Final Report

Soil-Root-Shoot Interactions in Potato

Ref: 11140035

Reporting Period: Oct 2017 – Jul 2021

Report Authors: Katharina Huntenburg and Ian C. Dodd

Date report submitted: August 2021

Additional copies of this report and other publications from the AHDB are also available at ahdb.org.uk, or can be obtained from:

Publications

AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel: 02476 692051
E-mail: Levy.Publications@ahdb.org.uk

CONTENTS

1. SUMMARY

1.1. Aim

This study aimed to further understand how potatoes sense their root environment and translate this into signals that are transmitted to the above-ground plant parts to regulate plant performance (photosynthesis), water allocation between plant organs and ultimately tuber yields.

1.2. Methodology

The study combines both greenhouse pot trials (where environmental conditions can be tightly regulated) and field trials to understand physiological mechanisms under typical cropping conditions. The pot trials elucidate general principles of stress signalling and physiological responses to drought and compaction in potato, while field experiments apply these findings in a more realistic setting. Consistent measurement methodologies (e.g. to determine plant and soil water relations) were adopted where practical to facilitate comparisons between experiments, and specialised instrumentation used (e.g. to collect xylem sap from potato roots; to non-destructively determine tuber growth and water content) to collect specific data sets.

1.3. Key findings

- Across a factorial combination of soil drying and compaction treatments, above ground biomass at full ground cover determines yield
- Drought stress reduces the size, but not number, of harvested tubers
- Field-grown plants maintained leaf water potential across a factorial combination of soil drying and compaction treatments, likely by increasing root growth (and thus water uptake) and restricting transpiration (water loss) by closing the stomata
- Drought stress increases leaf and xylem sap abscisic acid concentrations, thereby causing stomatal closure and limiting photosynthesis
- Strigolactones (SL) suppress lateral branching of potato shoots, but don't influence stomatal conductance irrespective of soil moisture
- Water content of tubers follows a diurnal pattern with decreasing water content in the day and increasing water content at night
- Tuber volume increases only at night even under well-watered conditions
- Water influx at night is necessary for tuber volume growth
- Mild drought stress stops tuber growth

1.4. Practical recommendations

- Breed for varieties that achieve full ground cover early in the season
- Ensure good soil conditions (moist, uncompacted, sufficient nutrition) in the first half of the season
- Irrigation later in the day may allow more water uptake into the tuber and less water loss through transpiration (leaf wetness duration needs to be considered)
- Irrigation closer to the ground can reduce evaporation from the canopy and increase crop water use efficiency
- Regular irrigation (to ensure sufficient soil water availability) is crucial during the tuber bulking period to ensure maximise yield
- Measuring plant hormones may inform breeding selection and irrigation management in the future, but more research is needed

2. INTRODUCTION

Climate change is expected to decrease tuber yields on all continents (e.g. in Eastern Europe, northern America and the lowlands of Africa) by the end of this century (Raymundo *et al.*, 2018). Unfavourable weather already limits UK potato production, since the major drops in national yields occurred in years with adverse rainfall conditions (AHDB, 2013). To mitigate drought effects, improved irrigation management strategies and breeding more drought tolerant varieties is necessary. Both these approaches rely on better understanding potato responses to drought stress. Understanding the physiological mechanisms regulating plant stress responses helps to predict crop behaviour and yield. Comparing controlled environment and field data may allow basic research to better explain the underlying mechanisms regulating crop yields.

As the soil dries, shoot growth is restricted earlier than root growth (Kramer and Boyer, 1995), with potato roots growing into deeper, moister soil layers to access additional water resources (Stalham and Allen, 2004). Thus, plants alter their root architecture and root-shoot ratio to provide sufficient water to the aerial plant parts, potentially avoiding leaf water deficits when water supplies are limited. Soil water deficit also alters root-to-shoot signalling to minimise water loss by the canopy. Transpiration can be restricted by stomatal closure in response to decreased guard cell turgor and/or hormonal signals from the roots (Davies et al., 2002). Strigolactones (SL) are phytohormones that are mainly produced in the roots (Visentin *et al.*, 2016) and affect shoot architecture and physiology (Saeed *et al.*, 2017). SL-deficiency increases shoot branching in many crops, including potato (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2010; Pasare *et al.*, 2013), increases stomatal conductance in tomato and Lotus (Liu *et al.*, 2015; Visentin *et al.*, 2016) and increases stomatal density in Arabidopsis (Ha *et al.*, 2014) under non-stress conditions. To secure yields, it is important to manage transpiration water loss and carbon capture through photosynthesis as the soil dries. How drought stress affects root-to-shoot signalling (including recently characterised hormone groups like strigolactones) in potato needs further investigation, utilising novel potato genotypes and methodologies for xylem sap collection, to better understand these physiological stress responses to allow more precise crop management (e.g. irrigation).

Potato tubers gain weight during the night and lose weight throughout the day (Baker and Moorby, 1969), and to optimise yield we need to understand what causes these diurnal fluctuations and find ways to minimise loss during the day and maximise gain at night. Nocturnal stomatal closure decreases transpiration, but day-time transpiration probably magnifies the difference in water potential between tuber and leaf. However, both Ψ_{tuber} and Ψ_{leaf} decrease during the day, and recover in the evening and overnight with a water potential difference between Ψ_{tuber} and Ψ_{leaf} of 0.3 – 0.4 MPa during the day and 0.1 – 0.2 MPa at night (Gandar and Tanner, 1976), with corresponding patterns of high xylem flux during the day and lower flux at night (Aliche *et al.*, 2020*a*). Thus, water supply to the leaves is the same under well-watered and drought stressed conditions during the day when the leaf transpires, but is lower under drought stress at night, when leaf water status recovers. Tuber contributions to the water flux towards the leaves overnight were hypothesized based on fluctuating tuber weight (Baker and Moorby, 1969), but not considered in more recent literature on stolon formation or fluxes of water and carbohydrates in drought-stressed potato plants (Lahlou and Ledent, 2005; Aliche *et al.*, 2020*a*,*b*). To determine whether tuber water content and tuber growth depend on soil water availability, an in-vivo study of the same tubers throughout the day and the night, with accompanying shoot physiological measurements, is necessary.

Altogether, this study aims to determine plant physiological responses to drought stress in potato and better understand the underlying root-to-shoot signalling mechanisms as well as water fluxes into and out of the tuber and their consequences for tuber growth.

3. MATERIALS AND METHODS

3.1. Field experiments

A 2x2 factorial combination of drought and compaction stress was set up at Cambridge University Farms in a randomized block design with 4 replicates in 2 consecutive years (Figure 1 for 2018 experiment). Plot dimensions were 9.0 x 4.5 m, with inter- and intra-row space of 0.75 m and 0.3 m respectively. Compaction was realized by driving a tractor repeatedly over the plots when they were irrigated to field capacity. After this treatment the field was left to dry and seedbeds were prepared under less wet conditions.

block 4	4-5 compacted well-watered no compost	4-6 compacted drought stressed compost	4-7 uncompacted well-watered compost	4-8 uncompacted drought stressed no compost
	4-1 compacted drought stressed no compost	4-2 compacted well-watered compost	4-3 uncompacted drought stressed compost	4-4 uncompacted well-watered no compost
block 3	3-5 uncompacted drought stressed no compost	3-6 uncompacted well-watered no compost	3-7 compacted drought stressed compost	3-8 compacted drought stressed no compost
	3-1 uncompacted drought stressed compost	3-2 uncompacted well-watered compost	3-3 compacted well-watered compost	3-4 compacted well-watered no compost
block 2	2-5 compacted drought stressed compost	2-6 uncompacted well-watered compost	2-7 uncompacted drought stressed no compost	2-8 uncompacted drought stressed compost
	2-1 compacted drought stressed no compost	2-2 uncompacted well-watered no compost	2-3 compacted well-watered no compost	2-4 compacted well-watered compost
block 1	1-5 compacted well-watered compost	1-6 compacted (accidentally) well-watered no compost	1-7 uncompacted well-watered compost	1-8 compacted well-watered no compost
	1-1 uncompacted drought stressed compost	1-2 compacted drought stressed compost	1-3 uncompacted drought stressed no compost	1-4 compacted drought stressed no compost

Figure 1: Field layout with plot number and treatments. Plots in grey were not included in this study. The arrow indicates compass point north.

Potatoes 'Maris Piper' were planted on 25th April 2018 and 5th April 2019 respectively. No irrigation was necessary until 12th June 2018 and 31st May 2019. Thereafter well-watered plots (WW) were irrigated when they reached a soil moisture deficit of 20 mm, whereas the deficit irrigated plots (D) were irrigated when they reached a soil moisture deficit of 60 mm. The irrigation boom was running at 30 $m*h^{-1}$ with nozzles hanging 1.5 m above ground. The usual time between irrigation intervals was 4 or 5 days.
Emerged plants were counted twice a week until 100% emergence was reached in almost all plots. Thereafter ground cover was taken weekly using the 2 middle rows in each plot.

