and Zeiger 2006a)) is important since it needs to be high enough to facilitate water transport via the xylem. When the plant transpires, the hydraulic conductivity will increase and as a result water is being ‘pulled’ into the plant’s xylem vessels (Steudle and Peterson 1998). During periods of drought the soil hydraulic conductivity will decrease and water uptake will become more difficult, resulting in reduced water uptake from the soil (Miyamoto et al. 2001). The hydraulic conductivity varies over the length of the root; there is an increase in hydraulic conductivity from the apex to the base (de Kroon and Visser 2003), and it can be different between different root types, such as tap root, lateral roots or fibrous roots (Mu et al. 2006).

Hydraulic conductivity is not only regulated by plant morphological factors, but also by several physiological factors. Abscisic acid (ABA) is a plant hormone that is involved in the regulation of stomatal closure and opening. When prolonged drought occurs ABA will accumulate in the plant, which will mediate stomatal closure (Ryan et al. 2016; Conesa et al. 2018; De Ollas et al. 2018). Once the stomata are closed water loss will be greatly reduced. ABA has been found to systemically reduce leaf hydraulic conductance of the stomata and xylem supplied ABA seemed to reduce overall plant hydraulic conductance (Dodd 2013; De Ollas et al. 2018). Where ABA regulated responses to hydraulic conductivity are physiological the actual closure of stomata is a morphological response facilitated by the shrinking and expansion of the guard cells. This process is in part regulated via osmosis, during dry periods there is less turgor, and the guard cells will shrink, resulting in stomatal closure and vice versa (Taiz and Zeiger 2006b).

When talking about efficient uptake of water, water use efficiency (WUE) is often used as a measure. WUE is the total dry matter produced per unit of water used, and in the case of sugar beet it can be calculated as a function of the whole plant or of the storage root (Rytter 2005; Bloch et al. 2006). Under water limiting conditions plants increase their WUE. However, if drought stress becomes severe the WUE will drop (Ober et al. 2005; Rytter 2005). Since there seems to be an optimum WUE it is possible to determine the water status of the plant based on the WUE. In case of a low WUE as a result of drought replenishment of the soil can improve the WUE (Hatfield et al. 2001).

Drought avoidance mechanisms

To prevent plants from suffering because of drought stress several defence mechanisms have evolved. These mechanisms involve root morphological adjustments and plant physiological adaptations. Some examples are; adjusting the rooting pattern, increase cortical aerenchyma, speed up maturity of the xylem vessels, or stomatal closure (McCully 1995; Hetherington 2001; Zegada-Lizarazu and Iijima 2004; Zhu et al. 2010). Since protection of crops from drought is in the interest of growers, irrigation methods are often used to prevent or limit drought stress in plants (Scott and Jaggard 2000). Since irrigation is not always available or cost-effective it is important to use breeding to improve drought tolerance.

When the evapotranspiration rate is high and the amount of rainfall is not sufficient to fully replenish the soil, the top soil is at risk of drying. This is a common occurrence in semi-arid regions. By growing deep roots, plants can gain access to water from soil layers below 30 cm (Zegada-Lizarazu and Iijima 2004). However, roots at depth are often subject to anaerobic conditions, as a result of reduced circulation of the airspaces in deeper soil layers (Unger and Kaspar 1994; Lipiec and Hatano 2003). And when conditions are suitable there might be other limitations, as seen in a study in sugar beet where water uptake from depth ceased before the potential gradient became too small (K F Brown et al. 1987).

Root morphology can be variable between different species and even within species. For example, some species and some genotypes of species have an increase in root cortical aerenchyma. This can lead to an increase in drought tolerance. Low amounts of cortical aerenchyma come with high metabolic cost, and with that limitations to root proliferation at depth (Zhu et al. 2010). High aerenchyma, on the other hand, has a low metabolic cost and therefore root proliferation at depth can be more widespread (Zhu et al. 2010). Besides the relatively lower metabolic costs, the aerenchyma can also provide a low resistance pathway for oxygen to reach depth (Vartapetian and Jackson 1997; de Kroon and Visser 2003).

Another root morphological trait that influences water uptake is the maturity of the xylem. It has been found that the conducting tubes of immature xylem are much smaller than those of mature xylem. Consequently, there is suboptimal water uptake in immature xylem compared to mature xylem. Most root tips and newly formed roots have a higher proportion of immature xylem and these root sections have reduced water uptake ability which can extend up to 20 cm from the root tip (McCully 1995). This could be an explanation for the effective rooting depth being different from the actual rooting depth (Ehlers and Goss 2003).

Photosynthetic assimilation reduces when water availability is limited. When the soil dries out the stomata will close and this results in a drop of the photosynthetic rates (Ober et al. 2005; Rivero et al. 2009). Stomatal closure can have negative impacts on plant growth such as wilting, leaf death and, eventually, death of the whole plant. Closure of the stomata is strongly influenced by ABA, which builds up in the leaves as the soil dries out. Therefore, this may be an adaptive system evolved to preserve water under drought conditions (Hetherington 2001).

Water limitations in crops are often resolved with additional irrigation. In sugar beet it is seen that irrigation is mainly applied from June-August (Scott and Jaggard 2000), however, drought early in the growing season (June-July) seemed to result in most yield reductions (Brown et al. 1987) suggesting that the June-July period would be most cost-effective for applying irrigation. Since irrigation is expensive and not always cost effective there is a new trend in irrigation which is deficit irrigation (Fereres and Soriano 2007). Deficit irrigation allows irrigation to replenish the soil until the evapotranspirational demands are almost met. This means the plants will implement drought prevention mechanisms such as reduced stomatal opening. Subsequently, water will still be extracted from the soil reservoir but at a lower rate than with full irrigation (Fereres and Soriano 2007). Sugar beet seems to respond positively to deficit irrigation when applied in the middle of the growing season e.g. June-August. By irrigating to supply for the evapotranspirational demand during this period yield losses were significantly reduced (Kirda 2002).

Root measurement techniques

Assessing root growth has always been a challenge and it still is today. Different methods have been developed over the years and they can be divided into two-dimensional root measurements and three-dimensional root measurements. Some 2D measurement techniques are: root washing, core break method, rhizotrons or minirhizotrons. 3D methods include X-ray CT and MRI scanning (Mancuso 2012). Each method has advantages and disadvantages, depending on the situation some methods might be better suited than others. However, most root measurements and subsequent analysis are time consuming, which is the reason more is known about the aboveground parts of the plant.

Out of the 2D techniques, the core break method is the least time consuming (Bennie et al. 1987). Soil cores are taken and by breaking them and counting how many roots are present the researcher can get an estimate of the root number at a certain depth (Kirkegaard and Lilley 2007). Root washing, on the other hand, takes the most time since the root samples need to be ‘washed’ out of the soil carefully and then usually a scanner is used to create an image of the spread-out roots which needs to be analysed afterwards. The advantage of this method over the core break method is that it provides the researcher with more information such as root length, diameter and density as opposed to just root number (Mancuso 2012).

Rhizotrons allow for roots to be visible/imaged through a see-through wall (made of plastic or glass). Roots that touch the see-through wall will be visible and therefore measurements can be taken (Mancuso 2012). Minirhizotrons work in a similar way, except for the fact that instead of a see-through wall, see-through tubes are places in the soil medium, usually at an angle, and a special camera can be used to image the roots touching the tubes’ surface (Mancuso 2012). Since this method is non-destructive, the same location in the soil can be visited multiple times to look at root proliferation and root decay (Cheng et al. 1991).

Like the (mini)rhizotrons method, X-ray CT and MRI scanning are both non-destructive and can be used to measure the same root system multiple times without disturbing the root system architecture (Tracy et al. 2012; Metzner et al. 2014). The main limiting factor in these studies is the sample size, the scanning resolution and the time it takes for image reconstruction (Mooney et al. 2011; Schulz et al. 2013). Both these 3D methods are also relatively expensive compared to the 2D methods. It can also be difficult to distinguish finer roots (Metzner et al. 2015).

Questions and aims

The aim of this project was to get a better understanding of water uptake in sugar beet. Previous studies on rooting in sugar beet have been limited, with very few reports since the 1980s (Brown and Biscoe 1985; Brown and Dunham 1986). Since this was the case our first question was to see what current varieties of sugar beet were capable of in terms of growing deep roots and taking up water from depth. A glasshouse experiment allowed us to regulate administration of water and closely monitor plant growth and health. This experiment allowed us to learn more about root growth in sugar beet under non-restricting soil conditions and under differing watering regimes.

The next experiment was set up similarly but this time soil constraints were added in the form of sub-soil compaction. In this setup root growth and water uptake were assessed. However, larger scale experiments were needed to look at root growth when beet are grown together. Therefore, large box experiments were designed, where sugar beet were grown in 2.6 m^3 boxes containing minirhizotrons at five different depths to monitor the root growth. These experiments allowed us to look at differences in rooting response under fully irrigated, droughted, early drought and late drought scenarios.

Ultimately field experiments were needed to look at sugar beet growth under ‘normal’ conditions, and thus two years of field experiments were held. These experiments allowed the study of root growth under irrigated and rainfed conditions as well as studying differences between varieties. All experiments helped us get a better understanding of root growth and water uptake in sugar beet with the help of various measuring techniques from root washing to X-ray CT and throughout all experiments overall plant health was monitored closely.

The experiments helped us to answer several questions on sugar beet rooting patterns and water uptake. By using a step by step approach, the following hypothesis were tested, often in a wider range of settings.

1. Sugar beet can produce deep roots and take up water from depth under unrestricted conditions (Chapter 2)

2. Water uptake by sugar beet from deep soil layers is limited by plant physiological restraints such as slow stomatal response and absence of secondary xylem (Chapter 2 and 3)
3. Roots proliferate in deeper soil layers in response to limited water availability in upper layers, when drought occurs later in the season the yield penalty will be higher (Chapter 3 and 4)
4. Different varieties have different rooting patterns, e.g. more root proliferation in deeper soil layers (Chapter 4)

Chapter 2 focusses on what sugar beet are capable of in terms of root growth and water uptake from depth. This glasshouse experiment left us with more questions about the timing of deep root formation and how the timing of drought stress affects yield. These questions are addressed in Chapter 3. Finally, Chapter 4 presents the data from two consecutive years of field experiments, where we again looked at root growth, but this time root growth in different varieties and we studied the effect of additional irrigation compared to rainfed on rooting traits and final yield. After explanations about the experiments and results there will be a general discussion and conclusion.

2. Assessing water uptake in sugar beet (*Beta vulgaris*) under different watering regimes

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Tamara F. J. Fitters – Main author and main researcher

Jennifer S. Bussell – Assisted with the LICOR measurements and setting up of the experiments. Additionally, she has helped with the writing of the manuscript by giving tips on the order of results and ways to display certain results

Sacha J. Mooney – Assisted with the English grammar and the readability of the manuscript

Debbie. L. Sparkes – Assisted with the experimental design and the English grammar and readability of the manuscript.

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

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 2006a). 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.

Material and methods

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.

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.

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}).

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.

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.

Results

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.

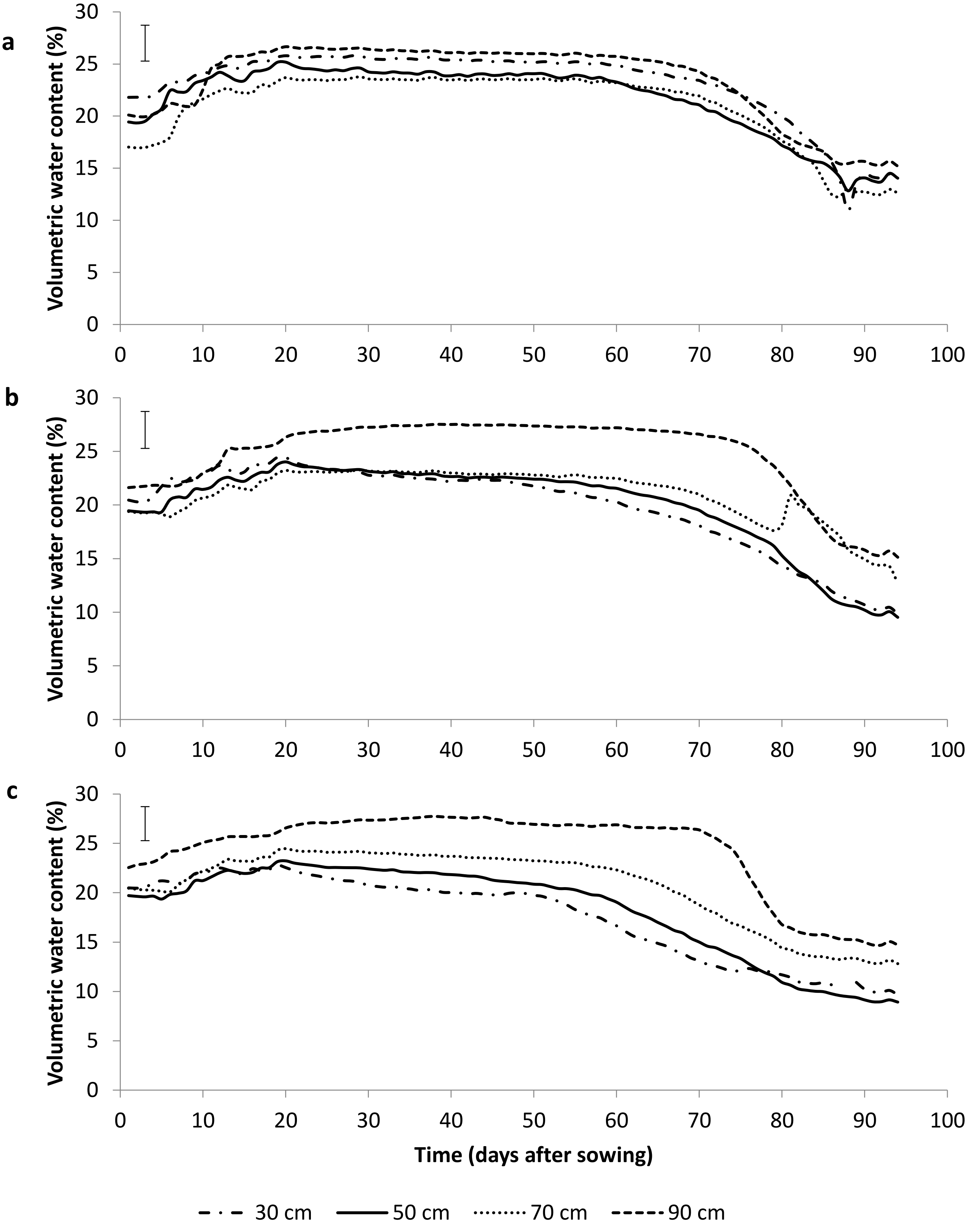

Figure 4 Volumetric water content (%) of a) control treated plants, b) average rainfall (AR) treated plants, and c) drought (DR) treated plants at different depths; 30cm, 50 cm, 70 cm and 90 cm. The bar shows the least significant difference (time*treatment*depth)

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.

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

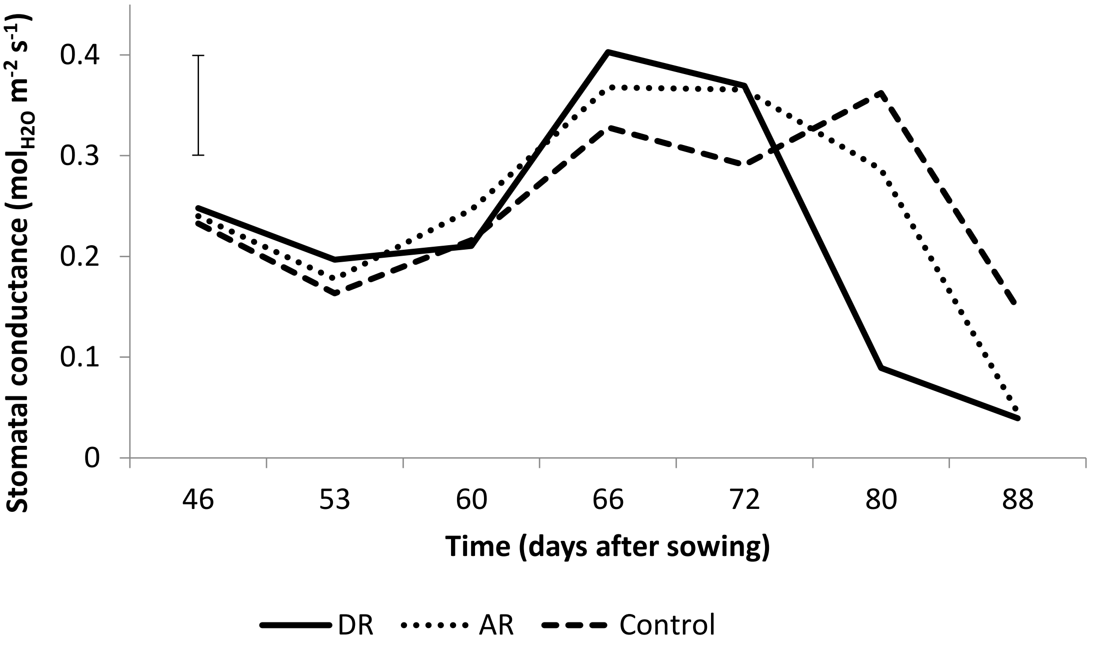

Figure 5 Mean stomatal conductance (mol_{H2O} m^{-2} s^{-1}) over time for each treatment; drought (DR), average rainfall patterns (AR), and control. The bar shows the least significant difference (time*treatment).

