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

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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 total root length. Experiments were conducted to assess root growth at different depths in response to specific watering regimes. Sugar beet plants were grown in wooden boxes ($2.16\ m^2 \times 1.2\ m$) 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.

1. Introduction

Worldwide, water availability is an increasing 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 $0.14\ m^3\ m^{-3}$ (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, 2018). 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). Sugar beet root architecture is normally conical with many roots at shallow depths and a decrease in root length with increasing depth (Brown and Biscoe, 1985). 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 mild to severe 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 (Johnson et al., 2001). Transparent 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

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minirhizotrons are an underestimation of root lengths depending on the measurement depth, and preferential root growth along the tube (Parker et al., 1991).

Several studies have looked into root growth in sugar beet (Brown and Biscoe, 1985; Brown et al., 1987), but over the past 30 years there have only been a few studies that focused on root growth in sugar beet which involved minirhizotrons. These studies were all done in field settings and the measuring depth varied from 0.7 m to 2 m depth. These studies focussed mainly on root response differences between tillage methods, nitrogen fertilizer (van Noordwijk et al., 1994; Vamerali et al., 1999), and very little was done on responses to varying water availability (Vamerali et al., 2009). Studies that look at sugar beet root growth with minirhizotrons in controlled conditions are relatively rare, but necessary to get a better understanding about root growth under non-restricting conditions. Controlled minirhizotron studies can help answer questions concerning changes in root growth and how these changes might affect the overall plant development and health.

To fill in any existing knowledge gaps, a minirhizotron experiment was done in controlled conditions. 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 development? 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).

2. Material and methods

2.1. Experimental design

Sugar beet were grown in six wooden boxes of 1.8 m × 1.2 m × 1.2 m (l x w x h) in 2016 and 2017. The soil medium was a sandy loam texture with an available PK content of 61 mg l^{-1} P and 850 mg l^{-1} K. and the boxes were emptied and filled with new soil between the two years. Assessment of penetration resistance showed that no compaction had occurred during filling, the resistance up to 75 cm was approximately 550 kPa. The boxes were arranged in a randomized block design with three blocks and were located in a polytunnel to exclude rainfall. The temperature fluctuated between −1 °C and 44 °C, with an average day temperature of 20 °C and an average night temperature of 11 °C. 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} (34.5% N) was applied on top of the soil as per field recommendation, no additional P and K was added. Each box contained ten horizontal minirhizotrons across the width of the box, two at each of the following depths: 30 cm, 50 cm, 70 cm, 90 cm, and 110 cm. To prevent over or under estimations the tubes were never placed in the same vertical plane. 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 by trickle tape to maintain field capacity until the different watering regimes were imposed.

2.2. Drought response experiment (2016)

Three sugar beet seeds (cv. Haydn) were planted at 3 cm depth at each plant position, three rows of eleven plants. At c.25 DAS (Days After Sowing) the boxes were thinned to one seedling per position. The two watering regimes were: 1. continuous irrigation (control), boxes were watered on demand depending on the temperature and rate of water uptake to maintain soil moisture levels around 0.35 $m^3 m^{-3}$. 2. drought from 57 DAS onward (DR) (BBCH growth stage 1.16). Exact amounts of water given can be found in the supplementary table.

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 in the middle 50 cm were imaged fortnightly through the minirhizotrons. The images (600 DPI) were taken at 1 cm intervals and then stitched together with ImageJ (Schindelin et al., 2012). The roots were traced manually after which the image was converted into a black and white format in ImageJ with the threshold color function (B&W) (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 by measuring the fresh weight, turgid weight and dry weight of leaf discs taken from the plants (Turner, 1981). At 131 DAS the DR plants were strongly suffering from drought and therefore the experiment was terminated and the plants were harvested. Leaf and root fresh weight and dry weight were determined, after drying at 75 ºC for at least seven days. Total plant water use efficiency (WUE) was calculated from the total plant dry weight divided by the total water uptake during the whole experiment.

2.3. 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) (start at BBCH growth stage 1.15), and no irrigation between 128–178 DAS ‘late drought’ (LDR) (start at BBCH growth stage 4.44). When re-watering, small amounts of water (equivalent to 15 mm of water per day) were given at first to avoid surface run-off. Exact amounts of water given can be found in the supplementary table.

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 after both treatments had a chance to replenish. Leaf and root fresh weight and dry weight of five beet in the middle of the front row were determined, after drying at 75 ºC 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.

2.4. 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.