3.1.1. Additional measurements 2018

One or two days after irrigation pre-dawn leaf water potential was measured and xylem sap was collected at 0.6 MPa overpressure for 2 minutes and kept on ice or dry ice (according to availability). A few hours after sunrise, leaf gas exchange was measured (LI-6400 Portable Photosynthesis System, LI-COR, Lincoln, USA) on one plant per plot and leaf water potential

(Ψ_{leaf}) of that same leaf determined with a Scholander-type pressure chamber. After measuring Ψ_{leaf}, leaf xylem sap was collected at 0.5 MPa overpressure and kept in liquid nitrogen or on dry ice. From the same plant tissue samples of young, developing leaves were taken, directly put into liquid nitrogen or kept on dry ice until storage at -80°C.
Weekly growth measurements included canopy height, stem length, number of leaves on the main stem and leaf length, leaf width and petiole length of leaf 10 (counted from the bottom) on three marked plants per plot.

3.1.2. Additional measurements 2019

Two weeks after full emergence, leaf gas exchange was measured (LI-6400 Portable Photosynthesis System, LI-COR, Lincoln, USA) on three plants per plot to determine possible treatment differences. In the same week, plant diameter of 3 plants per plot was taken to estimate plant growth (Figure 2) and penetrometer resistance to a depth of 100 cm was measured in each plot.

Figure 2: Plant size in uncompacted, well-watered treatment (control) two weeks after full emergence in May 2019. Extended tape is 35 cm.

3.2. Controlled environment experiments

Temperature:
Day: 22-25 °C
Night: 18-20 °C

Daylength: 12-14 hours

3.2.1. Drought stress experiments

3.2.1.1. Impact of strigolactones on stomatal conductance

Seed potatoes (*Solanum tuberosum* L.) of the variety ‘Desiree’ (hereafter called wild-type, WT) and its transgenic lines were kindly provided by Colin Turnbull of Imperial College London. The used lines were a DWARF14 knock-out obtained using a CRISPR-Cas9 construct (hereafter called d14), a CCD8-silenced line using a RNAi construct (hereafter called ccd8, Pasare *et al.*, 2013) with approximately 80 % reduction in gene expression (Pasare *et al.*, 2013) and a DWARF53- silenced line using a RNAi construct (hereafter called d53) with approximately 60 % reduction in gene expression. The transgenic lines were compared to the wild type (WT) with the respective empty vector construct. In a preliminary experiment the WT and the two empty vectors behaved similarly as the soil dried.

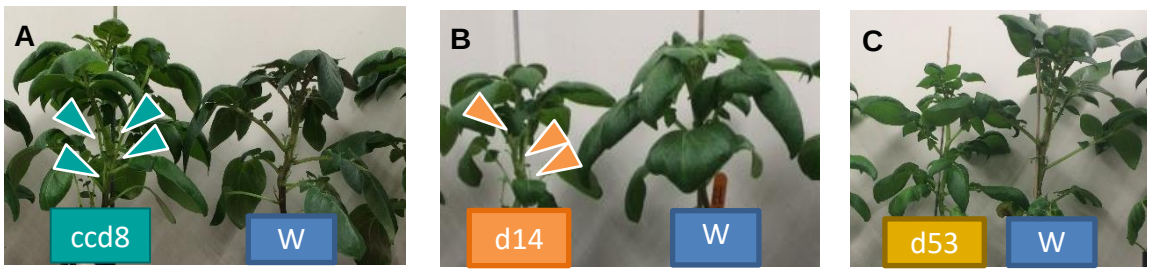

Figure 3: Phenotypes of strigolactone mutants in potato 'Desiree' and the wildtype (WT) in each of the experiments. Arrows indicate axillary outgrowth. (A) ccd8 mutant with axillary outgrowth, WT without outgrowth, (B) d14 mutant with axillary outgrowth, WT without outgrowth, (C) d53 mutant and WT with axillary outgrowth.

Three experiments under similar conditions were carried out. In each experiment, one transgenic line was compared to the WT (
Figure 3). Tubers were planted at 5 cm depth into cylindrical pots (9 cm diameter x 25 cm height) filled with standard potting compost (John Innes No. 2, Westland Horticulture Ltd, Huntingdon, United Kingdom). Plants were then grown in a controlled environment at 14-hour daylength (LED, B100 Valoya, Helsinki, Finland, 250-300 µmol m^{-2} s^{-1}) and 18/22 C (night/ day temperature). The first stem that emerged was retained, but all subsequent stems were excised to ensure uniform, single-stemmed plants that could be inserted in a pressure chamber to measure root water potential. All pots were watered every second day (with tap water) until emergence. After emergence, plants were watered daily with nutrient solution (Miracle-Gro®, half strength, Scotts Miracle-Gro Company LLC, Marysville, USA) to field capacity. When the plants reached 5-7 leaf stage (approximately four weeks after planting), water was withheld from half the plants (drought stressed, d) for seven days while the remainder were watered as described above (well-watered, ww).

Whole plant evapotranspiration was estimated by placing the pot on a balance at 1-hour intervals prior to other measurements. Stomatal density was measured from leaf imprints from leaflets of the youngest fully expanded leaves as previously described (Weyers and Johansen, 1985) using a Microscope with a 10x magnification ocular lens and 40x magnification objective lens (field of view = 0.0063 mm^2). Means were calculated for n = 5 plants using 5 leaflets per plant and 3 fields of view per leaflet. Stomatal conductance (g_s) of the abaxial surface of the youngest fully expanded leaf of four plants per treatment and day was measured using a transient time porometer (Model AP4, Delta-T Devices, Burwell, UK). Subsequently a young, still expanding leaf of the same plant was harvested and immediately frozen in liquid nitrogen for subsequent hormone analysis. These four plants per treatment were harvested each day (10.00am – 5.00pm) to measure leaf and root water potential (Ψ_{leaf} and Ψ_{root}, respectively) using a Scholander-type pressure chamber, as well as leaf area and fresh mass of the above ground plant parts. After measuring root water potential, 0.3 MPa additional pressure was applied to the root system to collect root xylem sap for 2 minutes. Abscisic acid (ABA) concentration of root xylem sap was determined by radioimmunoassay (Quarrie *et al.*, 1988). The xylem sap of *S. tuberosum* does not present nonspecific interference in the assay (Liu *et al.*, 2005). Soil moisture was measured concurrently (ML3 Theta-Probe, DeltaT Devices, Burwell, UK), by averaging measurements at the top and bottom of each pot.

3.2.2. Diurnal tuber volume and water relations

Potatoes (*Solanum tuberosum* cv. 'Maris Piper') were grown in cylinders of 40 cm height, 11.2 cm inner diameter and 3.9 L volume. The cylinders were filled until 9 cm below the edge to a bulk density of 1.5 g/cm^3 with demagnetized sandy loam and 200 ml fertilizer solution added (5 % Hakaphos Rot, content: 8 % N (5 % NO_3, 3 % NH_4), 12 % P_2O_5, 24 % K, 4 % Mg, 0.01 % B, 0.02 % Cu, 0.05 % Fe, 0.05 % Mn, 0.001 % Mo, 0.02 % Zn). Seed potatoes (TLC potatoes, Banchory, Scotland) were then placed on the settled soil and the pots were filled with loose soil until 2 cm below the edge. Plants were grown in a controlled environment at 14-hour light period (500 - 600 µmol*m^{-2}*s^{-1} at plant level, white LED, Cree LED, Durham, NC, USA), 20/ 16 °C day/ night temperature and 56 - 66 % relative humidity, RH (setpoint 60 %) at Forschungszentrum

Jülich, Germany. Every 2-3 days, pots were weighed to determine water loss (evapotranspiration) and re-watered to approx. 20 % soil water content. Plants emerged 11 to 18 days after planting. When they were 30 cm high, the apical bud was removed to limit height growth and therefore damage from robot handling during the experiment. Six weeks after planting (4 weeks after emergence), water was withdrawn from 4 plants for 2 days which were re-watered on the 3rd day, while 4 plants were watered daily with 150 ml from the top after the second block of physiological measurements. This irrigation volume was the mean daily water loss by evapotranspiration calculated over the week prior to the experiment. Drought stressed plants were re-watered basally by being placed in a tray of water for 20 min to ensure sufficient water uptake and prevent water loss through drainage caused by watering from the top after soil drying had decreased its water holding capacity.

Physiological measurements started 2 hours after supplementary lights were switched on and were carried out 30 minutes before each plant underwent MRI measurements in the morning and afternoon. Whole plant transpiration rate was measured by weighing the plants at 30-minute intervals. Two pots with soil, but without a plant, were weighed at a 4 h interval to estimate the evaporation rate from the soil without plant. Whole plant transpiration rate is estimated from the difference between evapotranspiration and soil evaporation. Furthermore, stomatal conductance and photosynthesis rates of a young, fully expanded leaf were measured using an infrared gas analyser (LI 6400XT, LI-COR, Lincoln, USA) with 1500 µmol PAR (10 % blue), 400 ppm CO_2, 300µmol/s flow rate, a Block Temperature of 23 °C and 50 % RH inside the cuvette. In the morning measurements (8.00 am – 11.30 am), the same leaf was measured as the previous afternoon. After leaf gas exchange measurements, one leaflet of this leaf was sampled and directly frozen in liquid nitrogen for subsequent ABA radioimmunoassay (Quarrie *et al.*, 1988). The apical three leaflets of the same leaf were subsequently used to measure leaf water potential with a pressure chamber (Plant Water Status Console, Soil Moisture Equipment Corp., Santa Barbara, USA). Fresh weight, dry weight and leaf area of the same leaflets were measured. On day 4, only the morning measurements were taken and thereafter whole plant fresh weight, dry weight and leaf area were measured. Fresh weight and dry weight of all tubers with a diameter larger than 5 mm were measured.