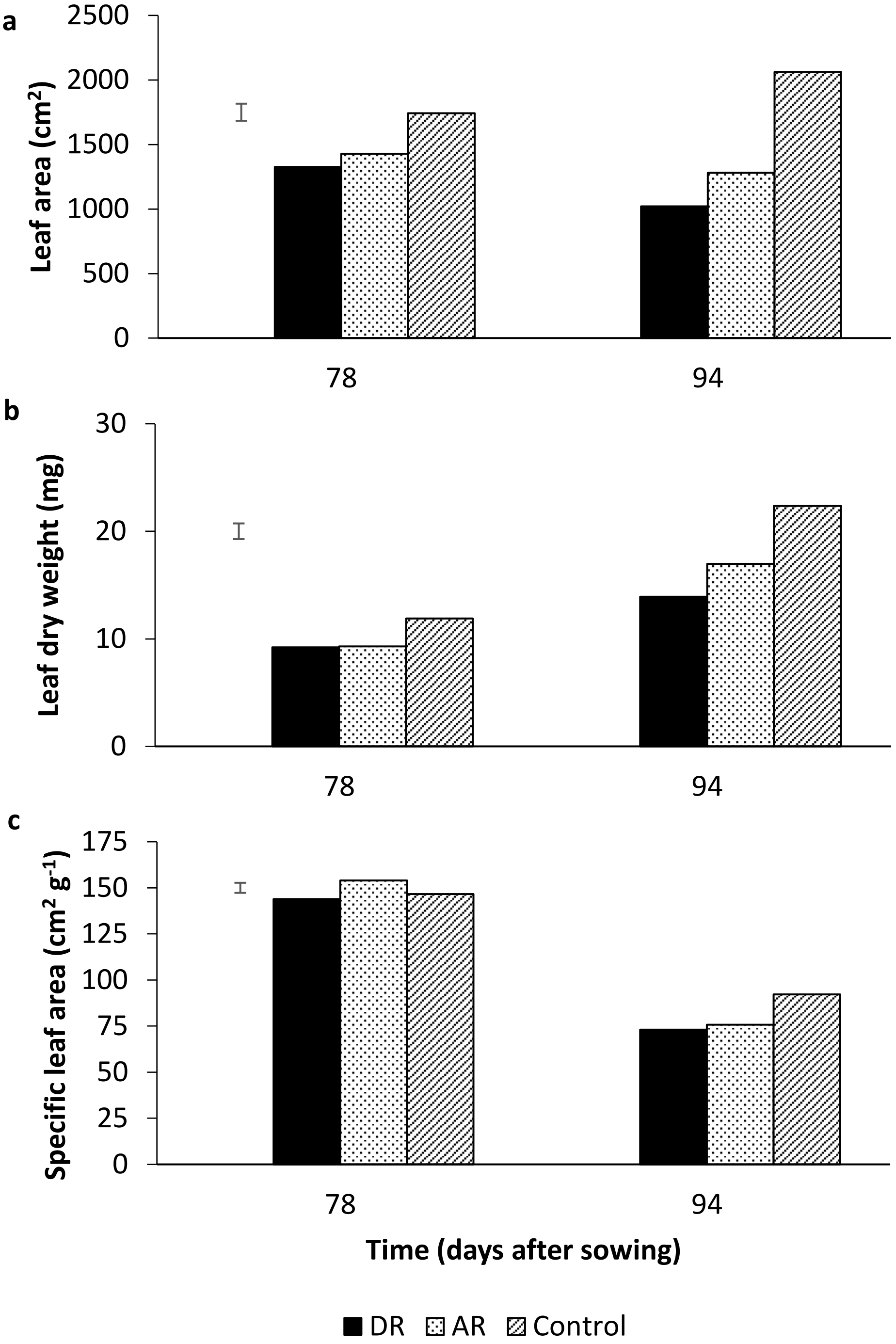

Figure 6 a) Leaf area (cm^2), b) Leaf dry weight (mg), and c) specific leaf area (cm^2 g^{-1}) at 78 DAS and 94 DAS. The bar shows the least significant difference (treatment).

and showed a strong decline in stomatal conductance when the water uptake was reduced from 0.29 to 0.04 mol_{H_2O} m^{-2} s^{-1}. The DR treated plants ended with the lowest stomatal conductance; at 72 DAS it was 0.37 mol_{H_2O} m^{-2} s^{-1} and at 80 DAS it had dropped to 0.09 mol_{H_2O} 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.

Canopy measurements

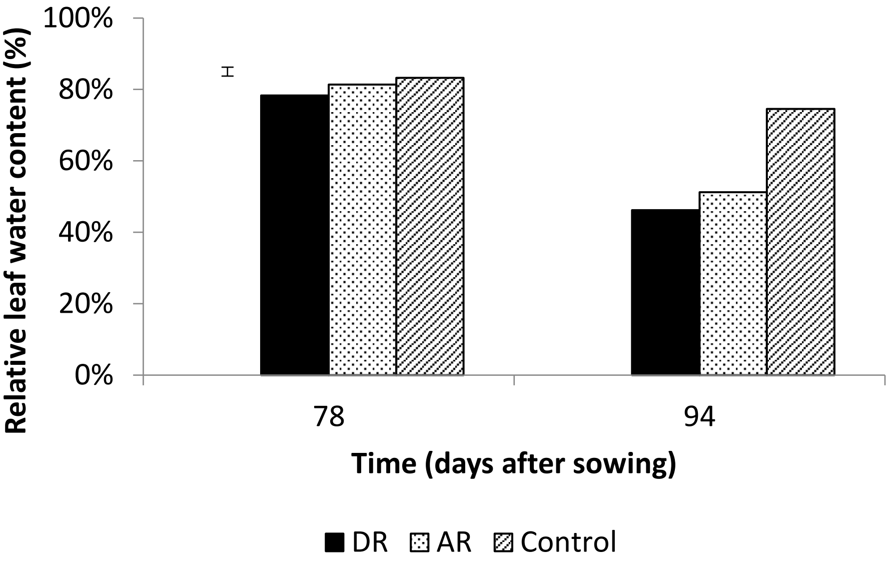

Figure 7 Relative leaf water content (%) at 78 DAS and 94 DAS. The bar shows the least significant difference (0.025) (treatment).

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 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.

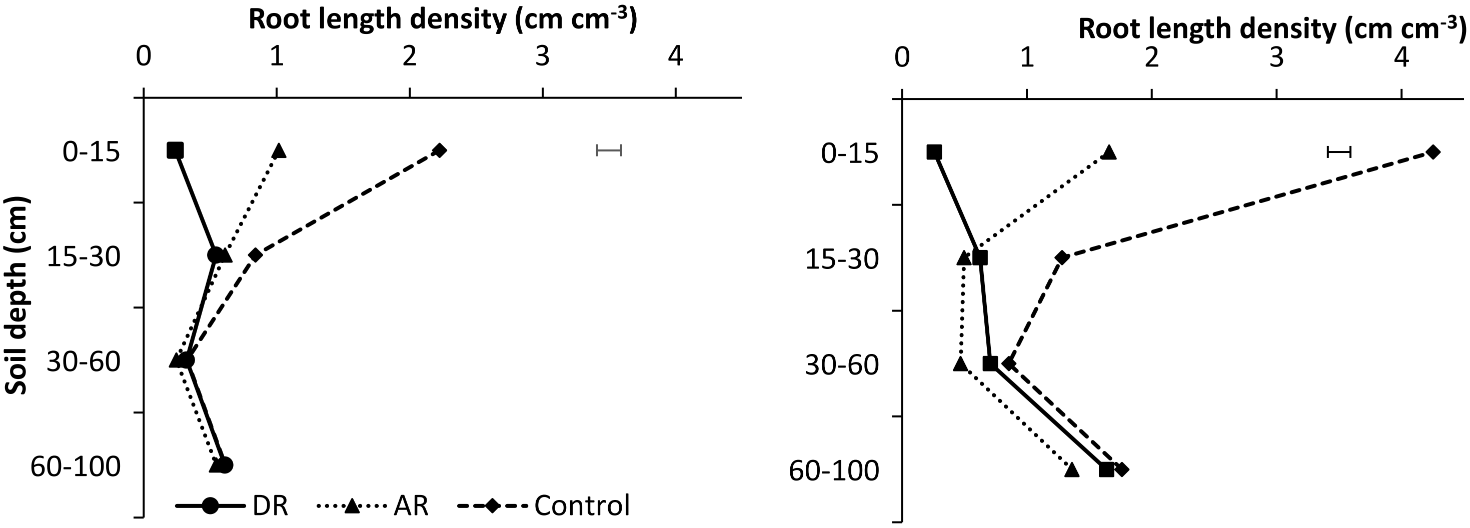

Figure 8 a) Root length density (cm cm^{-3}) at 78 DAS, and b) root length density (cm cm^{-3}) at 94 DAS. The three treatments have been divided into layers: 0-15 cm depth, 15-30 cm depth, 30-60 cm depth, and 60-100 cm depth. The bar shows the least significant difference (time*treatment*depth).

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 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 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.

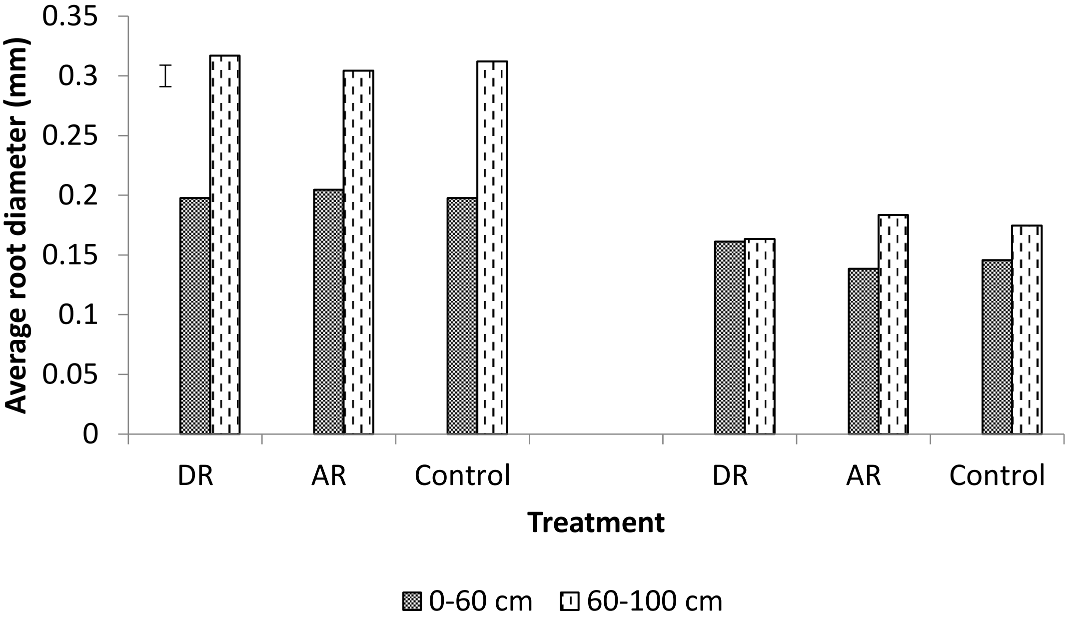

Figure 9 Average root diameter (mm) at 78 DAS (left) and 94 DAS (right). The three treatments have been divided into layers: 0-60 cm and 60-100 cm. The bar shows the least significant difference (time*treatment*depth).

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

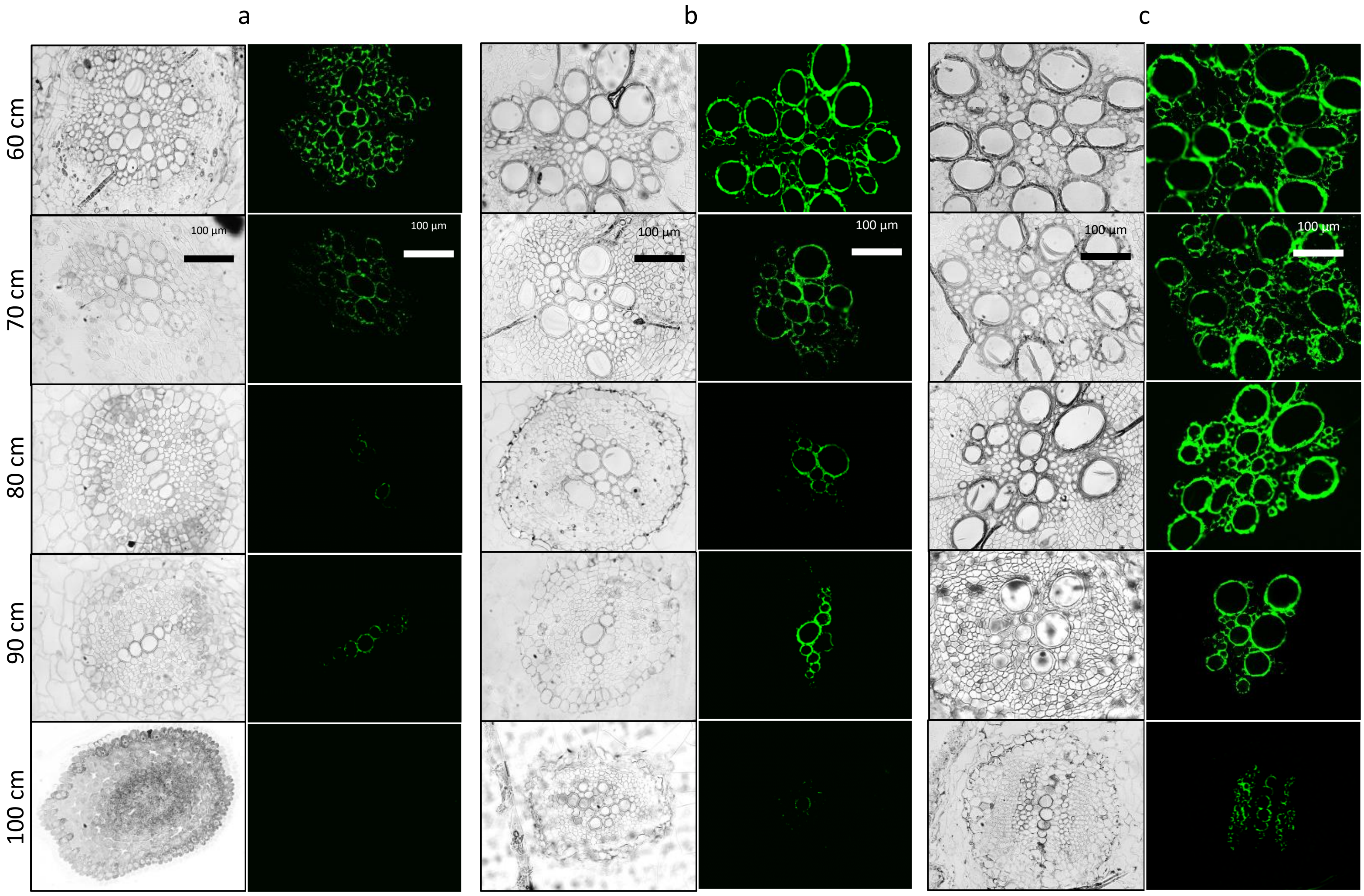

Figure 7 Cross section of the taproot in bright field and under the fluorescent microscope after staining with anti-rat FITC marker LM11 at 60, 70, 80, 90, and 100 cm depth. a) 48 DAS; b) 59 DAS; c) 69 DAS. The bar indicates 100 µm.

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.

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 (E S 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 reductions do not always seem to interfere with the photosynthetic assimilation, but they are thought to limit plant growth (Anderson and McNaughton 1973). This corresponds with our findings of a lower leaf area and dry weight in DR and AR treated plants compared to the control plants and also translates into a lower specific leaf area for the DR treated plants. Brereton et al. (1986) found that plants can adapt to negative soil conditions by reducing the leaf area expansion instead of reducing the stomatal conductance by reduced leaf water potential. The reduction in SLA, over time, was most likely a result of older, larger leaves dying and new, smaller leaves forming (Milford, Pocock, Riley, et al. 1985).

Considering the total amount of water taken up (on average 5600 ml for all treatments), there was limited water uptake from 90 cm depth (Figure 1) before the shallow soil layers were depleted, as previously observed in the field (K. F. Brown et al. 1987). Our findings suggest this is, at least in part, due to a lack of secondary xylem, as previously been seen by Mapfumo et al. (1993). In the control plants there were no significant changes in soil moisture content, since the extracted water was replenished almost immediately. Towards the end of the experiment, temperatures in the glasshouse had risen above a daily average temperature of 18°C (min 7°C, max 35°C), compared to an overall daily average of 14 °C, resulting in an increase in water uptake. During this period, the DR and AR treated plants took up more water from depth, where water was still available, while the control plants appeared to extract water throughout the whole column and not just from the shallow layer where the water was replenished immediately. There may be several reasons why water was not taken up from 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 1.3 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.

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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3. Sugar beet root growth under different watering regimes: A minirhizotrons study

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Sacha J. Mooney – Assisted with the English grammar and the readability of the manuscript

Debbie. L. Sparkes – Assisted with the experimental design and the English grammar and readability of the manuscript.

Sugar beet root growth under different watering regimes: a minirhizotron study

Tamara F J Fitters, Sacha J Mooney and Debbie L Sparkes

Abstract

The yield of sugar beet is often reduced by drought stress and it has previously been shown that water uptake, especially from deeper layers of the soil profile, may be limited by inadequate root density. Experiments were conducted to assess root growth at different depths in response to specific watering regimes. Sugar beet plants were grown in wooden boxes (2.16 m^2 x 1.2 m) in a polytunnel in two consecutive years. Minirhizotrons allowed regular monitoring of root growth at five different depths. Only when water in the upper soil layers had been depleted, did roots start proliferating in deeper soil layers. This development of the root system architecture, together with a lag between roots arriving at depth and actively taking up water, led to a delay in water being extracted from those deeper layers. During the period when roots were proliferating at depth, stomatal conductance reduced, indicating that the plants were suffering from water stress despite there still being water available. Even though new soil layers with high water availability were explored the stomatal conductance did not recover.

Keywords: minirhizotrons, root growth, sugar beet, water uptake, drought.

Introduction

Worldwide, water availability is becoming more and more of a problem for crops due to climate change. In addition to increasing average temperatures which will lead to higher water demand, there are likely to be more weather extremes resulting in periods with high water influx alternated with periods of drought (Rosenzweig et al. 2001; Kumar 2016; Kurnik and Hildén 2017). These dry periods can cause severe problems during critical stages of crop growth with a lower yield as a result (Araus et al. 2002; Ober and Luterbacher 2002; Pathan et al. 2014).

Sugar beet (*Beta vulgaris*) is grown in temperate regions all over the world and makes up 20% of the sugar production in the world, sugar cane providing the other 80% (FAO Investment Centre Division 2009). In the UK, sugar beet are mostly grown in East Anglia, where the soil type is predominantly sandy loam with an available water capacity of around 14% (Qi et al. 2005). Additionally, East Anglia is one of the drier regions in the UK with average annual rainfall being <600 mm in the past 10 years

(MetOffice 2018a). As a result, there is an average 10% yield loss due to low water availability which can exceed 25% in dry years (Jaggard et al. 1998).

Low water availability is not the only limitation to water uptake. Other factors that play a role are compaction and root tissue development. Compaction results in poor root growth, often at depth, and this, in turn, results in reduced water uptake from compacted soil layers (Kirkegaard and Lilley 2007). Root tissue development can be limiting when new roots are initially formed and the xylem tissue has not matured for optimum water uptake, as reported in grapevine and sugar beet (Mapfumo et al. 1993; Fitters et al. 2017).

Roots are known to have high plasticity and this allows them to adjust to environmental changes (York et al. 2016). During periods of drought, roots proliferate in soil layers with higher water availability (Li et al. 2002; Padilla et al. 2013). In sugar beet, roots can grow to over one metre deep and take up water from that depth if there are no soil constraints (Fitters et al. 2017). However, when there is compaction, sugar beet hardly show any root proliferation in deeper layers before drought occurs (Romano et al. 2012). Once drought occurs root proliferation at depth starts (Koevoets et al. 2016), but delays in root tissue development at that time can prevent immediate water uptake (Fitters et al. 2017).

Minirhizotrons have often been employed to look at root development over time in controlled conditions (Johnson et al. 2001). See through tubes are placed in the soil and a special camera is inserted into the tube to take images of the roots growing against the tube. The advantages of this method are that it is non-destructive and allows multiple measurements over time (Jose et al. 2001). Some disadvantages of measuring root length with minirhizotrons are an underestimation of root lengths depending on the measurement depth, and preferential root growth along the tube (Parker et al. 1991).

This study aims to answer the following questions: a) How do sugar beet roots proliferate over time at different depths under differing water regimes?; b) How does the timing of drought affect root growth and plant health? To answer these questions two experiments were conducted over two years. In the first year question a) was addressed by assessing well-watered vs drought conditions. In year two, early drought vs late drought were compared, addressing question b).

Material and methods

Experimental design

Sugar beet were grown in six wooden boxes of 1.8 m x 1.2 m x 1.2 m (l x w x h) in 2016 and 2017. The soil medium was a sandy loam texture and the boxes were emptied and filled with new soil between the two years. The boxes were arranged in a randomized block design with three blocks and were located in a polytunnel to exclude rainfall. The boxes were filled in stages to encourage consolidation by watering at each stage before adding more soil. This was done several times until the boxes were filled to the top. Each box had four volumetric soil moisture sensors, EC-5 (Decagon Devices, Labcell Ltd., Alton Hants, United Kingdom) fitted at four depths: 20, 50, 80 and 110 cm. Five Em5b data loggers (Decagon Devices, Labcell Ltd., Alton, Hants, United Kingdom) were used to log the half hourly readings from the soil moisture sensors. Solid fertilizer (Nitram; CF® fertilisers, Billingham, Cleveland, USA) equivalent to 120 kg ha^{-1} was applied on top of the soil. Each box contained ten minirhizotrons, two at each of the following depths: 30 cm, 50 cm, 70 cm, 90 cm, and 110 cm. Prior to the start of the experiment, field capacity (25% volumetric soil moisture content) was determined by watering the boxes to saturation and then letting them drain for two weeks. The boxes were watered daily to maintain field capacity until the different watering regimes were imposed.

Drought response experiment (2016)

Three sugar beet seeds (cv. Haydn) were planted at 3 cm depth at each plant location, three rows of eleven plants. At c.25 DAS the boxes were thinned to one seedling per position. The watering regimes were: continuous irrigation (control), drought from 57 DAS onward (DR).

The youngest fully expanded leaf was used for weekly stomatal conductance measurements (mol m^{-2} s^{-1}) using an AP4 Porometer (Delta-T Devices, Burwell, Cambridge, United Kingdom)(Parkinson 1985). All measurements were taken between 9.00 h and 13.30 h. Roots were imaged fortnightly through the minirhizotrons. The images were taken at 1 cm intervals and then stitched together. The roots were traced manually after which the image was converted into a black and white format in ImageJ (Schindelin et al. 2012). WinRHIZO (Regent instruments Inc., Québec, Canada) was used to determine the root length. Relative leaf water content (RWC) was measured at 83 DAS and 126 DAS (Turner 1981). At 131 DAS the plants were harvested and leaf and root fresh weight and dry weight were determined, after drying at 75 °C for at least seven days. Total plant water use efficiency (WUE) was calculated from the total plant dry weight and the total water uptake during the whole experiment.

Drought timing experiment (2017)

Three sugar beet seeds (cv. Haydn) were planted at 3 cm depth at each plant location, three rows of seven plants. At c.25 DAS the boxes were thinned to one seedling per position. The watering regimes were: no irrigation between 60 – 145 DAS, early drought (EDR), no irrigation between 128 – 178 DAS, late drought (LDR). When rewatering, small amounts of water (15 mm) were given at first to avoid surface run-off.

Stomatal conductance and root images were taken as described for 2016. Additional measurements were weekly SPAD measurements taken using a SPAD 502 plus meter (Konica Minolta, Tokyo, Japan). Between 132 DAS and 159 DAS the canopy temperature was recorded five times. A FLIR thermal camera (FLIR® Systems Inc., Wilsonville, Oregon, USA), was used alongside the software provided with the camera to assess the canopy temperature. Relative leaf water content (RWC) was calculated at 76, 102 and 124 DAS.

At 215 DAS the plants were harvested and leaf and root fresh weight and dry weight of five beet in the middle of the front row were determined, after drying at 75 °C for at least seven days. Total plant water use efficiency (WUE) was calculated from the total plant dry weight and the total water uptake during the whole experiment. Six storage roots, taken from the middle plants from each box were sent to the sugar factory to determine sugar yield.

Statistical analysis

A general ANOVA for a randomized block design was performed on plant biomass data, sugar yield data, and RWC measurements. For stomatal conductance, root length, soil moisture, canopy temperature, and SPAD data, a repeated measures ANOVA was performed. GenStat 15th edition (VSN International Ltd., Hemel Hempstead, United Kingdom) was used for the statistical analyses.

Results

2016 – Drought experiment

Under well-watered conditions there was considerable fluctuation in soil volumetric moisture content (Fig 1a). These fluctuations were caused by watering events and plants taking up water. However, the soil volumetric moisture content was kept above 0.25 m^3 m^{-3} for the majority of the experiment. When irrigation was halted at 57 DAS, there was a slow decline in soil moisture content at each depth (Fig 1b). Water was taken up at 20 cm immediately and, 8 days after drought started, the soil moisture content started to reduce at 50 cm as well. From 80 DAS substantial moisture reduction was also observed at 80 and 110 cm. Drought had strong impacts on water use efficiency (WUE); the WUE of the DR plants was 8.4 g l^{-1} compared to 6.7 g l^{-1} in the control plants.

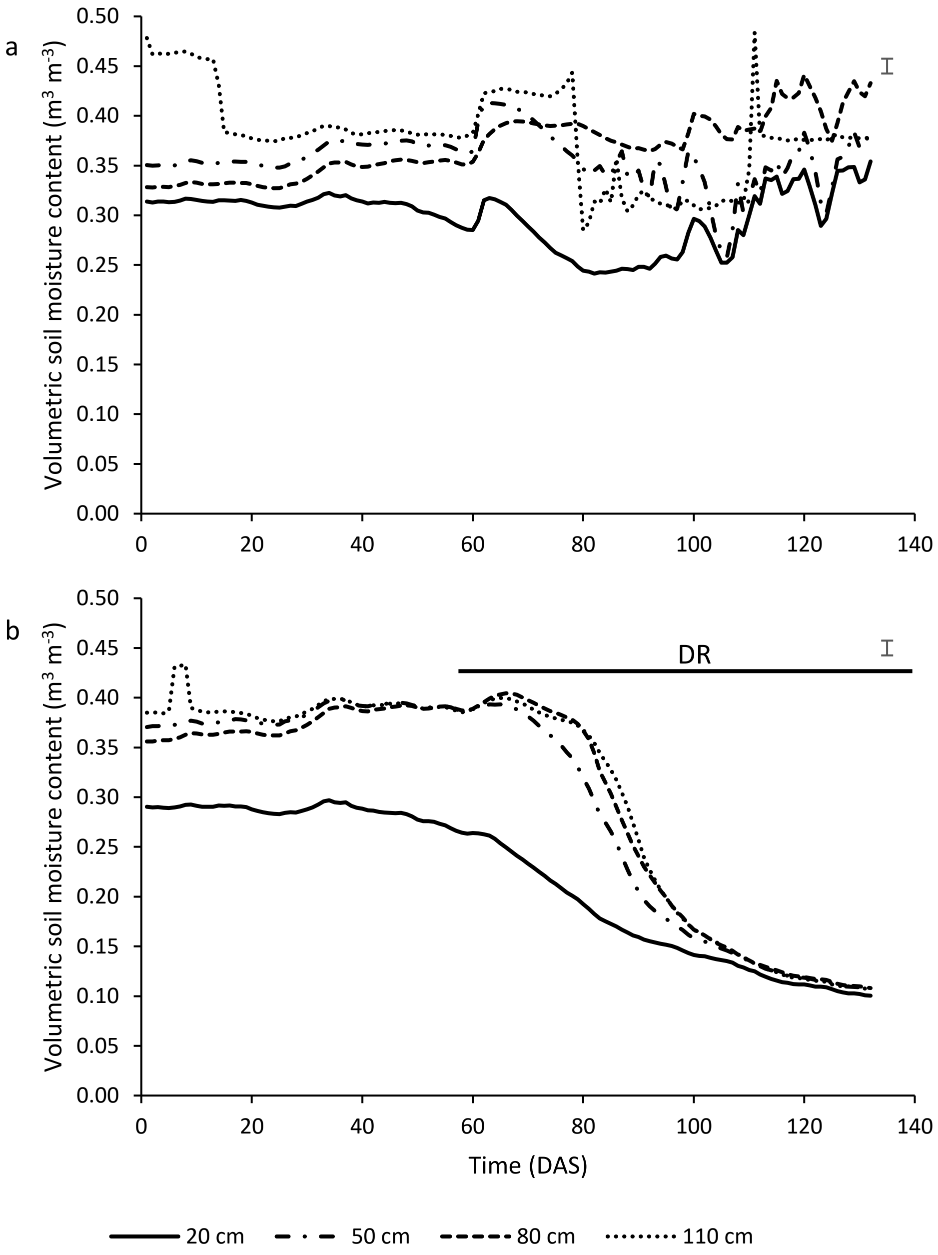

Figure 10 Volumetric soil moisture content (m^3 m^{-3}) over time at four different depths; 20, 50, 80 and 110 cm. Where a) Control and b) DR. The solid horizontal bar shows the duration of the drought treatment. The error bar shows the least significant difference (time*treatment).

Over time, there were clear changes in root length and distinct differences between the treatments (Fig 2). From 92 DAS there were significant differences in root length as a result of root proliferation at 110 cm in the DR treated plants ($p < 0.001$, DF = 48, l.s.d. = 161.8) (Fig 2). These differences persisted until the end of the experiment. The increase in roots at 110 cm coincided with reductions in soil moisture at the corresponding time.

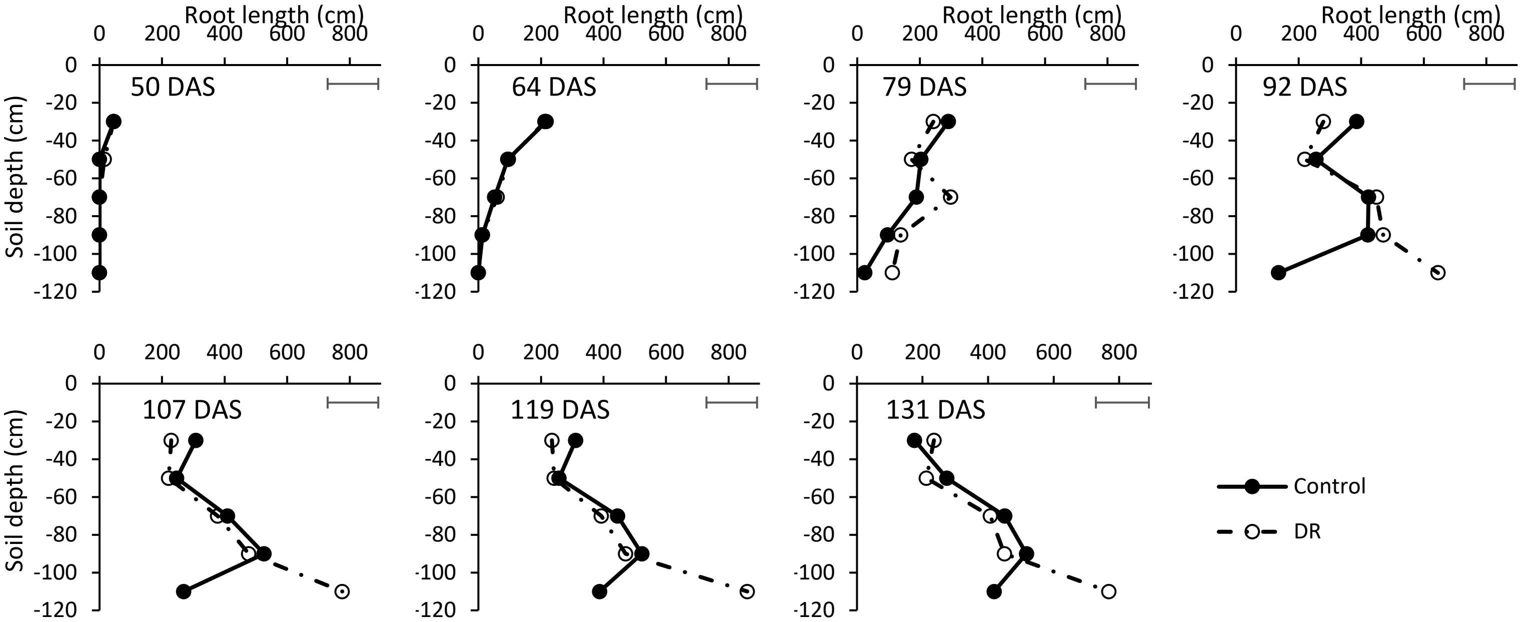

Figure 11 Root length (cm) at five different depths at consecutive time points. The error bar shows the least significant difference (time*treatment*depth).