Soil moisture was measured twice daily at 5, 20 and 35 cm below the soil surface using a simplified soil water profiler (SWaP, see van Dusschoten *et al.*, 2020) version at 180 MHz and with a Span of 60 MHz (Figure 11). The 5 cm measurement includes the tubers as indicated in Figure 11, Table 1 and therefore shows generally higher values than the lower two measurements. Mean soil moisture was calculated as the mean of three measurements (top, middle, bottom) per pot and timepoint.

Figure 4: Plants positioned in the holding magazine (A). The passing of plants from the rear when being moved into the magnet in the front can cause leaf damage, hence plants were wrapped in foil overnight (B).

MRI measurements were taken every 4 hours over 4 days and nights with a dedicated 4.7T magnet (Magnex Scientific Ltd., Oxford, England) with 400 $mT*m^{-1}$ gradient coils (MR Solutions, Guildford, England) and a 14 cm RF-coil (Doty Scientific, Columbia, SC, USA). Measurement setup and image processing were similar to that described (van Dusschoten *et al.*, 2016), but with the following adaptations (FOV = 132x132 mm, slice thickness was 1.2, Echo Time (TE) = 10 ms, image matrix = 256x256 using 2 averages). A 5 mm tube filled with a 5 mM $NiCl_3$ solution was inserted into the soil as internal reference to correct for non-biologically relevant signal fluctuations. Each measurement took about 30 min. A handling robot (MiniLiner 3.0, Geiger Handling GmbH & Co. KG, Jülich, Germany) picked the pots from a holding magazine and placed them into the magnet for measurements (Figure 4A). To prevent mechanical damage due to handling eight plants in a confined space, all plants were wrapped in foil from three sides during the night (8pm – 6am) (Figure 4B). In the morning, the foil was removed, and the plants were placed under a light panel (500 $\mu mol*m^{-2}*s^{-1}$, 14h/day, white LED, Cree LED, Durham, NC, USA) 5 m away from the magnet to keep environmental conditions similar to the measurement conditions. In the daytime plants were individually placed into the magazine shortly before the measurement.

To visualise images, the software package Mevislab (version 2.2.3, MeVis Medical Solutions, Bremen, Germany) was used. With this software, single tubers were separated from noise, roots and other tubers by setting intensity thresholds and distance thresholds for a region of interest with visual control of the generated mask. From the generated masks, tuber volume and water content (signal intensity) for each tuber and timepoint were extracted. Tuber volume and water content per unit volume (voxel) were normalised to the initial value before further analysis.

Image visualisation and analysis used the software package Mevislab (version 2.2.3, MeVis Medical Solutions, Bremen, Germany) to separate single tubers from noise, roots and other tubers as follows: First a binary mask is generated using a signal intensity threshold, with a binary closure completing any holes in this mask. To discriminate touching tubers and to remove roots, stolons and stems, this mask is eroded by a manually chosen distance. The mask within the tuber of interest is then selected manually and dilated by the previously selected distance to regain the original shape. As a final step, parts of the dilated volume which are closer to a non-selected part of the mask are removed. From these generated masks, tuber volume and water content (signal intensity) for each tuber and timepoint were extracted and normalised for temperature effects using a water filled reference tube. Tuber volume and water content per unit volume (voxel) were normalised to the initial value before further analysis.

Total signal intensity ≡ FW-DW

For statistical analysis and graphs, the software package R (Version 4.0.3, 2020, The R Foundation for Statistical Computing, Vienna, Austria) was used. Tubers were treated as pseudo-replicates, while plants were real replicates when calculating treatment means. Two-way repeated measures ANOVAs were carried out to evaluate the effects of drought stress and time after starting the treatment on all repeatedly measured variables. Assumptions of independent and identically (as Normal) distributed data were met. The assumption of sphericity is necessarily met because the factor drought stress has only 2 levels. For normalised tuber water content and normalised tuber volume, the start of the experiment (-5 hours) is not considered in the statistical analysis since all values were equal (=1). If significant differences occurred in the repeated measures ANOVA, pairwise comparisons were made using plant mean values (averaging across all tubers of one plant) for single time points.

3.2.3. Soil compaction experiment

Mineral soil (Norfolk Topsoil, Bailey's of Norfolk, Norwich, UK) in cylindrical pots (9 x 25 cm) was compressed using a torque wrench. For the compaction treatments, soil of a gravimetric soil water content of 0.05 $g*g^{-1}$ (low compaction) or 0.17 $g*g^{-1}$ (high compaction) was filled into the pot to a height of 3 cm and compressed with a force of 10 Nm. This process was repeated until the pot was filled to 5 cm below the top, resulting in a bulk density of 1.5 $g* cm^{-3}$ and 1.75 $g*cm^{-3}$ (low and high compaction respectively). For the control the same process was carried

out with dry soil, resulting in a bulk density of 1.4 g*cm^3 (uncompacted). ‘Maris Piper’ tubers of the size 20 – 30 mm were planted on the surface of the soil (following compression) and covered with 50 ml loose soil. After emergence, each pot was individually watered every second day to a water content of 1.8 g*g^1, using nutrient solution (Miracle Gro ®, half strength, Scotts Miracle-Gro Company LLC, Marysville, USA). Pot weights before watering were recorded daily to determine whole plant transpiration.

Ten weeks after emergence, two or six plants were harvested as described in Section 3.1.1. Stomatal conductance of the youngest fully expanded leaf was measured using a transient time porometer (Model AP4, Delta-T Devices, Burwell, UK). To collect root xylem sap, plant transpiration rate was estimated prior to gas exchange measurements by placing on a scale for 30 min. After measuring root water potential, additional pressure was applied to the root system (0.22 – 1.1 MPa) to collect root xylem sap at transpiration flow rate for 2 minutes.

3.3. Statistical Analysis

Statistical analysis was carried out using the software R (R Core Team, Vienna, Austria). For the field experiments two-way ANOVAs (for main effects of compaction, irrigation and their interaction) were carried out separately for every day, with Tukey’s HSD to determine significant differences between treatments. Regression lines were estimated using linear models.
Three-way ANOVAs (for main effects of genotype, treatment, measurement day and their interactions) determined the impact of strigolactones on stomatal conductance for each experiment (individual comparison of each genotype with the wildtype).
For the experiments on tuber volume and water relations tubers were treated as pseudo-replicates, while plants were real replicates when calculating treatment means. Two-way repeated measures ANOVAs were carried out to evaluate the effects of drought stress and time after starting the treatment on all repeatedly measured variables.

4. RESULTS

4.1. Field experiment

4.1.1. Plant Growth

The compaction treatment in 2019 did not result in significant soil compaction. Hence, the results for 2018 are shown. These results are published as an open access article in *Annals of Applied Biology* (Huntenburg *et al.*, 2021). Plants emerged 3 days earlier in uncompacted soil that in compacted soil and growth rates were lower than in the uncompacted treatment (Figure 5). Irrigation treatments commenced from calendar week 24 in 2018 with immediate effect on growth rates (Figure 5). Thus, soil compaction and deficit irrigation as single stresses significantly reduced plant growth in the beginning of the season and delayed achieving full ground cover (Figure 5). This reduced growth was a result of a lower number of leaves emerging and a lower leaf expansion rate (Figure 6).

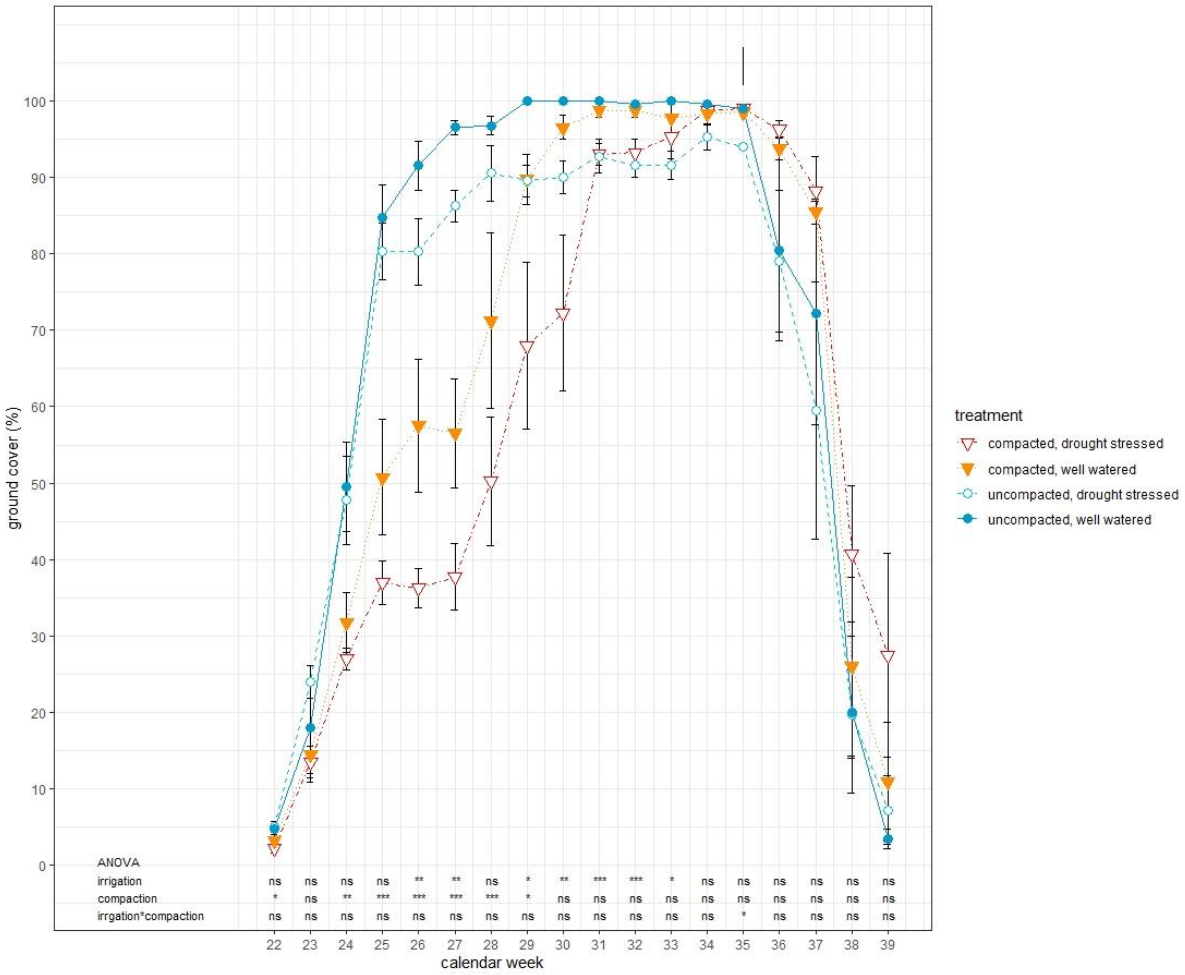

*Figure 5: Weekly ground cover of field grown 'Maris Piper'. Means ± SE of four plots per treatment with residual degrees of freedom df = 12. LSD (5%) for week 35 is given (black vertical line), because of significant interaction in this week. Statistical significance of irrigation, compaction and their interaction reported each week with: ns = not significant P > 0.05; * P < 0.05, ** P < 0.01, *** P <0.001.*

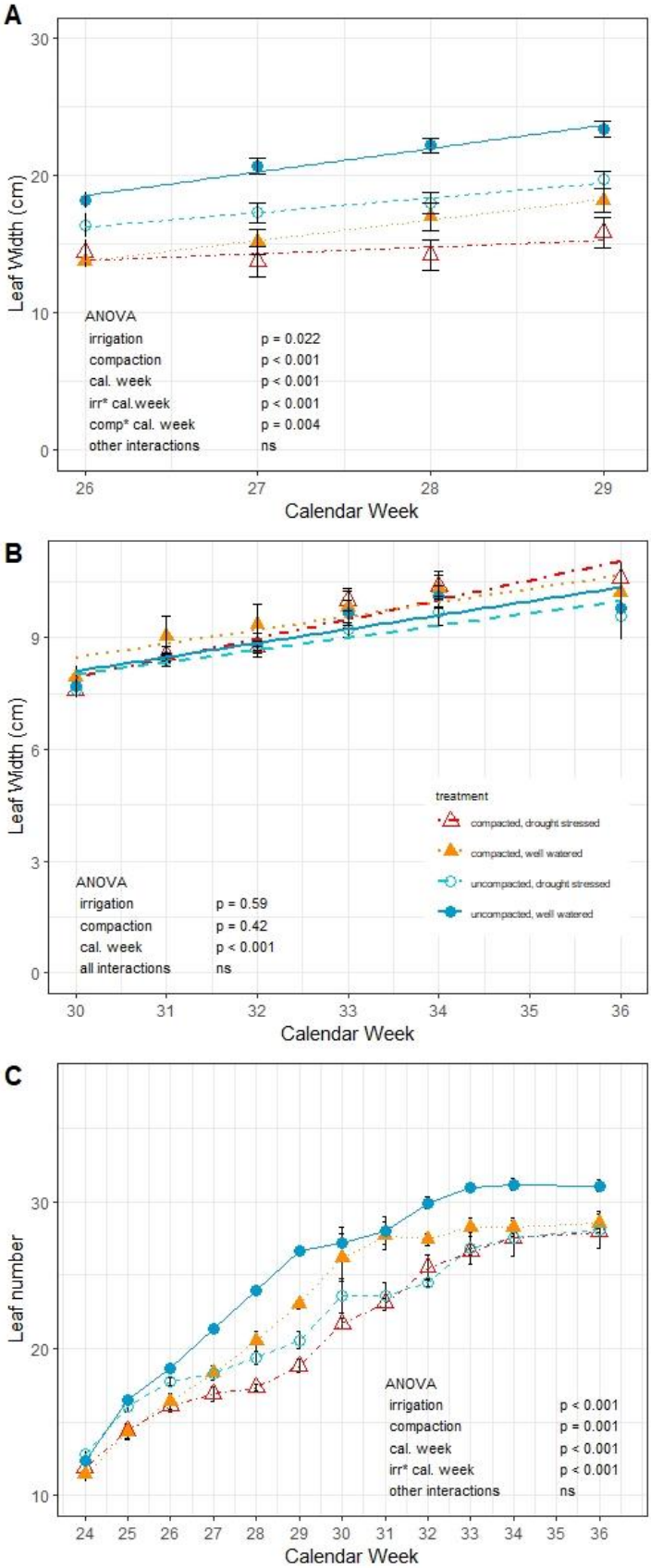

Figure 6: Leaf width in the first half (A) and the second half (B) of the season and weekly leaf number (C) of field grown 'Maris Piper'. Means ± SE of 12 plants per treatment (and measurement date) with residual degrees of freedom df = 12. Regression lines were fit using linear models.

4.1.2. Plant physiological parameters

To investigate whether differences in carbon gain (photosynthesis) affect shoot growth, leaf gas exchange was measured. Surprisingly, stomatal conductance and photosynthesis rates did not differ between treatments on most measurement dates (Figure 7A and B). Stomatal conductance (to water vapour) is linearly correlated with transpiration per unit leaf area. Irrigation as a main effect only had a significant impact in week 29, with deficit-irrigated plants having lower stomatal conductance and photosynthesis rates than well-watered plants ($p < 0.05$ for both parameters, Figure 7A and B). At this time, irrigation had been suspended for 17 days in the deficit irrigation treatments (Figure 7). In the last measurement week, both stomatal conductance and photosynthesis rates approximately halved as the canopy senesced. Thus, adverse soil conditions restricted shoot growth, but photosynthesis and transpiration per unit leaf area (stomatal conductance) were only affected after a prolonged period without irrigation.

As leaf gas exchange did not differ between treatments, plant water status was also measured. For pre-dawn and daytime leaf water potential, generally there were no treatment differences (Figure 8). In week 26, pre-dawn leaf water potential was measured the night before and the night after irrigating the well-watered plots. Before irrigation, pre-dawn water potentials were similar, but after irrigation the pre-dawn water potential of the well-watered plants was significantly higher ($p < 0.0001$, $F_{(1,10)} = 33.96$), by 0.05 MPa than of the drought-stressed plants. In week 29, drought-stressed plants had not been irrigated for 17 days when measured pre-dawn and soil moisture had been low for this period in drought-stressed treatments (Figure 8). Hence, the difference in pre-dawn water potential between irrigation treatments in this week is a result of low water availability in drought stressed plants. In week 30, all plots were irrigated the day before measurements. Thus, there was a clear response in pre-dawn water potential to an irrigation event in well-watered plants, but a decrease in drought stressed plants only became apparent after a prolonged period at low soil moisture. In week 32, compacted treatments had higher pre-dawn water potentials than uncompacted treatments. Daytime leaf water potential did not differ between treatments throughout the whole season, with values between -0.9 and -1.4 MPa (Figure 8B). Thus, pre-dawn leaf water potential better discriminated the treatments than daytime leaf water potential.

To understand hormonal responses, leaf tissue ABA levels were measured from samples taken directly after gas exchange measurements. In week 26, samples were taken the day after well-watered plants were irrigated for the first time and ABA levels in drought stressed plants were 60 % higher than in well-watered plants with mean values of 758 ng ABA*g^{-1} DM and 473 ng ABA*g^{-1} DM for drought-stressed and well-watered treatments, respectively (Figure 7C). On all following measurement dates, no treatment differences were detected (Figure 7C). Drought-stressed treatments had the highest values in weeks 26 and 35. Comparing the treatments via repeated measures ANOVA showed no significant impact of any factor, likely due to the high variability of the data. Thus, any treatment differences in leaf ABA concentration were transient and not maintained through the growing season.

Tuber yield

Reduced plant growth and therefore lower biomass at full ground cover was correlated with lower tuber yield per hectare (Figure 9). Soil compaction and drought decreased the yield by 31 % and had synergistic effect of co-occurring stresses (Figure 10A). These findings illustrate that drought stress and soil compaction substantially decrease yield and that a large proportion of this variation can be explained by canopy growth ($R^2 = 0.71$,Figure 9).

Interestingly, significant differences in tuber size distribution were observed between irrigation treatments, but not between compaction treatments. Drought-stressed plots had more tubers between 30 mm and 50 mm and fewer tubers >60 mm ($p < 0.05$ for all comparisons) than well-watered plots (Figure 10B). Total tuber number was also higher in drought-stressed treatments than in well-watered treatments (28 tubers per m^2 vs. 24 m^2 tubers respectively, $p = 0.036$, $F_{(1,12)} = 5.57$). Hence, lower yield of the drought-stressed treatments resulted from smaller, rather than fewer, tubers.