From 97 DAS, 40 days after irrigation stopped for the DR treated plants, the control plants had a higher stomatal conductance that the DR plants (p = 0.005, DF = 10, l.s.d. = 0.272) (Fig 3). At

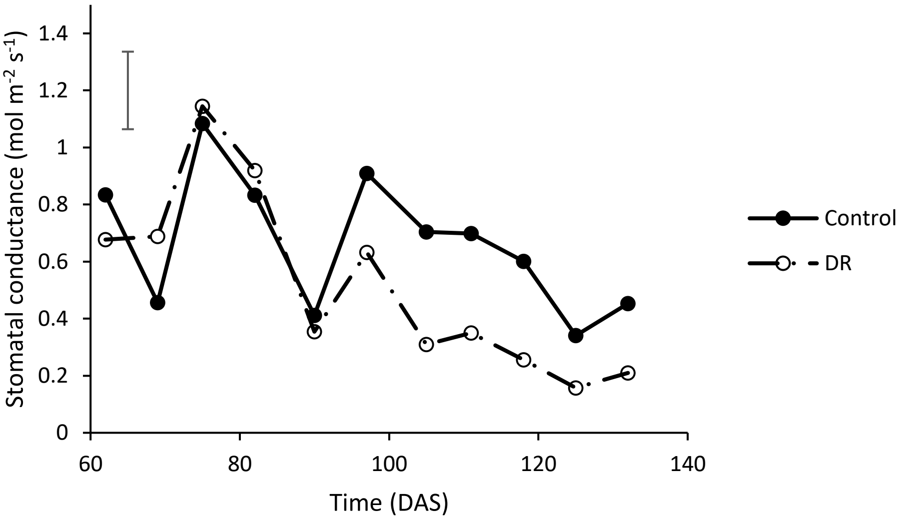

*Figure 3 Stomatal conductance (mol m^{-2} s^{-1}) drought had started at the time of the first measurement. The error bar shows the least significant difference (time*treatment)*

approximately the same time (92 DAS), roots started to proliferate at 110cm depth (Fig 2).

At the end of the experiment there were large differences in dry weights. The control plants had a significantly higher overall dry weight of 217 g against 124 g in the DR plants (p<0.001; DF=1;

lsd=36.57). A leaf dry weight of 107 g ($p<0.001$; DF=1; lsd=14.03) and root dry weight of 110 g ($p=0.04$; DF=1; lsd=49.5) compared to 67 g and 57 g in the DR plants respectively. The final root water content was three times as much in the roots of the control plants as in the DR treated plants and the leaf water content was 3.8 times as high in the control plants.

Relative leaf water content (RWC) measured at 83 DAS did not show any significant differences despite the DR plants not receiving any water for 26 days. At 127 DAS the DR treated plants had a significantly lower RWC ($p=0.026$; DF=1; lsd=9.83), 76% compared to 89% in the control plants. At this time the actual water content of the control plants was 1.3 ml and that of the DR treated plants was 0.7 ml ($p=0.016$; DF=1; lsd=0.364).

2017 – Drought timing

When watering stopped, in both the EDR and LDR treatments, there was a slow decline in moisture content at each depth (Fig 4a-b), with water being taken up from the top layers before being depleted from the bottom layers. When watering restarted there was no immediate increase in volumetric soil moisture content: it took approximately 30 days before replenishment was seen. After rewatering for 70 days the EDR treated plants had only managed to replenish to 91% of the starting volumetric water content (Fig 4a). The LDR plants were rewatered for 37 days, after which the soil moisture content was replenished to 78% of the starting value (Fig 4b). No differences in WUE were found between the different treatments.

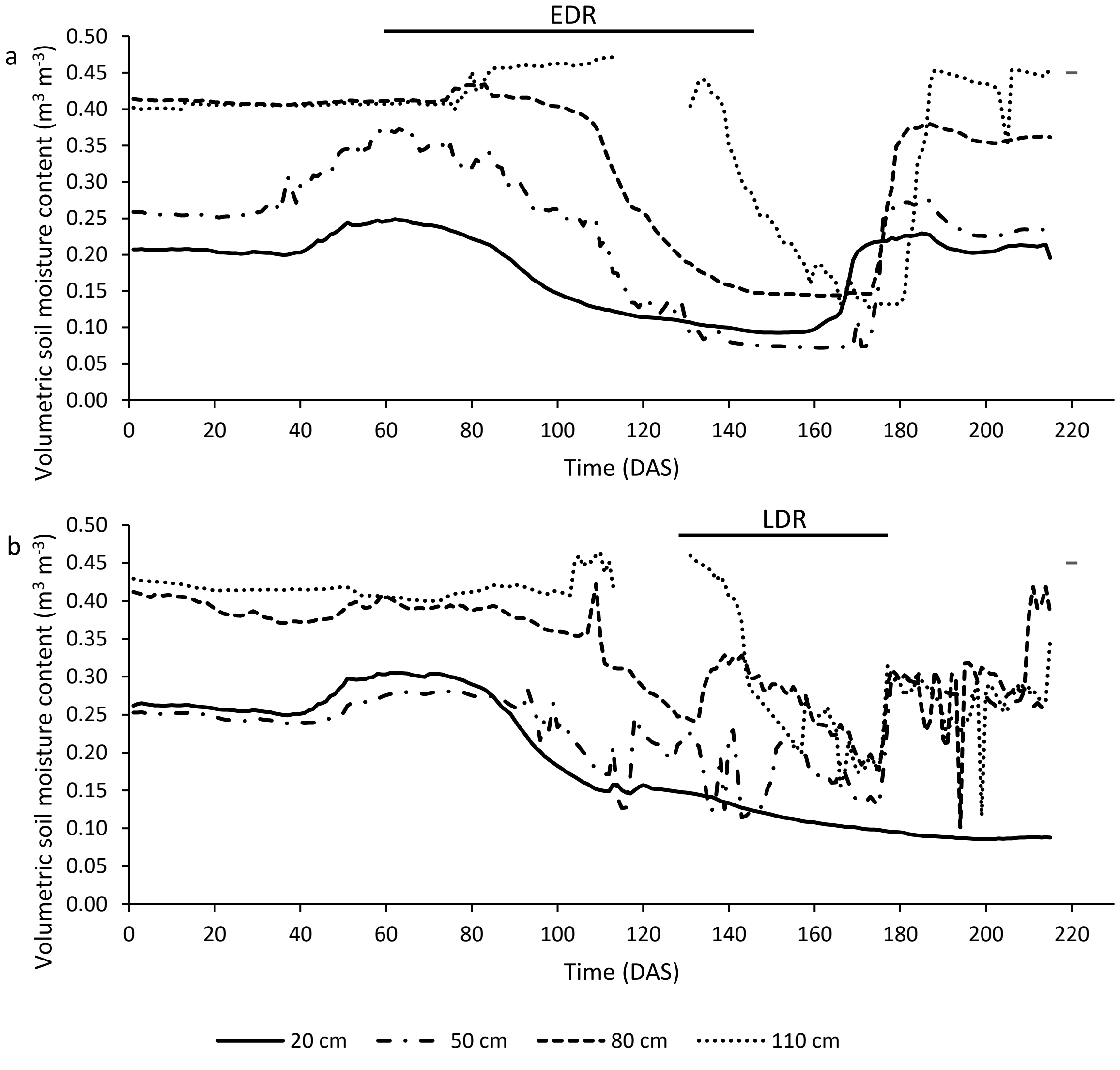

Figure 4 Volumetric soil moisture content ($m^3\ m^{-3}$) over time at four different depths: 20, 50, 80 and 110 cm. a) the EDR treated boxes, b) the LDR treated boxes. The solid horizontal bars show the timing of early (EDR) and late (LDR) drought. The error bar shows the least significant difference. Due to sensor failure there was a loss of data at 110 cm depth in both treatments between 115 and 130 DAS.

The first differences in root length appeared at 101 DAS (Fig 5), when the EDR treated plants had a significantly higher root length at 70 cm, 41 days after drought started. Eleven days later the LDR plants had caught up and the differences disappeared (Fig 5). At 90 cm a similar trend was seen between 127 and 133 DAS, where the EDR plants had started proliferating at this depth first and after 20 days the LDR plants had caught up (Fig 5). At 140 DAS most differences were found at 110 cm depth (Fig 5). However, when irrigation of the LDR plants stopped at 128 DAS, root proliferation at 110 cm depth was seen, resulting in the difference between treatments disappearing (Fig 5). At 203 DAS the LDR treated plants had a higher root length overall (Fig 5).

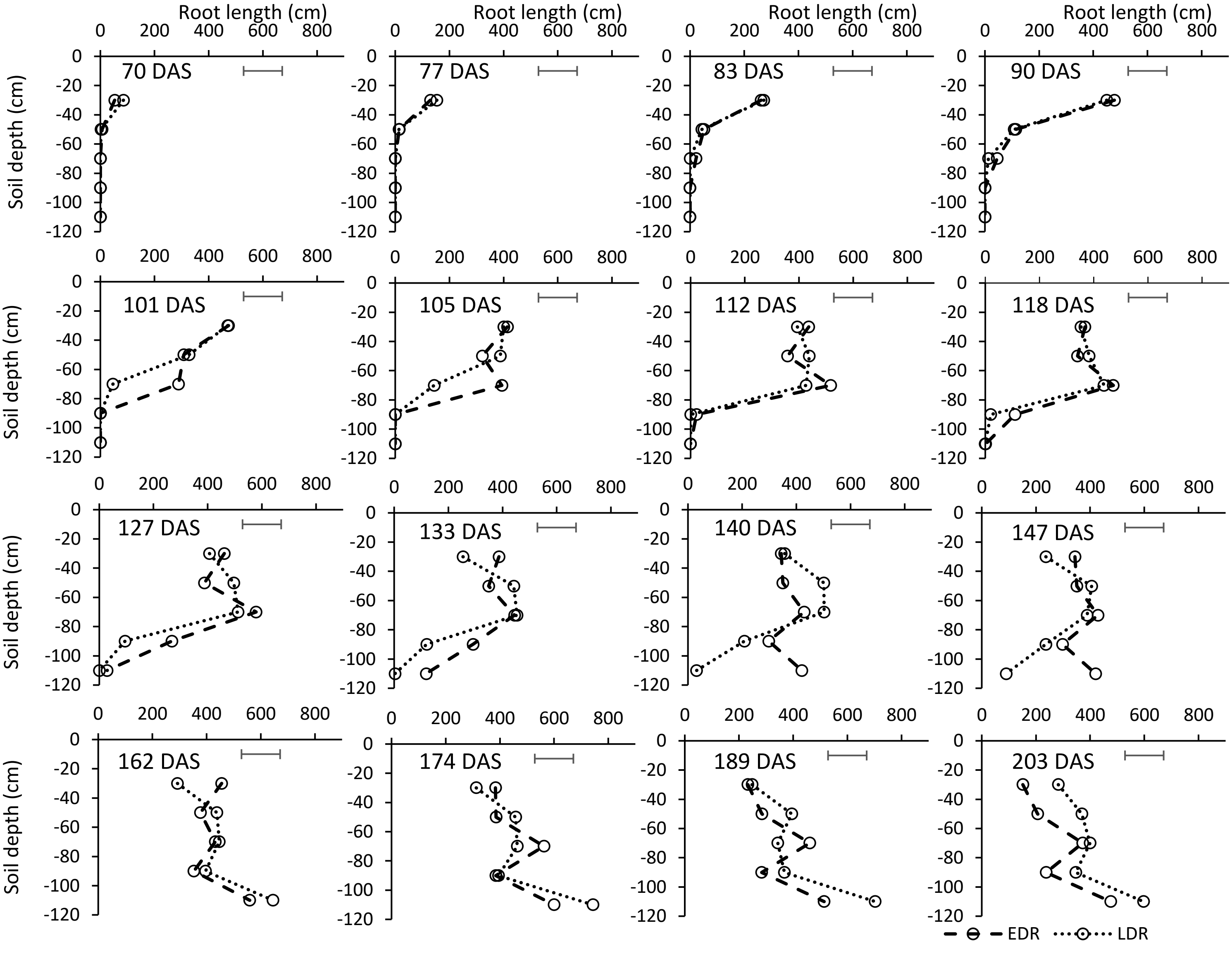

Figure 5 Root length (cm) at five different depths at successive time points. The error bar shows the least significant difference (time*treatment*depth)

The first significant differences in stomatal conductance were observed at 110 DAS which coincided with a decrease in soil moisture content at 80 cm in the EDR treated plants (Fig 6). At 145 DAS the EDR plants were rewatered and from then there was an increase in stomatal conductance. From the moment the irrigation of the LDR treated plants stopped at 128 DAS the stomatal conductance became more like the EDR treated plants. After rewatering of the LDR there was again an increase in stomatal conductance and at 186 DAS there were no more significant differences between the two treatments.

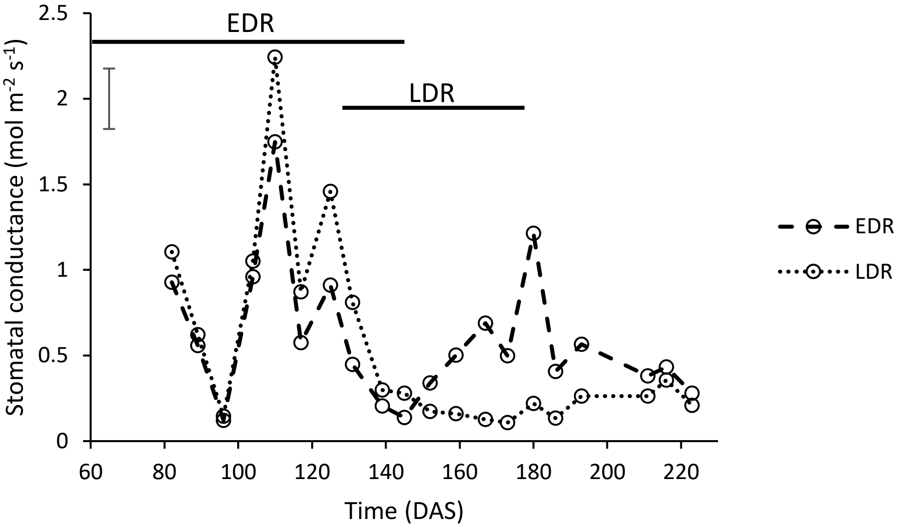

Figure 6 Stomatal conductance (mol m^{-2} s^{-1}) over time for each treatment: EDR and LDR. The solid horizontal bars show the timing of early (EDR) and late (LDR) drought. The error bar shows the least significant difference.

Weekly SPAD measurements showed no differences in SPAD until 110 DAS when the EDR treated plants had a significantly higher SPAD than the LDR treated plants (Fig 7), likely caused by a concentration effect as a result of reduced water content. At 159 DAS the LDR treated plants' SPAD values went up as a result of drought stress while the EDR treated plants' SPAD values stabilised and even declined. After rewatering the SPAD values of the LDR plants declined again.

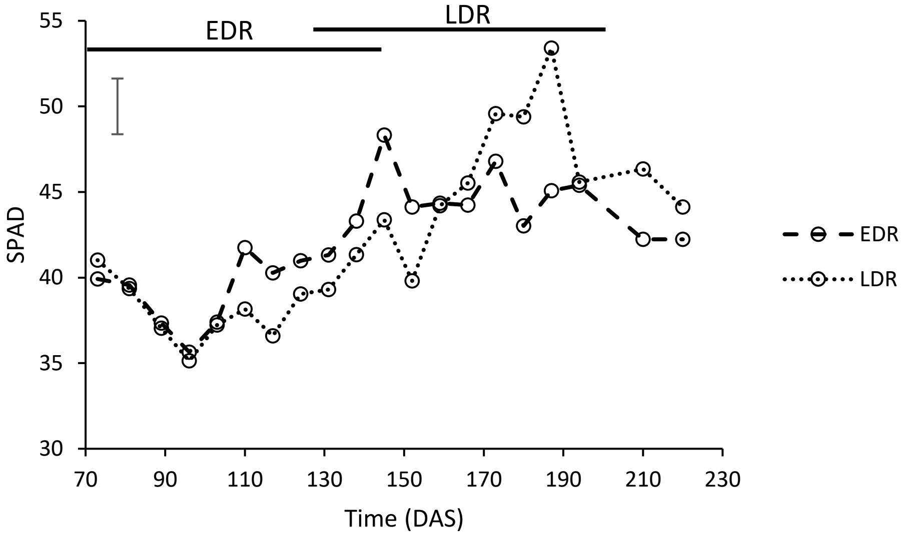

Figure 7 SPAD over time for each treatment: EDR and LDR. The solid horizontal bars show the timing of early (EDR) and late (LDR) drought. The error bar shows the least significant difference.

At the end of the experiment there were no significant differences in total dry weight or root dry weight. However, the EDR plants had a significantly higher leaf dry weight (p=0.005; DF=1; lsd=43.26), with the EDR plants having 278 g and the LDR plants only 139 g of leaf DW. When looking at the RWC there were no significant differences until 124 DAS, when the EDR treated plants had a significantly higher RWC of 80% (p<0.001; DF=1; lsd=0.323), compared to 77% in the LDR plants. When the sugar content was determined at the end of the experiment it showed no significant differences.

Around the time the EDR plants were rewatered, regular canopy temperature measurements were taken (Fig 8). When rewatering occurred, the canopy temperature dropped significantly below that of the LDR treated plants which were at that point subjected to drought.

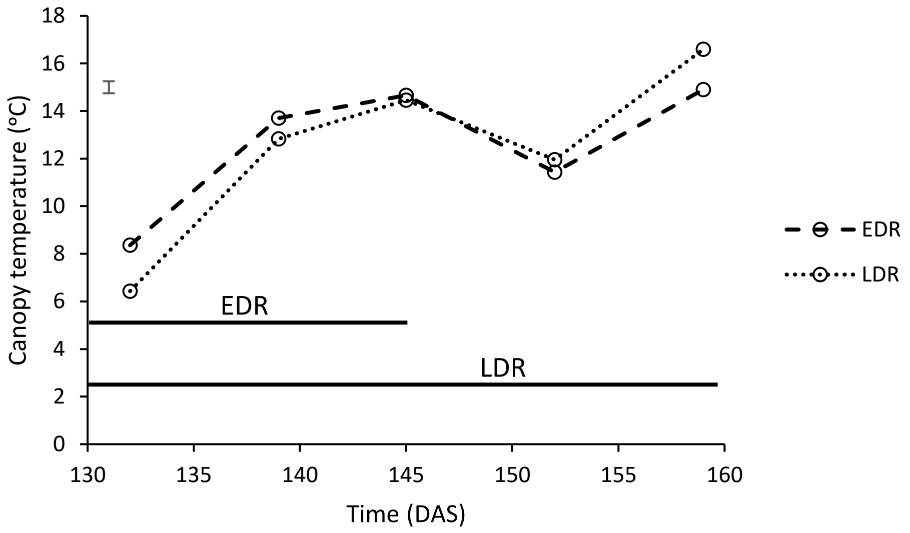

Figure 8 Canopy temperature (°C) from 132 DAS to 159 DAS. The moment that the EDR treated plant have replenished and the LDR treated plants are starting to suffer from drought. The solid horizontal bars show the timing of early (EDR) and late (LDR) drought. The error bar shows the least significant difference.

Discussion

To benefit water uptake under drying soil conditions, root proliferation at depth has often been observed in crops (Asch et al. 2005; Comas et al. 2013; Lobet et al. 2014). Our findings show that in sugar beet grown under both prolonged and short periods of drought, deep roots were developed. In 2016, the soil moisture decreased rapidly from the top of the profile which resulted in roots mostly being formed at depth. In some layers a delay was observed between roots reaching a certain depth and water uptake from that depth. A possible explanation for this could be that the xylem had yet to mature before optimum water uptake could take place (Mapfumo et al. 1993; Fitters et al. 2017). In 2017, this delay between roots reaching a depth and there being water uptake was more pronounced. It is likely that the higher root imaging frequency in 2017 resulted in there being earlier detection of roots at each depth and therefore a more precise estimation of when roots reached a given depth could be made. Water availability had a strong effect on the rooting patterns. In both years most differences were found at 110 cm, with drought stressed plants showing strong root proliferation at this depth after approximately 20 days of drought. Similar rooting patterns under drought conditions were seen in other crops such as maize, wheat and sunflower (Wasson et al. 2012; Lynch 2013; Lilley and Kirkegaard 2016). The use of minirhizotrons in this study allowed close monitoring of root growth at the different depths (Johnson et al. 2001). As a result, differences caused by water limitations were seen in root growth before above ground measurements gave an indication of water being limited.

A decrease in water availability does not always result in an immediately quantifiable plant response (Pang et al. 2011). When drought occurred, stomatal conductance remained unchanged for at least 39 days in the case of the 2016 plants and the LDR plants in 2017. The EDR plants took longer with 50 days, possibly because no action during a short drought period was more beneficial for the plant in comparison with immediate stomatal closure (Crute et al. 2009). Another possible reason could be that the water demand was not as high since these plants were smaller when drought started compared to the EDR plants and the plants in 2016. However, when the drought persisted, stomata were closed which resulted in lower stomatal conductance at the cost of reduced photosynthesis. This is often seen as a plant protection mechanism to preserve water (E S Ober et al. 2005; Rivero et al. 2009). However a previous study in soybean observed that the reduction in photosynthesis was not immediate (Daryanto et al. 2016). Daryanto et al. (2016) found that at first, photosynthesis decreased less rapidly compared to stomatal conductance. This might be since several photosynthetic processes are influenced by stomatal conductance and not the other way around (Medrano et al. 2002). Stomatal conductance was regulated in short term by the plant evaporative demand. The long term regulation was led by conditions of water extraction from the soil (Tardieu and Davies 1993).

Stomatal conductance is often used as an indication of drought stress. Flexas and Medrano (2002) stated that a stomatal conductance below 0.1 mol m^{-2} s^{-1} is a sign of severe drought. In our experiments, the stomatal conductance never fell below 0.1 mol m^{-2} s^{-1}, suggesting there was no severe drought stress but rather mild to moderate drought stress. This theory is supported by the relative leaf water content (RWC) values. In neither year were there significant differences in RWC except at 127 DAS in 2016, where a reduction was seen after plants had not been watered for 70 days. Another canopy indicator for drought stress is canopy temperature, with drought stressed plants having a higher canopy temperature compared to well-watered plants (Jackson et al. 1981; González-Dugo et al. 2006; Grant et al. 2006; Panigada et al. 2014). In 2017, the canopy temperature was measured for a short period around the time of rewatering of the EDR plants. As soon as rewatering occurred there was an immediate response in canopy temperature, even before changes in stomatal conductance or RWC were seen. Therefore, the canopy temperature method could prove to be a quick and simple measurement for early detection of water stress.

Despite the drought in these experiments being mild to moderate, there were strong impacts on water use efficiency (WUE) under prolonged drought in 2016. The DR treated plants had a WUE of 8.4 g ml^{-1}, compared to 6.7 g ml^{-1} in the control plants. Previous studies have shown that drought results in lower WUE in C3 cereal crops and sugar beet (Araus et al. 2002; Bloch et al. 2006; Rinaldi and Vonella 2006). However, in 2016 the WUE was higher in the DR treated plants indicating there was no drought. Blum (2005) pointed out that a higher WUE does not necessarily mean a higher yield potential. High WUE as a result of lowered water uptake can be misleading and the drought might have actually had a negative effect on yield. In 2016, water uptake was reduced severely in the DR treated plants and this resulted in a decrease in root dry weight but the ratio between the two turned out higher than that of the control plants. In 2017 there were hardly any differences between the treatments but, overall, WUE was much lower than in 2016. A possible explanation for this could be a difference in plant density. Previous studies in sorghum and sugar beet showed that a higher plant density resulted in higher yield notwithstanding all plants had similar amounts of available water (Blum 1970; Sögüt and Arioglu 2004). Since the plant density was higher in 2016, this could explain why WUE was lower in 2017.

Root dry weight was used as an indicator for yield and continuous drought had a stronger negative impact compared to short periods of drought. The DR treated plants had a significantly lower root dry weight at the end of the experiment compared to the control plants. In 2017, no significant differences were found between the root dry weight of the EDR and LDR treated plants. Similar results were found in wheat when different germplasms were grown under full irrigation, early drought, late drought and continuous drought; there were hardly any differences between grain yield of early and late drought but continuous drought had a significantly lower yield than the fully irrigated plants (Ginkel et al. 1998). Even though EDR and LDR did not result in significant differences in root weight there were strong differences in leaf dry weight. Continuous drought resulted in 48% less leaf dry weight compared to the control plants. Possibly because of rapid leaf senescence of older leaves under drought.

Wetting and drying cycles can have a substantial impact on the particle mobilization and the rate of organic matter decomposition (Majdalani et al. 2008; Zhu and Cheng 2013). It was found that certain amount of water can mobilize more particles when the irrigation is interrupted (drying) (Zhuang et al. 2007). This could have happened to some degree during the irrigated period in all treatments, where water was given several times a week for a set period. The process of slow drying between two irrigation events can strongly affect the soil aggregation and microbial activity (Zhu and Cheng

2013). This leads to changes in rhizosphere respiration, which in turn, affects the decomposition speed of the soil organic matter. This suggests that the treatments that were irrigated could have had an increased decomposition rate and therefore root death might have been more pronounced in irrigated treatments. The root images taken showed more root browning in the irrigated boxes which could have been an indication of increased decomposition rates. At the same time there might have been differences of this effect throughout the box as a result of fixed partial root zone irrigation. Since trickle tape is used to irrigate the boxes there is an area close to the soil surface between the trickle tapes that never got replenished. Previous studies showed that the amount of micro-organisms was substantially lower in the dry soil sections of partially irrigated systems (Wang et al. 2006; Hu et al. 2011). This could be an indication for reduced root growth as well. However, it was thought that the depletion zone did not stretch to below 20 cm. There has even been studies to show that partial root zone drying can lead to improved water use efficiency (Kang and Zhang 2004).

Conclusion

When water availability was reduced sugar beet responded by proliferating their roots at depth. There was little root proliferation in the top 30 cm as a result of drought stress. When new roots were formed there was often a delay before water was actually taken up. It would therefore have been beneficial if roots had already been in place before drought stress occurred. Continuous drought had the most negative effects, resulting in a drastic reduction in stomatal conductance and leaf and root dry weight. Shorter periods of drought followed by rewatering showed only temporary decreases in stomatal conductance and there were no strong impacts on root dry weight or sugar yield. The differences observed in root dry weight between the years could be attributed to the difference in plant density in the experiments, with there being a higher plant density in 2016 than 2017. Future studies might look at the effects of drought stress with different intensities, timings and durations and possibly even the effects of low water availability under different plant densities.

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4. Sugar beet root response in the field under different water availabilities

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Tamara F. J. Fitters – Main author and main researcher

Sacha J. Mooney – Assisted in understanding the X-ray CT data. Additionally, assisted with the English grammar and the readability of the manuscript

Debbie. L. Sparkes – Assisted with the experimental design and the English grammar and readability of the manuscript.

Sugar beet root response in the field under different water availabilities

Abstract

In the UK, there are reports of yield losses due to drought in almost all years. The root system could play a role in mitigating this drought stress by improving water uptake. There might be varietal differences when it comes to rooting patterns but so far none have been reported in sugar beet yet.

This study shows the results of two years of field experiments, looking at rooting patterns and overall plant health under both rainfed and irrigated conditions. In the first year three different varieties were used, and the second year five different varieties were used.

No significant yield differences were found between the rainfed and irrigated treatments, probably due to drought stress only being mild. There were however differences in overall plant health and rooting patterns when plants were only rainfed.

Varietal differences were observed, they were most strong when plants were subjected to mild drought stress. During the first year the dry period happened late in the growing season while the second year the dry period happened early in the season. However, the timing of drought had no impact on the final yield, possibly because the drought stress had only been mild.

Varietal differences observed might indicate the possibility of breeding for certain root traits to mitigate drought stress in sugar beet in the future.

Keywords: Root growth, water uptake, drought, irrigation, stomatal conductance, soil constraints

Introduction

Sugar beet (*Beta vulgaris*) yields across the UK, are still rising despite stagnating yields in other crops (Supit et al. 2010). Previously Jaggard et al. (2007) showed that there is an average 10% yield loss to drought in UK sugar beet which can increase up to 25% loss in dry years. The main sugar beet growth area in the UK is in East Anglia, which is also an area where rainfall is relatively low compared to the rest of the UK.

UK sugar beet is generally sown in March and harvested anytime between September and the following March (Draycott 2006a). During this time a storage root is produced in which sugar is stored in the form of sucrose, the beet normally has a sugar content of 17% (Draycott 2006b). Growers aim to reach canopy closure as early as possibly since radiation interception is directly correlated to yield (Jaggard et al. 2009). If drought occurs at any time it will result in negative effects on yield, but drought during the June-July period seemed most disadvantageous (K F Brown et al. 1987). To prevent yield losses due to drought it is important to look at the root growth in sugar beet.

Annual rainfall in the sugar beet growing area was estimated at 600 mm and the soil type is predominantly sandy loam with a maximum available water capacity of 130 mm in the top 100 cm (Scott and Jaggard 2000). At the start of the growing season it is assumed that the soil is at maximum water holding capacity, however during the critical growth period (June-August) crop demand exceeds the combined supply from soil and rainfall (Scott and Jaggard 2000). It was found that sugar beet in the UK need 319 mm water between June and August. The sandy loam soils most sugar beet is grown on, have a water availability of 130 mm in the top 100 cm of the soil (Scott and Jaggard 2000). The average amount of rainfall in the sugar beet growing area between June and August is 116 mm, resulting in a maximum water availability of 246 mm, which is less than the optimum amount (Jaggard et al. 2007). Climate change models predict an even bigger water deficit as a result of reduced precipitation during the summer months (Richter and Semenov 2005).

Recent climate prediction models indicate that if the current trend continues there will be an increase in temperatures in Western-Europe including the UK of 1.5-3 °C by 2050 (Olesen et al. 2011). Alongside rises in temperature it is predicted that the mean precipitation per rainy day will increase resulting in periods of severe rain alternated with period of extreme heat and drought (Moriondo et al. 2011; Olesen et al. 2011) resulting in more rain in winter and less rain during the crop growing period (Jenkins et al. 2009). These predicted climate projections are thought to have a strong negative impact on crop yield as a result of drought stress, in the long run. Crops will suffer from drought more often and it is important to consider possible adaptations to prevent reductions in yield.

Root systems and their limitations in relation to water uptake are being increasingly explored. Root plasticity is found to play a great part in enhancing the water availability for plants (Ho et al. 2005; Padilla et al. 2013). When roots detect water, they can adjust their growth so there will be more root proliferation in the soil regions with high water availability and lower root proliferation in soil regions with low water availability. However, this is not always possible and there are many limitations to root growth such as compaction and low nutrient availability (Clark et al. 2003; Hodge 2004; Carminati et al. 2013).

It has been found in the 80s that sugar beet can grow roots over one metre deep but there is hardly any water uptake from depth (Brown and Biscoe 1985). Recent studies have shown that sugar beet indeed grow deep roots and, under unrestricted conditions, they can take up water from deeper layers (Fitters et al. 2017). There were, however, delays between roots being produced at depth and water uptake from the deep layers. It was found that the xylem need time to mature before efficient water uptake takes place (Mapfumo et al. 1993; Fitters et al. 2017). Field grown sugar beet are often subjected to soil constraints, such as compaction, which negatively affects root growth and thus water uptake (K F Brown et al. 1987; Lipiec et al. 2012).