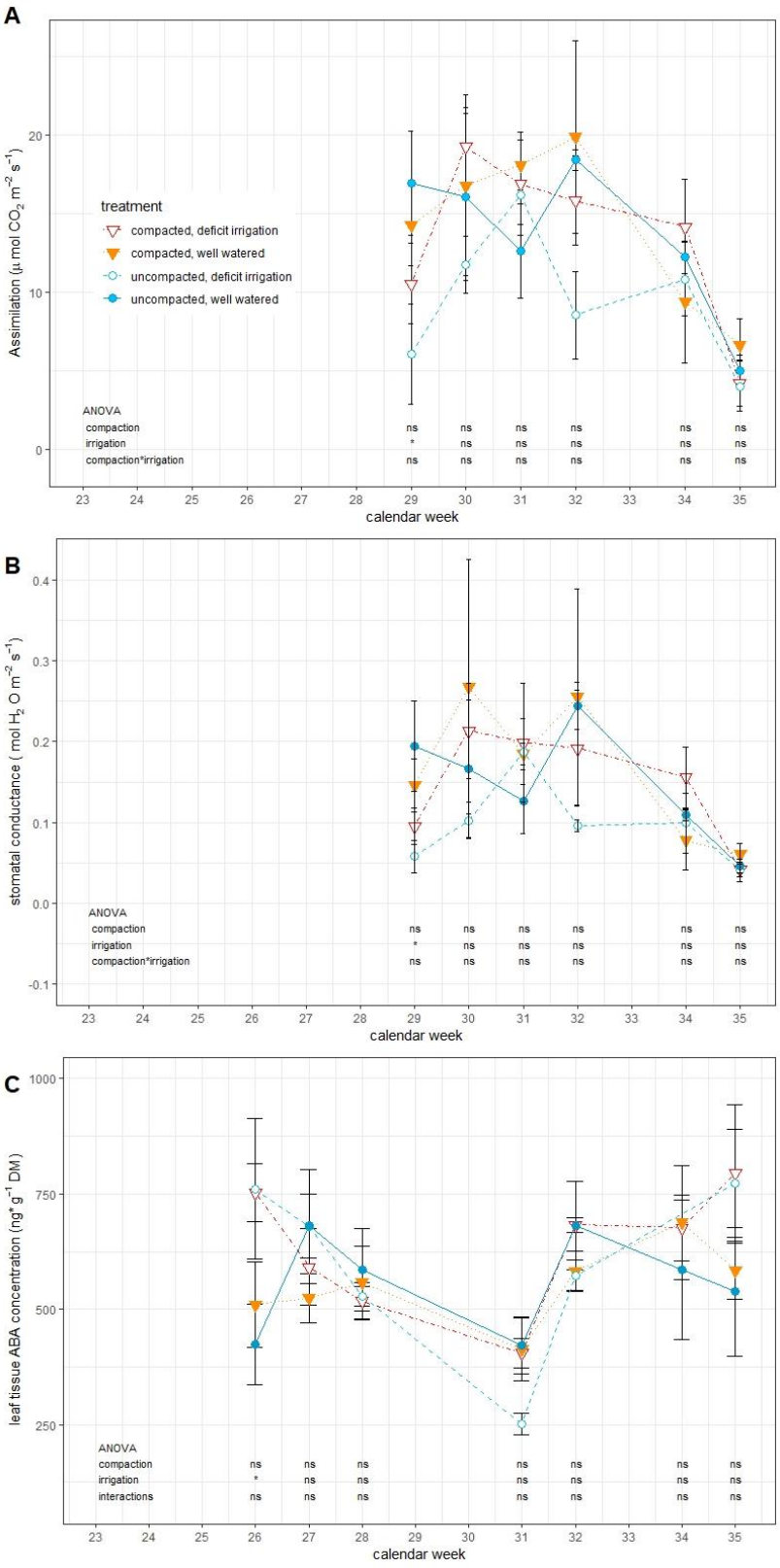

*Figure 7: Stomatal conductance (A), photosynthesis rates (B) and leaf tissue ABA levels (C) measured in field grown Maris Piper under different compaction and irrigation treatments. Means ± SE of 4 plants per treatment and measurement day with residual degrees of freedom df = 8 for stomatal conductance and photosynthesis rate and df = 6 for leaf tissue ABA levels. Asterisks indicate significant differences between treatments on a distinct day with: ns = not significant P > 0.05; * P < 0.05, ** P < 0.01, *** P <0.001.*

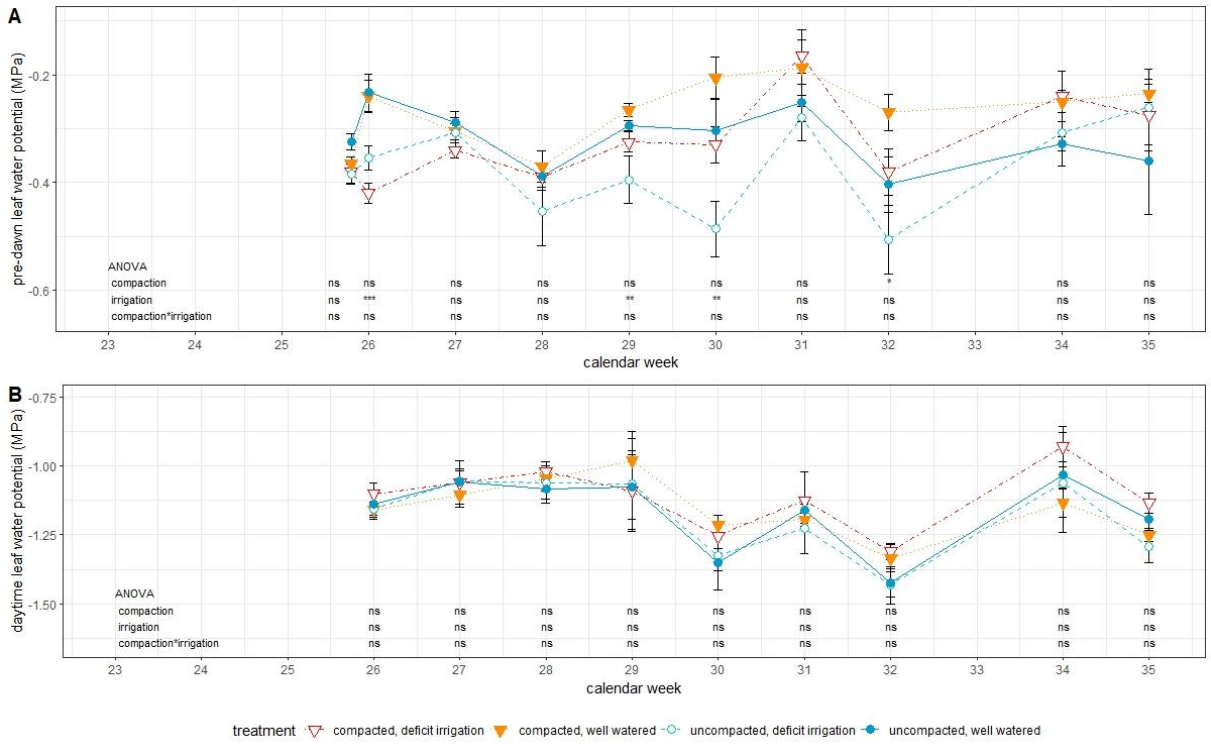

*Figure 8: Development of pre-dawn (A) and daytime (B) leaf water potential of potato 'Maris Piper'. Means ± SE of 4 plants per treatment and measurement day with residual degrees of freedom df = 10. Asterisks indicate significant differences between treatments on a distinct day with: ns = not significant P > 0.05; * P < 0.05, ** P < 0.01, *** P < 0.001.*

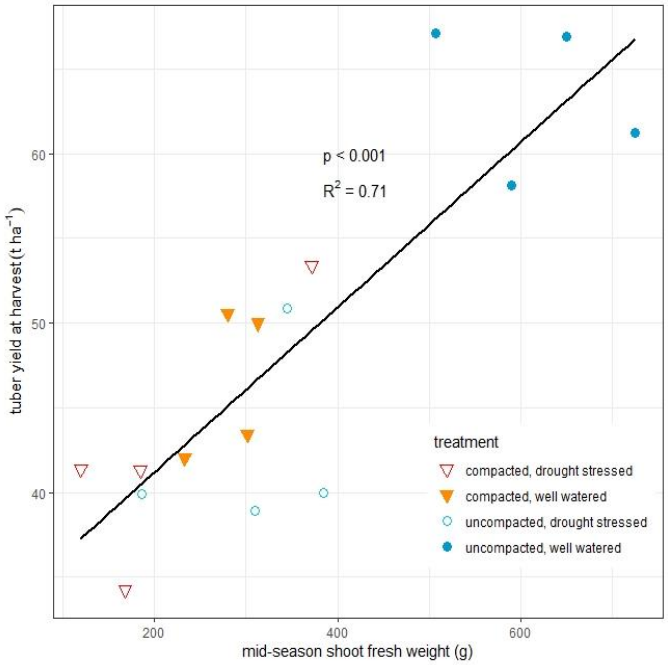

Figure 9: Relation between biomass at full ground cover (measured in calendar week 31) and final yield for field-grown 'Maris Piper'. Each data point represents one plot with three plants harvested for above ground biomass and yield calculated as in from 10 plants harvested per plot (2.5m²). Regression line was calculated using a linear model (y = 0.05 (± 0.0008) x + 31.42 (± 3.24)). Error bars omitted for clarity, residual standard error = 5.7 on 14 degrees of freedom.