Sugar beet varieties are thought to be genetically similar resulting in little to no differences in traits between different varieties (Ober et al. 2004). At the same time it has been observed that other crops such as maize and wheat show great genetic variation in their root distribution under varying water availabilities (Ginkel et al. 1998; Hund et al. 2009). Some key limiting factors to water uptake such as compaction have previously been identified but there might be more factors that limit water uptake in sugar beet (Brown and Biscoe 1985). To verify this, this study aimed to answer the following questions: How does the distribution of roots differ between sugar beet varieties when grown in the field?; does water availability affect the root distribution?; and what are the key limiting factors for root growth? Two years of field experiments were held to gather data to answer these questions.

Material and methods

Experimental design

Two field experiments were conducted in 2016 and 2017 at the University of Nottingham Farm, Sutton Bonington Campus (52°50 N, 1°15 W). Both experiments were arranged as split-plot designs with four replicate blocks, each plot was a large plot so one halve could be used for destructive measurements while the other halve was grown until harvest. Irrigation was on the main plot and variety on the sub-plot. In 2016 the varieties were: Aurora, Haydn and Hornet. In 2017, the same three varieties were grown and two more were added: BTS340 and Darnella. Irrigation was applied when wilting occurred: in 2016 irrigation was applied between 124-162 days after sowing (DAS) and in 2017 irrigation was applied between 70-97 DAS. In 2016, the average temperature during the experiment was 14.3 °C (Min: 3.4 °C, Max: 23.8 °C). In 2017, the average temperature was 14.3 °C (Min: 4.1 °C, Max: 23.6 °C). Rainfall distribution differed between the years with mostly spring precipitation in 2016 and summer precipitation in 2017 (Fig. 1a-b). In most months the precipitation was higher than the average monthly precipitation between 1982 and 2010, especially in 2016 when three times the average amount of rainfall was received in June (Fig. 2). The soil type for both years was Dunnington Heath Series, classified as a stony sandy loam (Cranfield University 2018). In 2016 the stony sandy loam was

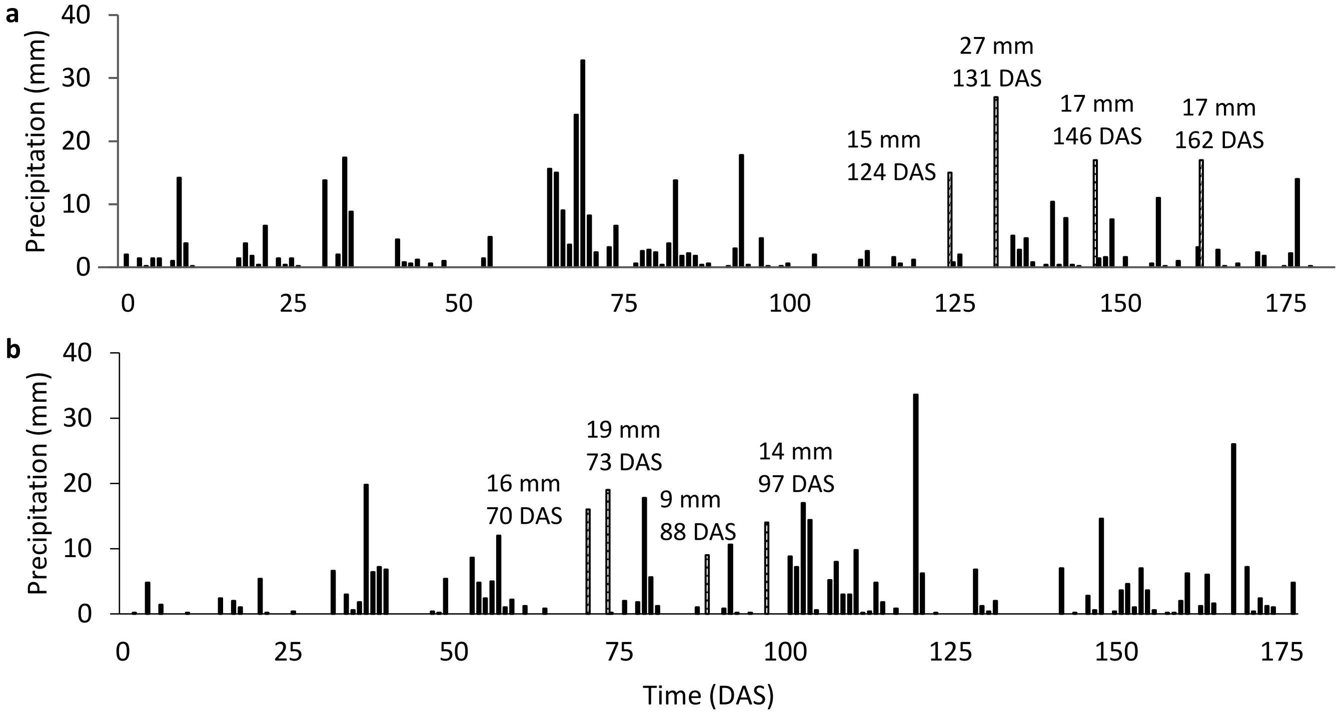

Figure 12 a) Precipitation during the experiment in 2016, the dashed bars represent the irrigation moments with at 124, 131, 146 and 162 DAS. b) Precipitation during the experiment in 2017, the dashed bars represent the irrigation moments at 70, 73, 88 and 97 DAS. Each irrigation moment includes information on the exact amount of water given.

overlying a clay subsoil at 50 cm. The fields were fertilized with 120 kg ha^{-1} of N in both years in accordance with the UK's RB209 standard for sugar beet (Defra 2010).

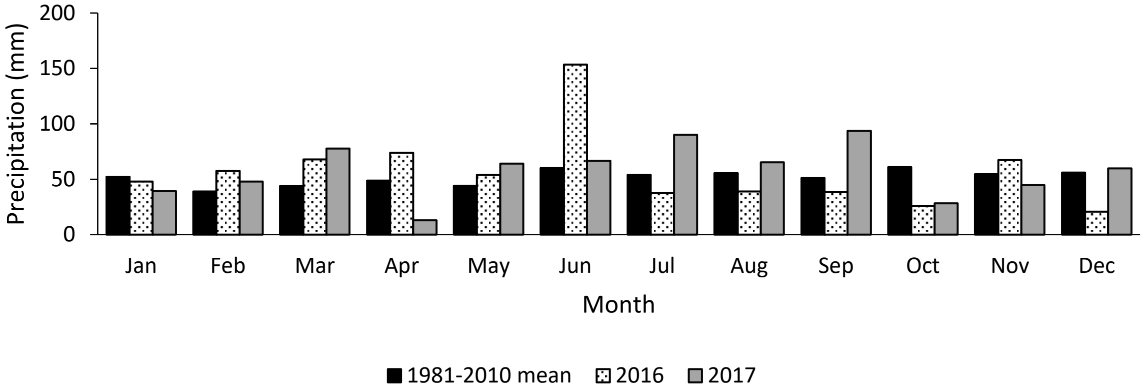

Figure 13 Average monthly precipitation (mm) between 1981-2010 and the monthly precipitation for both 2016 and 2017.

Measurements

In 2016, fortnightly stomatal conductance (mol m^{-2} s^{-1}) measurements were taken from the time when the fifth leaf had fully expanded. All measurements were taken between 9.00 and 13.00 h with an AP4 Porometer (Delta-T Devices, Burwell, Cambridge, United Kingdom). At the time of canopy closure (106 DAS) ten beet were harvested. The fresh weight was measured and, after drying for at least 5 days at 80 °C, the dry weight was measured. Three cores (ø 4.6 cm) were taken from each plot with a tractor mounted corer that could core up to 1 m depth. Each core was then divided into sections: 0-15 cm, 15-30 cm, 30-60 cm and 60-100 cm depth. Roots from each section were extracted 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) (cm cm^{-3}) was then calculated (Camposeo and Rubino 2003). At 179 DAS the experiment ended and again the fresh and dry weight of ten beet was measured. Alongside three cores were taken, and the roots were washed and measured as described above. Three rows were harvested and taken to the sugar factory to determine the sugar yield.

In 2017, the stomatal conductance (mol m^{-2} s^{-1}) was measured as described for 2016. The first destructive measurement was done at canopy closure (91 DAS). Three cores were taken, and the roots were extracted and measured as in 2016. At 147 DAS more cores were taken, and the roots were measured. At 177 DAS the experiment ended, and the beet were harvested, and the fresh and dry weights were measured as described for 2016. The sugar beet were sent to the BBRO tarehouse at Wissington Sugar Beet factory to determine the sugar yield.

In 2016, one core in each destructive plot was taken at the time of canopy closure (106 DAS). The core was then cut up in sections of 10 cm at the following depths: 10-20 cm, 35-45 cm and 55-65 cm. These sections were then scanned with a Phoenix X-ray CT machine (GE Measurement & Control Solutions, Wunstorf, Germany). X-ray CT scanner was set at 140 kV and 160 µA. The resolution was set to 40 µm and each section took 15 minutes to scan.

After the grey-level X-ray CT images were resized to 650 x 650 pixels to exclude non-soil areas, the images were processed with ImageJ 1.52a software (Schindelin et al. 2012). First the images were converted to 8-bit after which the *Mean* filter was used and next *Sharpen* was used. After enhancing the image, the threshold was set to the preset *IJ_IsoData*. Lastly, the image was converted to black and white after which the black area, representing pore space, could be analysed.

Statistics

Data was analysed by ANOVA using Genstat 17th edition (VSN International 2011). Four replicates were used for treatment measurements in both years, within each treatment varietal differences were studied and for the root data different depths within those varieties were studied. Correlations between all variables were calculated and tested for each year.

Results

2016

At the time of canopy closure, 106 DAS, no irrigation had been applied, and therefore there were no differences in rooting traits between rainfed and irrigated plots. There were, however, varietal differences and differences with increasing soil depth (Fig. 3). Haydn had a significantly higher RLD compared to Hornet and Aurora at 0-15 cm and a significantly higher RLD than Hornet at 60-84 cm (Fig 3a. $p < 0.001$, DF = 9, l.s.d. = 0.327).

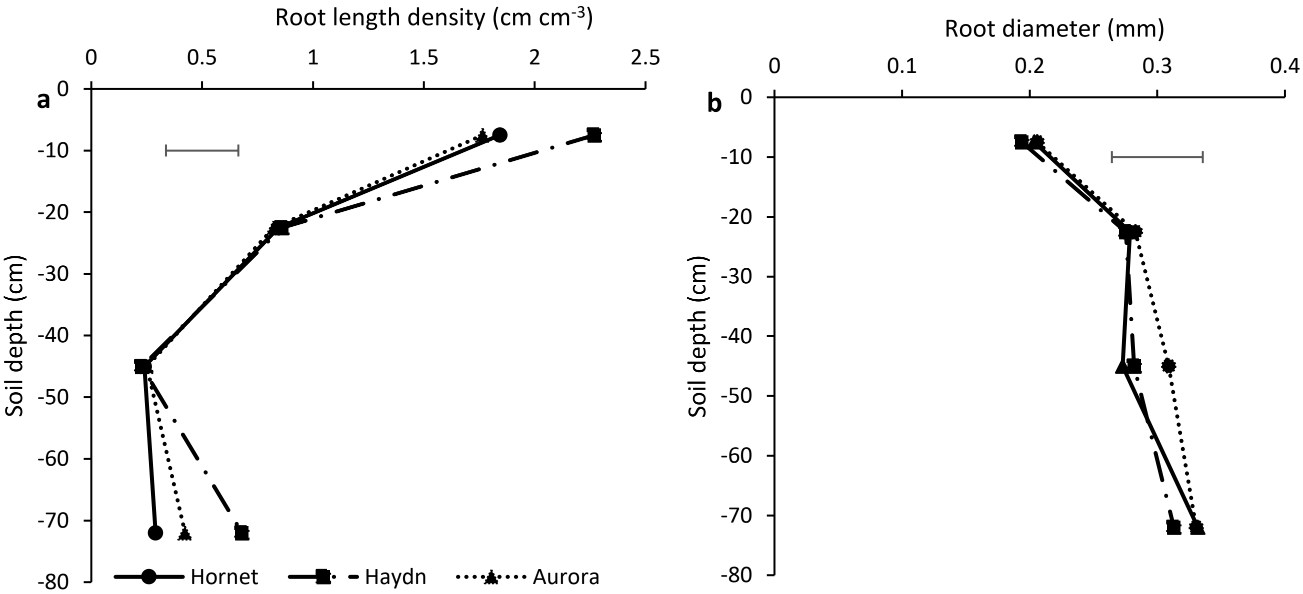

Figure 14 a) Root length density (cm cm^{-3}) at 106 DAS at four different depths at consecutive points. b) Average root diameter (mm) at 106 DAS at four different depths at consecutive points. The error bar shows the least significant difference (variety*depth).

Overall there was a higher RLD at 0-15 cm compared to deeper soil layers. The average root diameter showed that the roots in the 0-15 cm section were significantly thinner compared to roots in deeper layers (Fig. 3b). Hornet had significantly lower average root diameter at the 30-60 cm section compared to Aurora ($p < 0.001$, DF = 9, l.s.d. = 0.071).

At the end of the experiment the RLD and average root diameter were determined again (Fig. 4) and an overall variety*treatment interaction was found ($p = 0.011$, DF=2, l.s.d. = 0.318). Under irrigated conditions both Haydn and Aurora had a decrease in RLD. However, Hornet showed an increase in RLD under irrigated conditions, albeit no significant increase. With increasing depth there was a significant decrease in RLD of all varieties (Fig. 4a). Hornet showed the strongest decrease in RLD with depth, the RLD was half that of Aurora in the 30-60 cm section ($p < 0.001$, DF = 6, l.s.d. = 0.389). At 170 DAS the soil had dried out significantly and therefore coring was only possible up to 60 cm. The differences previously found in the average root diameter had completely disappeared at 170 DAS (Fig. 4b).

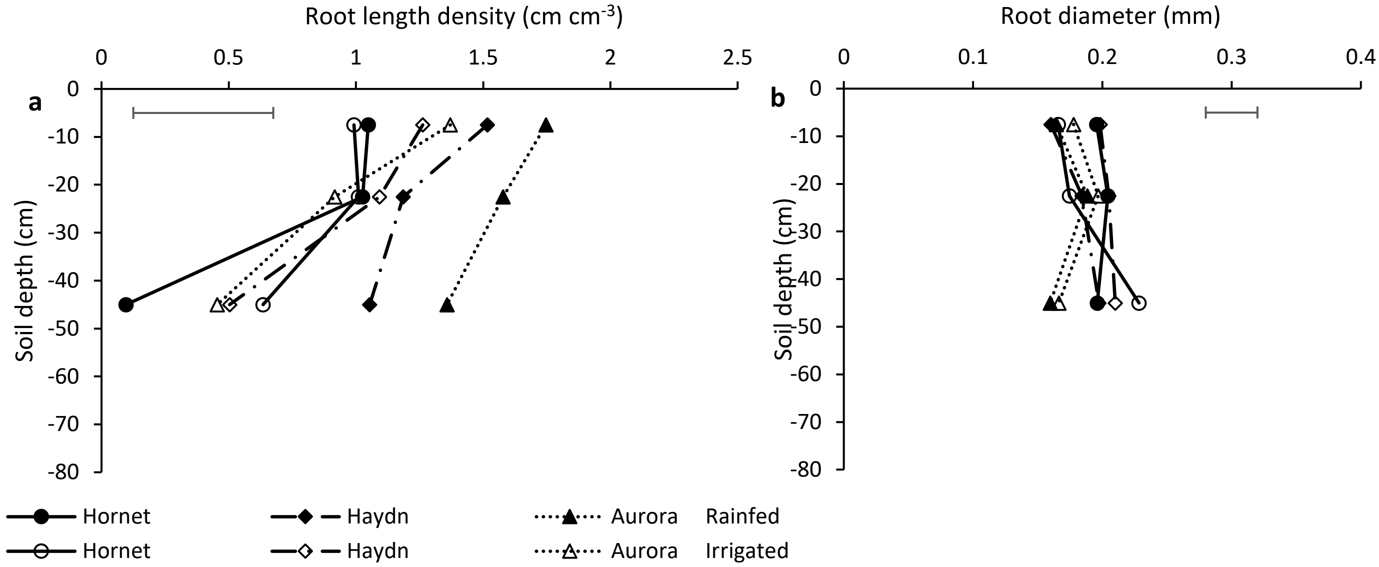

Figure 16 a) Root length density (cm cm^{-3}) at 170 DAS at four different depths at consecutive points. b) Average root diameter (mm) at 170 DAS at four different depths at consecutive points. The error bar shows the least significant difference (treatment*variety*depth).

-When first irrigation was given at 125 DAS there was a subsequent increase in stomatal conductance at 131 DAS (p = 0.039, DF = 1, l.s.d. = 0.061) as seen in Figure 5a. Throughout the experiment there were significant varietal differences (Fig. 5b) with Hornet having a consistently lower stomatal conductance compared to Haydn and Aurora, which showed similar values (p = 0.024, DF = 2, l.s.d. = 0.075).

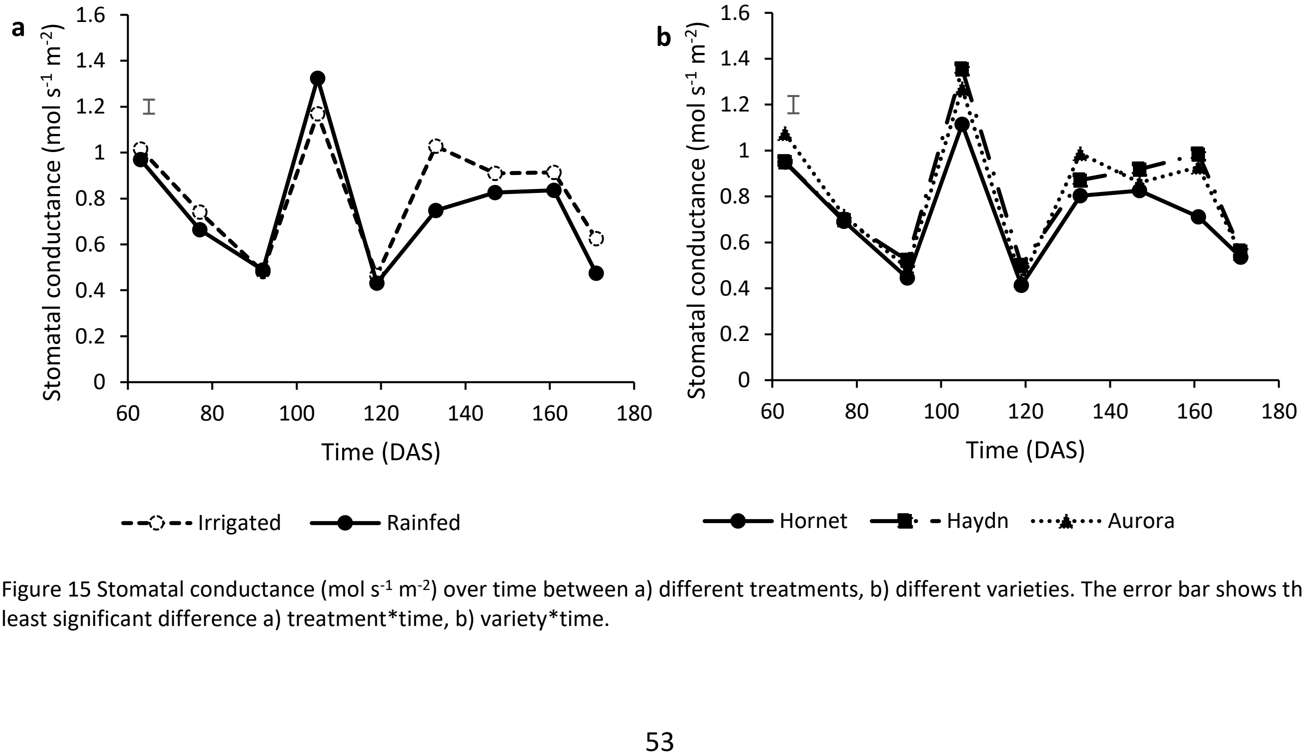

Figure 15 Stomatal conductance (mol s^{-1} m^{-2}) over time between a) different treatments, b) different varieties. The error bar shows the least significant difference a) treatment*time, b) variety*time.

Root and canopy fresh and dry weights were measured at canopy closure (97 DAS) and at harvest (170 DAS). At 97 DAS treatments had not been applied yet and there were no significant differences found between the varieties in either root or canopy fresh and dry weight. At 170 DAS irrigation had been applied and this resulted in a significantly higher water content in the root of irrigated beet (p = 0.012, DF = 1, l.s.d. = 0.563). However, there were no significant differences in the root or canopy dry weights (Table 1).

The change in water content between irrigated and rainfed sugar beet translated through to the percentage of sugar in the beet with the irrigated beet having a significantly lower percentage of sugar compared to the rainfed beet (p < 0.001, DF = 1, l.s.d. = 0.324), indicating the sugar in the irrigated beet had been diluted (Table 1). This was confirmed when there were no significant differences found between the treatments when looking at actual sugar yield (tonnes ha^{-1}). The actual yield did show significant varietal differences with Hornet having a higher sugar yield compared to Aurora (p = 0.044, DF = 2, l.s.d. = 0.954) (Table 1).

Table 2 Root dry weight (kg), root water content (%), sugar content (%), and sugar yield (tonnes ha-1) at 170 DAS. The mean, overall l.s.d. and P*-values for the variety, treatment and variety*treatment are found at the bottom of the table.*

Treatment	Variety	Root dry weight (kg)	Root water content (%)	Sugar content (%)	Sugar yield (tonnes ha^{-1})
Rainfed	Hornet	0.82	20.8	17.9	14.7
	Haydn	0.84	20.4	17.8	13.6
	Aurora	0.85	20.0	17.3	13.4
Irrigated	Hornet	0.84	20.9	17.2	15.1
	Haydn	0.82	21.7	16.8	14.2
	Aurora	0.75	20.9	16.8	14.0
Mean		0.82	20.8	17.3	14.2
l.s.d.		0.126	0.975	0.561	1.349
variety		*P* = 0.710	*P* = 0.185	*P* = 0.065	*P* = 0.44
treatment		*P* = 0.368	*P* = 0.012	*P* < 0.001	*P* = 0.174
variety*treatment		*P* = 0.399	*P* = 0.208	*P* = 0.371	*P* = 0.979

Overall the X-ray CT analysis showed that there was a significant decline in the number of pores with increasing depth (p = 0.002, DF = 2, l.s.d. = 30.29) (Fig 6a). There were some interaction effects between the different varieties and depths, most likely the result of variation within the field.

The pore size distribution did not show any significant differences but there was, however, a trend in the 25:75 ratio when comparing Hornet at 10-20 cm depth to Aurora 35-45 cm depth and Haydn 55-65 cm depth (p = 0.081, DF = 6, l.s.d. = 30.8).

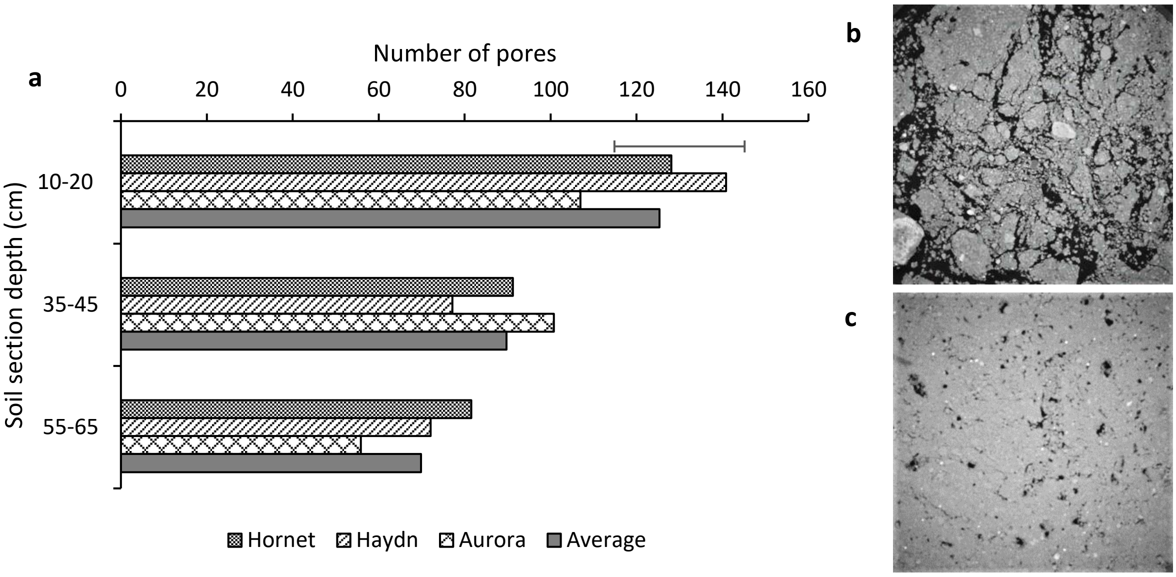

Figure 17 a) Number of pores in different soil sections for the three different varieties and the average number of pores at each depth. The error bar shows the least significant difference (Depth). b) An X-ray CT image from the 10-20 cm section growing Haydn. c) An X-ray CT image from the 55-65 cm section growing Aurora.

Due to high variation in total pore area (µm^2), average pore size (µm) and porosity (%) there were no significant differences.

2017

Roots extracted at canopy closure showed no differences between the treatments at all. However, similar to 2016, there were differences between the varieties (Fig. 7a). Overall Aurora had the lowest root length density (RLD) and Haydn and BTS 340 had the highest RLD (p = 0.002, DF = 4, l.s.d. = 0.186). There was an even stronger variety*depth interaction where Aurora had a very low RLD in the 30-60 cm section compared to Haydn and BTS 340. Hornet and BTS340 showed an increase in RLD at 15-30 cm compared to 0-15 cm, while Aurora, Darnella and Haydn had a decreased RLD (p < 0.001, DF = 10, l.s.d. = 0.322). BTS 340 had its highest RLD at 30-60 cm section and its lowest RLD in the 0-15 cm section while Aurora and Darnella showed the opposite pattern of having the highest RLD at 0-15 cm section and the lowest RLD at 30-60 cm. When looking at the average root diameter, there were no differences whatsoever. Over time differences became less pronounced and the rooting pattern changed. There was an overall increase in RLD at the 0-15 and 30-60 cm sections at 147 DAS (Fig. 7b). There was a significant variety*depth interaction where BTS340 had a very high RLD at 30-60 cm compared to the RLDs found by all varieties at 15-30 cm (p = 0.039, DF = 10, l.s.d. = 0.669). No diameter differences were found at either time point.

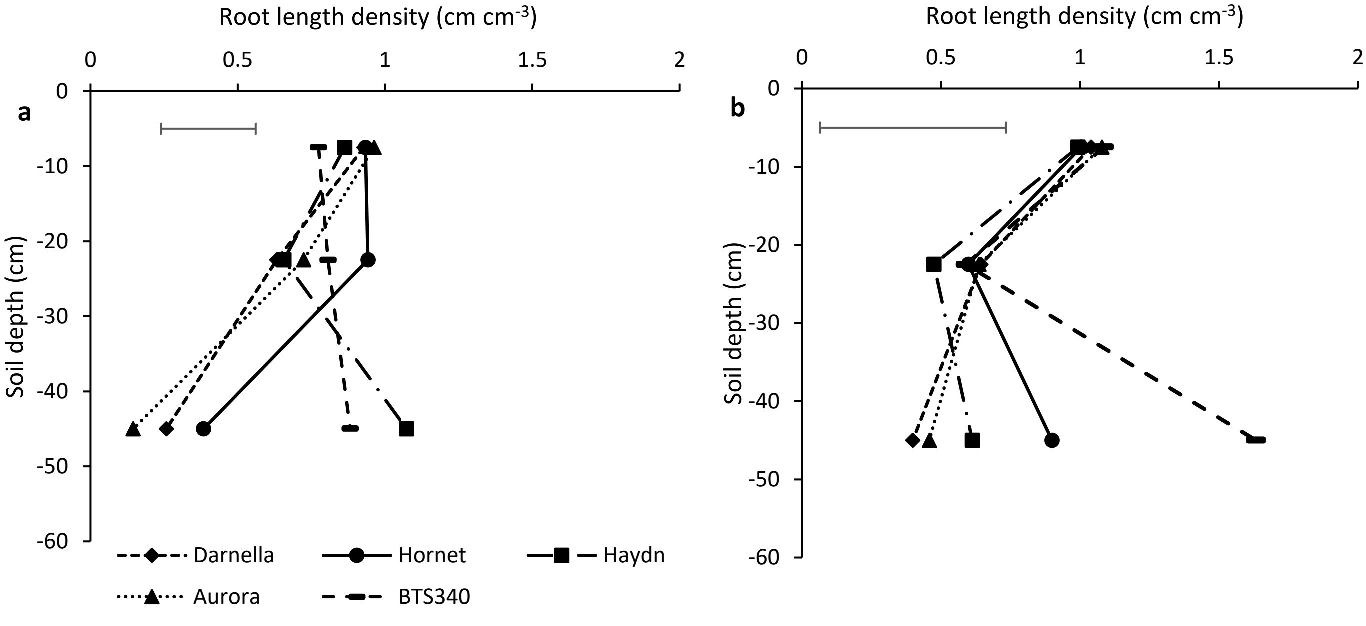

Figure 18 a) Root length density (cm cm^{-3}) at 91 DAS at four different depths at consecutive points. b) Root length density (cm cm^{-3}) at 147 DAS at four different depths at consecutive points. The error bar shows the least significant difference (variety*depth).

Irrigation was first given at 70 DAS, and from this moment onward there were differences in stomatal conductance with the irrigated plots having a higher stomatal conductance compared to the rainfed plots. After rainfall was sufficient to meet the water demand (See Fig. 1 at 108 DAS) these differences reduced again (Fig. 8). Overall the irrigated plants had a significantly higher stomatal conductance (p = 0.045, DF = 1, l.s.d. = 0.104).

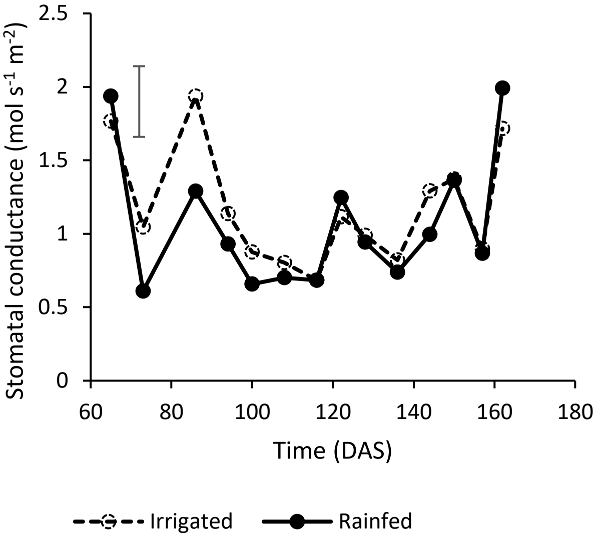

Figure 19 Stomatal conductance (mol s^{-1} m^{-2}) over time for irrigated and rainfed beet (averaged across varieites). The error bar shows the least significant difference treatment*time.

At 155 DAS beet were harvested and the fresh and dry weight were determined. The fresh and dry weight at 155 DAS showed both differences between treatments as well as varieties. Leaf dry weight was significantly higher in plants that had received irrigation ($p < 0.001$, DF = 1, l.s.d. = 32.49). The total plant water content showed that the irrigated plants had a significantly higher water content ($p < 0.001$, DF = 1, l.s.d. = 464.1). Varietal differences were mostly seen in leaf and root water content. Aurora had a significantly higher leaf water content compared to Darnella (p = 0.025, DF = 4, l.s.d. = 277.9). The same thing was not found in the root dry weight. Darnella had a significantly higher root water content compared to Haydn (p = 0.045, DF = 4, l.s.d. = 534.4) (Table 2). Aurora only had a slightly higher water content than Haydn indicating that the trend seen in leaf water content was almost reversed in the root water content. There were no treatment effects found in sugar content (%) or actual sugar yield (tonnes ha^{-1}). However, varietal differences were found; Hornet had a significantly higher sugar content (%) than Darnella, BTS340 and Aurora (p = 0.007, DF = 4, l.s.d. = 0.371) (Table 2). As for the actual yield, Darnella and BTS340 had a significantly higher yield than Hornet, Haydn and Aurora ($p < 0.001$, DF = 4, l.s.d. = 0.784) (Table 2).