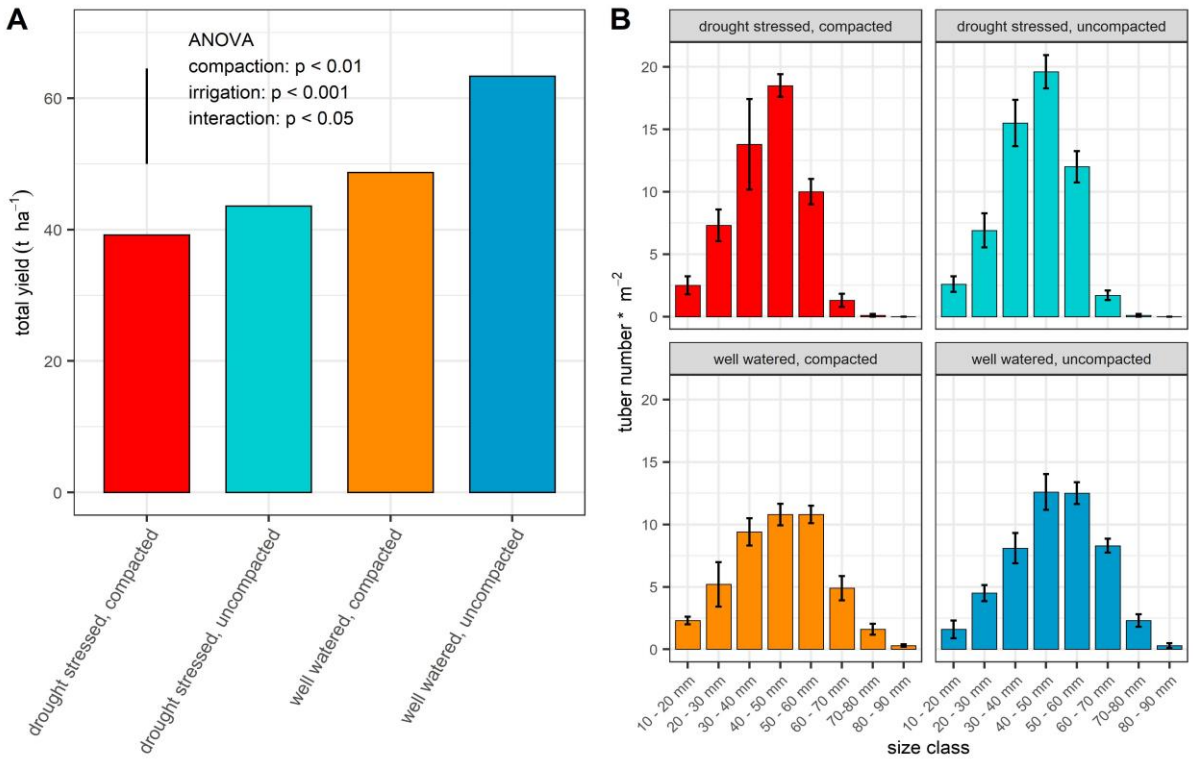

Figure 10: Total yield (A) and tuber size distribution (B) of field grown 'Maris Piper'. Means (± SE) of four plots per treatment, yield calculated and tubers counted of 10 plants per plot (2.5m²). Vertical line in (A) shows LSD (5%), residual degrees of freedom = 12.

4.2. Controlled environment experiments

4.2.1. Drought stress experiments

4.2.1.1. Impact of strigolactones on stomatal conductance

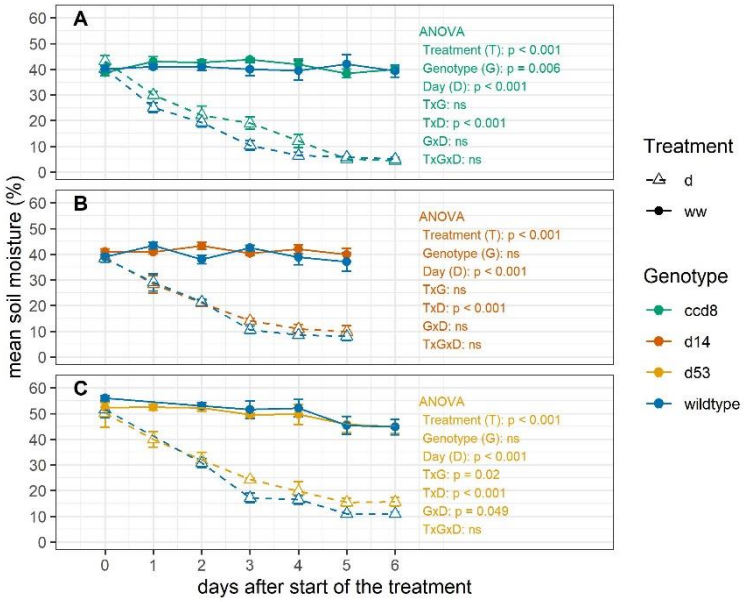

Figure 11: Soil moisture of well-watered (filled circle) and drought stressed (open triangle) potato plants in three strigolactone (SL) mutants compared to the wildtype. Mean ± SE of 4 plants per treatment, genotype and measurement day. P Values from ANOVA reported in each panel.

Daily irrigation maintained mean soil moisture at high levels in well-watered plants. Withholding water continuously decreased soil moisture throughout the measurement period in drought stressed plants of all genotypes (Figure 11). The d14 line did not show any differences in soil drying to the wildtype (Figure 11A). The ccd8 line had a higher soil moisture on day 3 after the start of the treatment, however the values did not significantly differ between genotypes in the following days (Figure 11B). The drought stressed plants of d53 showed a higher soil moisture on day 3 after the start of the treatment than the drought stressed plants of the WT, but values were similar in both genotypes before and after this day (Figure 11C). Thus, genotypes that were compromised in either SL biosynthesis or signalling dried the soil at comparable rates to the WT.

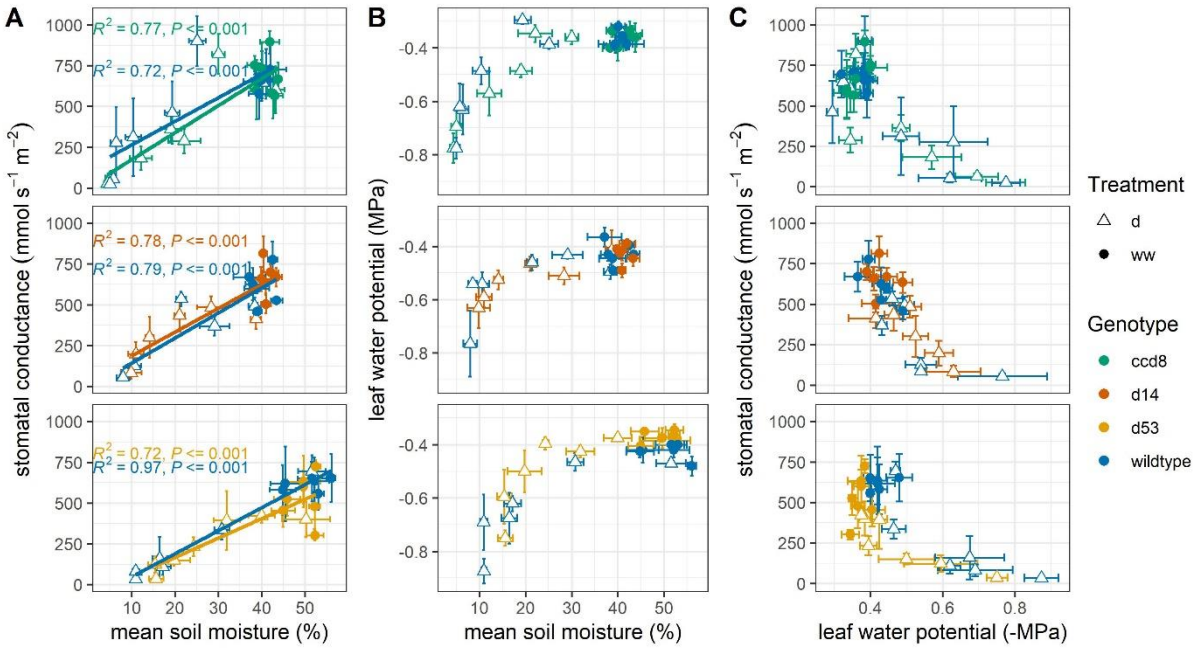

Figure 12: Correlation between stomatal conductance and soil moisture (A), leaf water potential and soil moisture (B) and stomatal conductance with negative leaf water potential (C) for well-watered (filled circle) and drought stressed (open triangle) potato plants in three strigolactone (SL) mutants compared to the wildtype. Each point represents mean ± SE of 4 plants per treatment, genotype and measurement day.

Stomatal conductance decreased linearly with soil moisture in all genotypes (Figure 12A). Leaf water potential was maintained at -0.4 MPa at 20 - 50 % soil moisture (Ψ_{soil} = -0.3 - -0.1 MPa) and declined rapidly at < 20 % soil moisture (Ψ_{soil} < -0.3 MPa) in all genotypes (Figure 12B). Stomatal conductance declined with leaf water potential as the soil dried, but similarly in the transgenic lines and the WT ($p > 0.05$ for all individual comparisons). In well-watered plants (Ψ_{leaf} > -0.4 MPa), stomatal conductance was not correlated with leaf water potential (Figure 12C, R^2 = 0.13). Stomatal conductance decreased linearly with Ψ_{leaf} at < -0.45 MPa in all genotypes (Figure 12C, R^2 = 0.52). There was no significant difference between genotypes in the response of stomatal conductance to leaf water potential ($p > 0.05$ for all individual comparisons). Thus, leaf water relations of all genotypes showed consistent responses to soil drying.

Stomatal density on the abaxial side of the leaf was similar in the wildtype and the SL hypersensitive genotype (d53), but lower than the WT in the SL deficient (ccd8) and insensitive (d14) genotypes (Figure 13A). Adaxial stomatal density was similar between the WT and the SL deficient (ccd8) and SL insensitive (d14) genotypes (Tukey test, $p < 0.01$), while the hypersensitive genotype (d53) showed a higher stomatal density than all three other genotypes (Figure 13B). However, adaxial stomatal density was 78 % lower than the abaxial stomatal density for the WT and d53 and 85 % lower than the abaxial stomatal density for ccd8 and d14, respectively. Thus, abaxial stomatal density potentially has a stronger effect on whole plant transpiration.

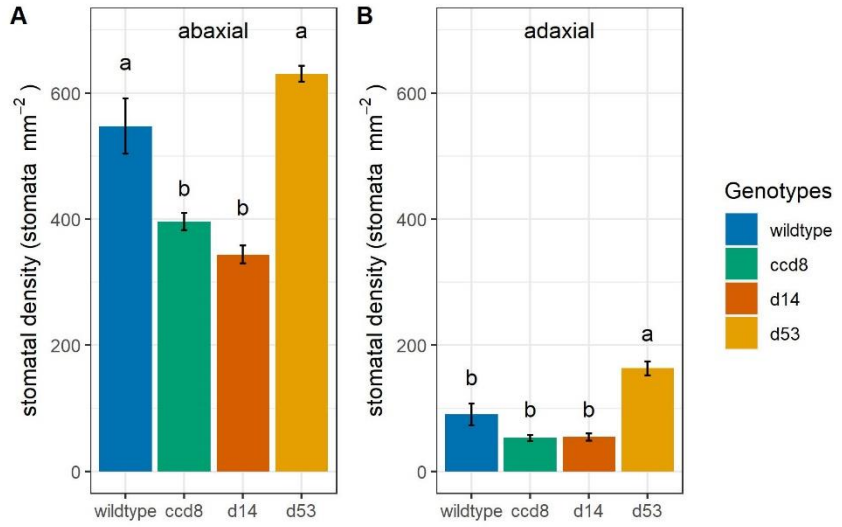

Figure 13: Number of stomata on the abaxial (A) and adaxial (B) side of the leaf. Means ± SE of 5 plants (n = 5) with 5 leaflets per plants measured at 3 different points. In each panel, different letters represent significant differences according to Tukey Test (p < 0.01)

Root xylem sap ABA concentration was similar for the ccd8 silenced line and the wildtype ($p > 0.05$). In both genotypes the logarithm of the ABA content in the xylem sap increased linearly with decreasing soil moisture without difference between the slopes (Figure 14A). Thus, there was no difference in ABA export from the roots between the SL deficient line (ccd8) and the WT. Stomatal conductance declined with increasing ABA content (presented in log(pmol ml^{-1}) for clarity at lower concentrations) (Figure 14B). Slopes of the regression lines did not differ between genotypes ($p > 0.05$, Figure 14B). Hence, the SL deficient line (ccd8) and the WT show similar stomatal responses to increased ABA levels.

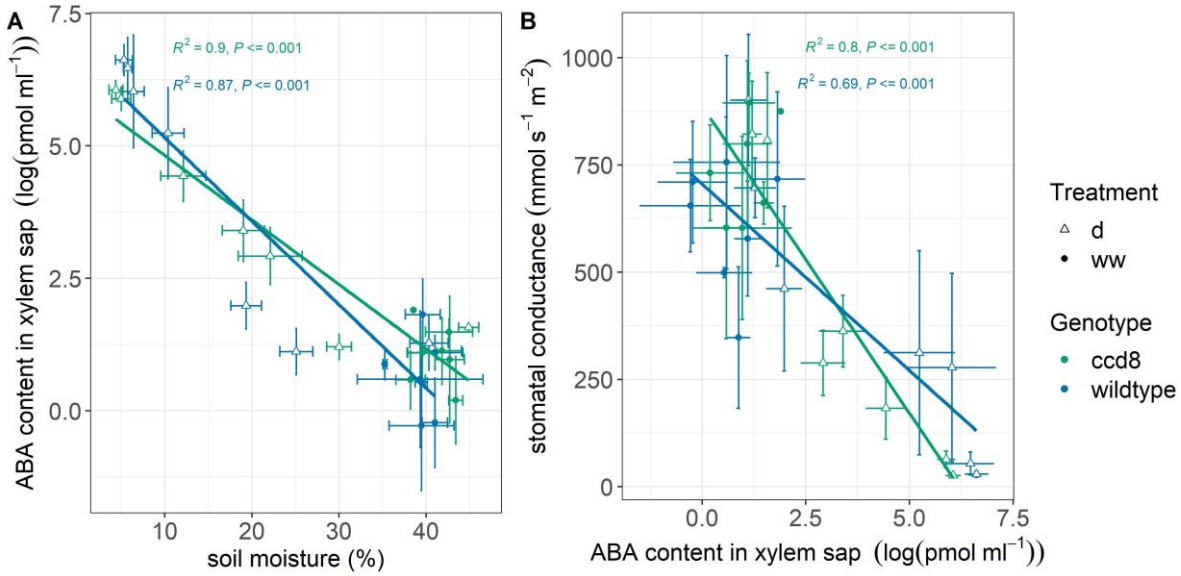

Figure 14: Relation between (A) soil moisture and logarithm of root xylem sap ABA concentration and (B) logarithm of root xylem sap ABA concentration and stomatal conductance in well-watered (closed circle) and drought stressed (open triangle) plants of the wildtype (blue) and the ccd8 silenced line (green). Each point represents Mean ± SE of 2-4 plants per treatment, genotype and measurement day.

4.2.2. Diurnal tuber volume and water relations

Mean soil moisture was similar in both treatments at the beginning of the experiment and was maintained between 8.2 - 11.3 % for well-watered plants (Ψ_{soil} = -0.07 – -0.03 MPa respectively). These values did not significantly differ over time (1-way ANOVA, p = 0.057). Nineteen hours after start of the treatment (HAS), soil moisture of drought stressed plants was almost halved compared to well-watered plants (p < 0.001, Figure 15E). After re-watering the drought stressed plants, the soil held 3.8% and 5.4% more water than the well-watered plants at 47 HAS (p = 0.004) and 67 HAS (p = 0.0009) respectively. Hence soil moisture differed between the treatments during the drying period, and re-watering was sufficient to restore soil moisture in drought stressed plants.

Afternoon leaf water potential was similar between treatments on day 1 (-1 HAS) but was 0.24 MPa lower in drought stressed than well-watered plants on day 2 (23 HAS, p = 0.001). After re-watering, there were no significant treatment differences (p = 0.06 and p = 0.31 for 47 and 67 HAS respectively, Figure 15C). Similarly, soil drying decreased stomatal conductance and photosynthesis rates in drought stressed plants (Figure 15A and B), both of which recovered to well-watered levels upon re-watering (p = 0.56 and p = 0.62 for stomatal conductance and photosynthesis rate respectively at 47 HAS). Leaf tissue ABA content of drought stressed plants was 57% higher than well-watered plants on day 2 (t-test, p = 0.0011), but did not differ after re-watering (p = 0.056 and p = 0.31 for 47 HAS and 67 HAS respectively). Thus, soil drying decreased plant water status and gas exchange and increased foliar ABA concentration on day 2, but re-watering re-hydrated leaves and restored leaf gas exchange to the level of well-watered plants within the 3 h between re-watering at 44 HAS and the measurement at 47 HAS.

Since mean soil moisture was markedly higher in drought stressed plants after re-watering than in well-watered plants, moisture distribution in the pot was examined more closely. Moisture was always highest close to the soil surface (Figure 16), likely because tubers in that layer influenced the measurement. Treatment differences were obvious in the lower layers after 0 HAS, when well-watered plants were re-watered and had a higher soil moisture than drought stressed plants until drought stressed plants were re-watered (individual t-tests, p < 0.05, Figure 16B and C). After re-watering drought stressed plants from the bottom of the pot at 43 HAS, the water content at the bottom and at the top of the pot increased drastically, reaching values higher than in well-watered plants (Figure 16A and C), while the soil moisture in the middle of the pot remained lower than in the well-watered plants (Figure 16B). This suggests redistributed water from the soil at the bottom of the pot into the tubers at the top of the pot.

Relative tuber water content (water content per volume unit) fluctuated diurnally (Figure 17A), but differently in well-watered and drought stressed plants (RM ANOVA p = 0.01). In well-watered plants, relative tuber water content decreased significantly over the day until re-watering and then increased significantly overnight at a higher rate than it decreased in the day (Table 1). In contrast to well-watered plants, tuber water loss of drought stressed plants exceeds subsequent water uptake (Table 1). Re-watering increased the water content of drought stressed tubers, which was subsequently not significantly different from well-watered plants (47 – 63 HAS, p > 0.05). This confirms that some of the water taken up by the roots was indeed transferred into the tubers.