Table 3 Root dry weight (kg), root water content (%), sugar content (%), and sugar yield (tonnes ha^{-1}) at 155 DAS. The mean, overall l.s.d. and P*-values for the variety, treatment and variety*treatment are found at the bottom of the table.*

Treatment	Variety	Root dry weight (kg)	Root water content (%)	Sugar content (%)	Sugar yield (tonnes ha^{-1})
Rainfed	Hornet	0.87	75.9	17.2	16.9
	Haydn	0.92	74.8	17.0	17.6
	Aurora	0.90	77.6	16.7	17.4
	Darnella	0.93	78.2	16.6	19.8
	BTS340	0.98	77.3	16.6	18.7
Irrigated	Hornet	0.99	76.7	17.2	17.5
	Haydn	0.90	75.9	16.8	16.4
	Aurora	0.83	77.1	16.7	17.2
	Darnella	1.03	78.1	16.5	19.6
	BTS340	1.10	76.1	16.6	19.4
Mean		0.94	76.8	16.8	16.0
l.s.d.		0.204	1.700	0.524	1.109
variety		$P = 0.144$	$P < 0.001$	$P = 0.007$	$P < 0.001$
treatment		$P = 0.304$	$P = 0.939$	$P = 0.690$	$P = 0.898$
variety*treatment		$P = 0.579$	$P = 0.280$	$P = 0.998$	$P = 0.171$

Discussion

Differences in root traits of plants have often been observed between genotypes of the same species (Hund et al. 2009; Romano et al. 2012). Alongside the genotypical variation there is also root plasticity which can result in different rooting patterns in response to environmental factors (Ober et al. 2004; Ho et al. 2005; Padilla et al. 2013). Together, genetic and environmental factors determine the root architecture (Dorlodot et al. 2007). Since current UK sugar beet varieties have all originated from one monogerm plant around 1948 there is limited genetic variation between the varieties (Bosemark 2006). In our study we did see differences between the varieties indicating that even small genetic variations could lead to substantial differences. Especially in 2016 there was a clear difference between the varieties at 170 DAS, indicating that mild drought stress exaggerated these differences.

Under non-drought stressed conditions, differences were less pronounced indicating plant responses become more noticeable when stress levels increase (Chaves et al. 2008).

Previous studies have shown that there are often differences in the rooting patterns at depth with deeper soil layers often containing less roots or roots of thicker diameter (K F Brown et al. 1987; Lipiec et al. 2012; Colombi and Walter 2016; Fitters et al. 2017). Both years there were reductions in root length density of most varieties with increasing depth. During the first coring moment in 2016 the RLD was almost four times as high in the top 15 cm compared to the 60-84 cm section. This difference was reduced by the time of harvest when the top 15 cm had only twice the RLD found at the 30-60 cm section, which was at that time the deepest that coring was possible. Kashiwagi et al. (2006) found that in chickpea, the RLD at deeper soil layers correlated better with yield than the RLD from shallow layers. This suggests that even though the RLD was substantially higher in the top soil section this might not have any effects on the final yield. However, in this study, no correlation between RLD at any depth and sugar yield was found. This was most likely due to the limited drought stress present in both years. The weather data shows that the amount of precipitation in almost all months of both the 2016 and 2017 growing season was higher than the long-term average between 1981-2010 (MetOffice 2018b). There were not many root diameter differences but the average root diameter became slightly higher with depth at both coring moments, most likely as a result of increased penetration resistance with depth (Lipiec et al. 2012). Our X-ray CT data showed that the number of pores decreased with increasing depth. This corresponded with a lower root length density in the 60-84 cm soil section. A previous study in Australia showed that with increasing depth more roots grow in existing pores (White and Kirkegaard 2010), since there were less pores at deeper layers, this might explain why less roots were found. The other parameters measured did not show any significant differences due to great variability.

In 2017, differences in RLD at different depths were less clear, Darnella, Hornet and Aurora showed a clear decrease in RLD with depth at 91 DAS, but Haydn and BTS340 showed a mild increase in RLD with depth. At 147 DAS Darnella and Aurora still showed the same pattern, yet Hornet, Haydn and BTS340 now showed a pattern with the lowest RLD at 15-30 cm and a higher RLD at both the 0-15 cm and 30-60 cm sections. Dardanelli et al. (1997) stated that the root system architecture is very variable under slightly different environmental conditions. This indicates that the differences that are found between rooting patterns of varieties used in both 2016 and 2017 can be very different even though the environmental factors were only slightly different. The average diameter was very similar at the different depths. This was likely the result of low soil physical constraints (Clark et al. 2003) unlike in 2016 when a heavy clay soil was found at 50 cm depth.

Increased water availability did not lead to big differences in root traits most likely because water availability was rarely limiting in either year. From February to June 2016, the amount of rainfall was higher than the long term average and in June three times the average rainfall was received (MetOffice 2018b). Because of this no irrigation was given until September. In 2017 the amount of rainfall was higher than average from February until September, with the exception of April. Since April had seen four times less rainfall compared to the average of 1981-2010 some irrigation was given in May, where several days of no rainfall at all were alternated with days with heavier rainfall (MetOffice 2018b). In 2016 at 170 DAS irrigation resulted in a lower RLD in Aurora and Haydn compared to rainfed plots. It is more common to see a higher RLD as a result of drought since roots are thought to explore the soil more under water limiting conditions (Asch et al. 2005; Comas et al. 2013). However, Camposeo and Rubino (2003) found that higher water availability resulted in higher RLD, mostly at shallow soil depths but lower RLD at deeper depths. Since 2017 had been a relatively wet year there were no RLD differences as a result of additional irrigation.

Despite there not being root responses to the additional irrigation given there were differences in stomatal conductance. As soon as irrigation was given an increase in stomatal conductance was observed in both years. This corresponds to the many studies that found that stomatal conductance decreases when water availability reduces (Miyashita et al. 2005; Steduto et al. 2007). The stomatal conductance never reached values of 0.1 mol m^{-2} s^{-1} or lower, which indicates that there was no severe water stress at any stage (Flexas and Medrano 2002). Even though the yield in the rainfed plants was lower than in the irrigated plants, the difference was not enough to be significant. Previous studies showed that an increase in CO_2 levels mitigated the negative effects of reduced stomatal conductance (Long et al. 2004; Long et al. 2006). Varietal differences in final yield were observed but no relation to the RLD was found.

Conclusion

During this study it was found that distribution of sugar beet roots differed between varieties. When the number of pores decreases the root length density decreases. Different watering regimes only affected the root growth when mild drought occurred later in the 2016 season, there was no water stress in 2017. Root growth seemed mostly limited by soil physical constraints. Low number of soil pores could have been the reason for a lower root length density which in turn could limit water uptake from deeper soil layers. Overall it was found that sugar beet yield was not affected by the mild drought stress that had occurred, any differences in yield were attributed to varietal differences that did not show in any of the measured rooting traits or stomatal conductance.

Acknowledgements

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5. General discussion

At the start of this PhD we formulated the following hypotheses:

1. Sugar beet can produce deep roots and take up water from depth under unrestricted soil conditions
2. Water uptake by sugar beet from deep soil layers is limited by a) soil compaction and/or b) plant physiological constraints.
3. Roots proliferate in deeper soil layers in response to limited water availability in upper layers
4. Late drought has a stronger negative impact on yield than early drought
5. Different varieties have different rooting patterns, e.g. more root proliferation in deeper soil layers.

As the experiments progressed we gained more insight in the capabilities and limitations to root growth and water uptake in sugar beet. Here, we will give an overview of the discussion points we have come across and compare our results with published literature.

All experiments showed that a reduction in available water resulted in increased root proliferation, mainly at depth. This was most clear in the glasshouse and box experiment (Chapter 2 and 3) but even the field experiment showed that there was a slightly higher root length density when plants were rainfed compared to fully irrigated (Chapter 4). Similar rooting patterns under drought were seen in wheat, maize and oilseed rape as well (Shaw et al. 2002; Asch et al. 2005; Comas et al. 2013; Lynch 2013; Lobet et al. 2014). Chapter 3 showed that this increase in root growth, in response to moisture deficit, was observed both under prolonged and short periods of drought. It was seen in spring barley, wheat and oilseed rape that a root length density (RLD) of 1 cm cm^{-3} was needed to take up 90% of the available soil moisture (Carvalho et al. 2014; White et al. 2015). Our glasshouse experiment (Chapter 2) and the field experiments (Chapter 4) showed that the RLD was close to, or more than, 1 cm cm^{-3} in the top section of the soil by the end of each field experiment and in the fully irrigated and simulated rainfall treatments in the glasshouse experiment. However, the drought treatment in the glasshouse experiment had a RLD below 0.93 cm cm^{-3} but still managed to take up 90% of available water, indicating that the minimum threshold for sugar beet RLD is lower than that of barley, wheat and oilseed rape.

The field experiments (Chapter 4) were the only two experiments where multiple varieties were grown. Differences in rooting traits between plants of the same species but with a different genotype have been observed before (Hund et al. 2009; Romano et al. 2012). However, UK sugar beet varieties originate from one monogerm plant used for breeding in the late 1940s, and hence the likelihood of differences between varieties is small (Bosemark 2006). The field experiments did show some differences between the varieties, especially when there was some mild drought stress in 2016. Under non-stressed conditions differences were less pronounced indicating stressed plants exhibit root architectural differences more clearly (Chaves et al. 2008). However, under non-stressed conditions we hardly found any differences. It was also seen that, in the field, there were a low number of pores found at depth. Previously it has been observed that with increasing depth more roots grow in pores (White and Kirkegaard 2010). Since fewer pores were found in deeper soil layers, this would likely have limited root growth resulting in a lower root length density.

When looking at changes in soil moisture content, it was seen that the stomatal conductance dropped when the soil became depleted of moisture, as has previously been observed (Pang et al. 2011). There was, however, a delay in water being taken up from depth. Both experiments showed that water uptake did not happen from deep layers until the shallower layers had been depleted of water, even though plants were showing signs of drought stress. This has been seen in the field before as well (Brereton et al. 1986). Chapter 2 shows that a possible reason for this delay in water uptake from depth was a lack of secondary xylem, resulting in inefficient water uptake from depth until the xylem had matured, as also seen by Mapfumo et al. (1993). In a root anatomy experiment (Chapter 2) it was found that it takes approximately three weeks for a root that forms at depth (below 60 cm in this experiment) to develop secondary xylem. This corresponds with our findings in the glasshouse experiment where we found a three week delay between roots being observed at a certain depth and water uptake from that depth. In the box experiment (Chapter 3) a delay between roots arriving at deeper soil layers and roots taking up water from that depth was also found. However, the delay in water uptake in the box experiment seemed shorter at first, most likely since the exact moment of roots reaching a certain depth and water being taken up from that depth was not determined since root observations were taken every 14 days. During the second year, measurements were taken more regularly and the delay seen was close to three weeks again. Therefore, results from the box experiment support our hypothesis that the delay between roots arriving and water uptake was caused by the absence of secondary xylem in newly formed roots.

When water availability was limited the water use efficiency (WUE) increased, as was seen in both the glasshouse experiment (Chapter 2) and the box experiment (Chapter 3). These findings have been seen before in sugar beet and various other C3 crops (Araus et al. 2002; Bloch et al. 2006; Rinaldi and Vonella 2006). However, the glasshouse experiment and the 2016 box experiment also showed that when drought stress is prolonged and severe, there will be a reduction in WUE. One of the reasons for the improvement in WUE is thought to be a partial closure in stomatal conductance, (Araus et al. 2002). In all our experiments it was seen that lower water availability resulted in lower stomatal conductance, but whether this was a plant protection mechanism or a water conservation mechanism is not clear (E S Ober et al. 2005; Rivero et al. 2009). In the glasshouse experiment (Chapter 2) the net photosynthetic assimilation was measured and showed that the pattern in net photosynthetic assimilation follows the same pattern as the stomatal conductance, indicating that the improvement of WUE was the result of stomatal closure rather than maintaining photosynthetic assimilation. Flexas and Medrano (2002) previously showed that a stomatal conductance of 0.10 mol m^{-2} s^{-1} or lower is an indication for severe drought stress in C3 species. During the glasshouse experiment (Chapter 2) the stomatal conductance dropped below 0.10 mol m^{-2} s^{-1} towards the end of the experiment indicating severe drought and this could explain the drop seen in photosynthetic assimilation. However, during the box experiment and field experiment (Chapter 3 and 4), the stomatal conductance never dropped below 0.10 mol m^{-2} s^{-1}, in either year. The stomatal conductance did drop drastically when watering was restricted resulting in moderate stress rather than severe stress. It is thought that leaf gas exchange is controlled by soil moisture content (Gollan 1985; Tardieu and Davies 1993), which seems to correspond in part with our findings. Under increase in water availability generally, an increase in stomatal conductance was seen (Chapter 2, 3 and 4). SPAD was measured during the glasshouse experiment and the box experiment and under drought stress an increase in SPAD was observed. This was most likely caused by a concentration effect. Previous studies have shown that drought generally results in a decrease in SPAD values. It is possible that sugar beet show a different response compared to other crops, this is something that should be tested in the future.

In the glasshouse experiment and 2016 box experiment, storage root dry weight was used as an indication of yield, in the 2017 box experiment and the field experiment amount of sugar was used as the yield indicator. Under drought the yield was severely reduced as had been seen in the glasshouse experiment and 2016 box experiment (chapter 2 and 3). However, when water was replenished after drought or when drought was mild there were no yield penalties (chapter 3 and 4). Similarly it was found in wheat that continuous drought had a strong negative impact on yield while shorter period of drought followed by replenishment did not result in strong yield penalties (Ginkel et al. 1998). While under drought stress followed by replenishment there were small differences in yield, strong differences were seen in leaf dry weight, probably due to high leaf senescence under drought. It has also been seen previously that wetting and drying cycles can have a negative effect on nutrient mobilization and organic matter decomposition (Majdalani et al. 2008; Zhu and Cheng 2013), resulting in different final leaf dry weights.

All these experiments have helped to answer our research questions regarding current sugar beet varieties and their capabilities in terms of rooting and water uptake. We now know that sugar beet are able to grow deep roots in both unrestricted and mildly restricting soils, especially when water availability in shallow soil layers becomes scarce. If water is available from deeper layers, and more shallow layers are being depleted, water will be taken up from depth. However, we have also seen that there is a delay between roots arriving at a certain depth and water being efficiently taken up from this depth. The varieties in the field experiment showed differences in root distribution, which were especially strong when mild drought stress occurred. Reduced water availability generally had a negative impact on yield, but the duration and timing of drought made a difference in how strong a yield effect was seen. Our experiments have allowed us an exclusive look into root growth at different depths and under different settings and in chapter four we also compared different varieties.

Ongoing research will continue to look the relation of water availability and sugar beet growth and yield. The BBRO have several projects currently running (BBRO 2017):

- Understanding soil plant interactions to improve sugar beet productivity
- Economics of sugar beet irrigation in England
- Understanding water use efficiency of sugar beet
- Variety trials programme

The first project looks at a wide range of soil plant interactions in relation to plant development and yield. This includes water availability, optimum nitrogen levels and differences in soil structure. The second project aims to quantify the response of sugar beet to irrigation and the economics of irrigation. The third project aims to identify sugar beet traits that are linked to conservation of water. The last project is one of the projects that is held every year to determine the agronomic performance of the different sugar beet varieties. These trials can find differences between varieties and depending on the environmental factors of each year they could identify varieties that are more tolerant to low water availabilities.

It would be good if future work involved research into mitigating low water availability. More field scale studies should be done to examine the effect of our current climate in relation to sugar beet yield and root growth. Important here is that trials are done on different soil types and with multiple varieties. Our research showed that there are varietal differences that could potentially help mitigate low water availability, either by deep root growth or by differences in stomatal closure rates. Sugar beet are grown on different soil types and each soil type has a different water availability, hence the need for trials on a variety of soil types. Another question we were left with is the effect of soil constraints on root growth; there could be several soil constraints that alter root growth and subsequent water uptake or just water movement throughout the soil in general. Some of the X-ray CT data we have from the field suggests there is a lower pore count with increasing depth and this might mean roots grow along existing root channels at those deeper soil layers, which results in poor root-soil contact, thereby limiting water uptake. More research into this and possible solutions such as tillage or perhaps the use of cover crops is needed to gain more insight into this issue. More research into traits that affect the rate of water uptake and water loss is important as well. The rate in which primary xylem matures to secondary xylem could vary between varieties; although we did not test that in this study. Given sufficient genetic variation, breeders could select varieties based on their RLD and/or speed at which secondary xylem is formed. Varietal differences indicate there is potential for breeding of certain traits that help mitigate low water availability. It might be possible to increase the number of measurements that is currently done on field trials to include stomatal conductance measurement and canopy temperature measurements. Even though these measurements take time they can give a good indication of the water status of the plant. Soil moisture measurements can help locate where water is taken up from. Any differences found could be an indication that there is potential to breed for a certain trait. Our findings can be utilised to inform future work to improve sugar beet drought tolerance even further.

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