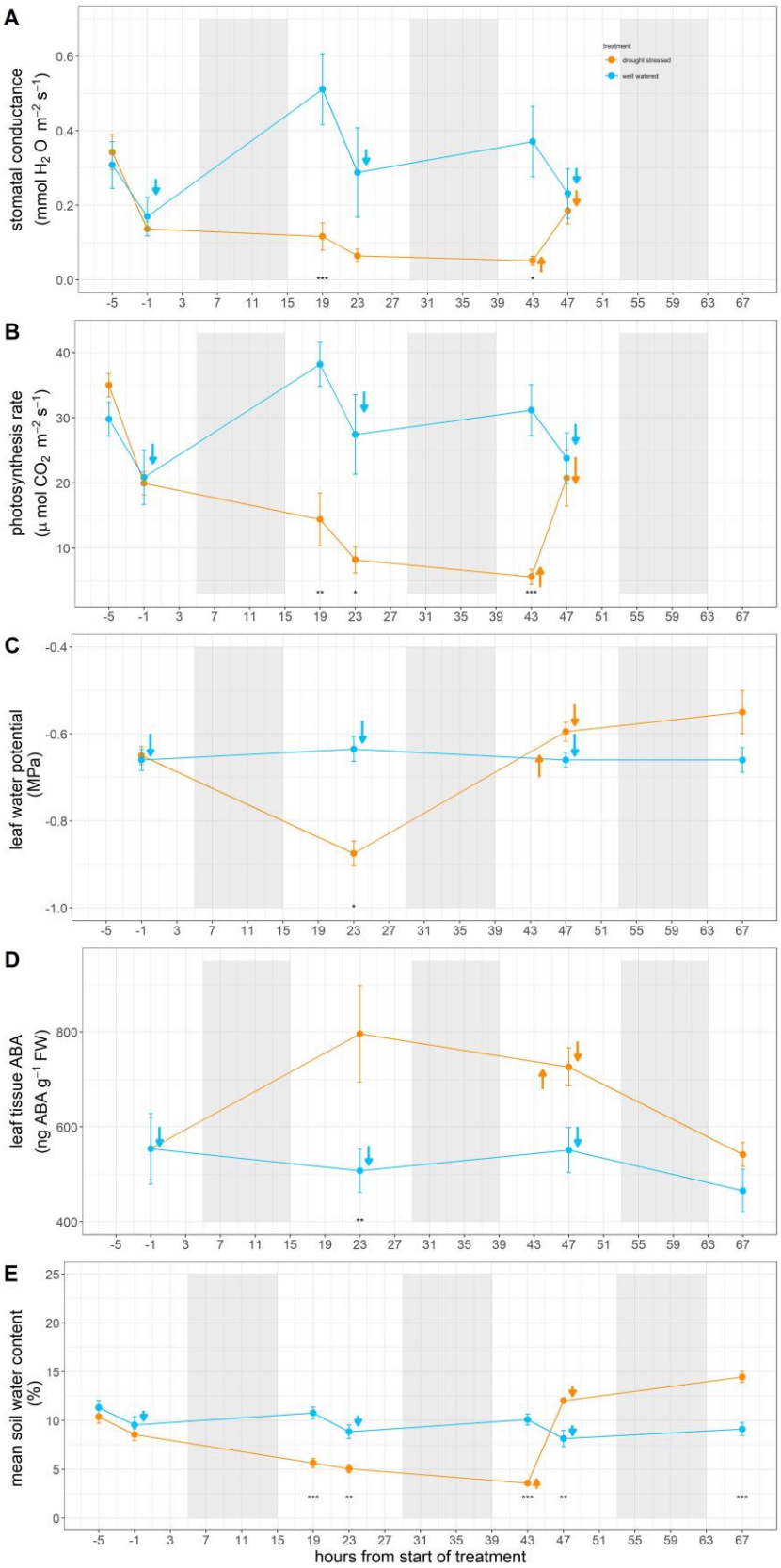

*Figure 15: Stomatal conductance (A), photosynthesis rate (B), afternoon leaf water potential (C), leaf tissue ABA content (D) and mean soil moisture (mean of 3 positions per pot) (E) of well watered (blue circles) and drought stressed (orange triangles) plants. Means ± SE of 4 plants per datapoint. Grey bars indicate hours without light. Asterisks indicate s Asterisks indicate significant differences between treatments at that timepoint according to t-test with * p < 0.05, ** p < 0.01, *** p < 0.001.*

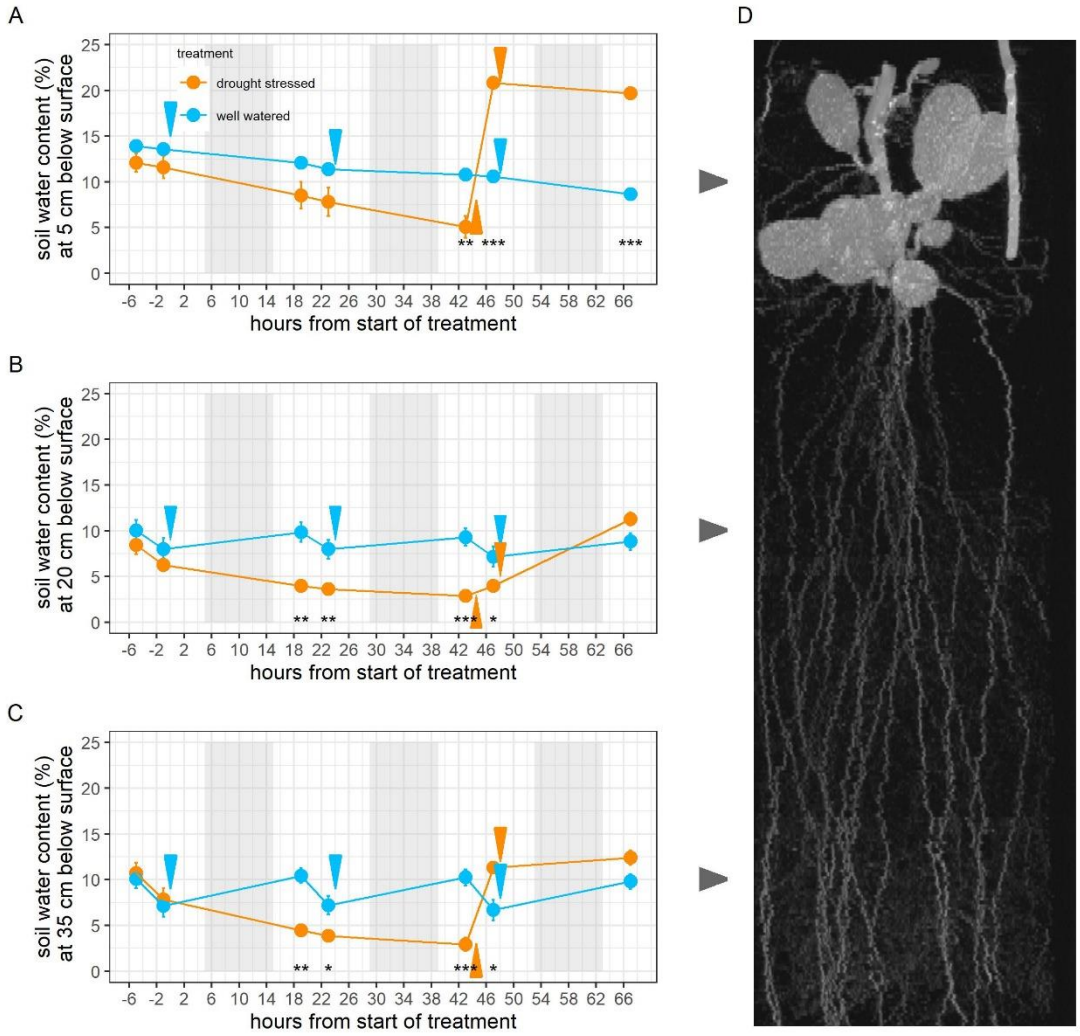

*Figure 16: Soil moisture of well watered (blue) and drought stressed (orange) plants measured at 5 cm (A, also contains tubers), 20 cm (B) or 35 cm (C) below the soil surface and image of root and tuber distribution of a sample plant (D). (A – C) Means ± SE of 4 plants per datapoint. Grey bars indicate hours without light, arrows indicate when plants were watered from the top (downward arrow) or the bottom (upward arrow) in the treatment corresponding to the colour. Asterisks indicate significant differences between treatments at that timepoint according to t-test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (D) Grey arrows at right indicate locations of soil moisture measurements.*

Table 1: Diurnal differences in relative tuber water content of well-watered and drought stressed plants.

	Well-watered			Drought stressed		
	Time (HAS)	∆ Rel. water content	t-test	Time (HAS)	∆ Rel. water content	t-test
Start of the experiment *Day*	-5	- 4.8 %	p = 0.0029 **	-5	- 8.0 %	p = 0.022 *
Lowest value day 1 *Night*	-1	9.5 %	p = 0.027 *	3	6.9 %	p = 0.086
Highest value day 2 *Day*	15	- 8.6 %	p = 0.033**	15	- 11 %	p = 0.023 *
Lowest value day 2 *Night*	23	8.5 %	p = 0.0101 *	27	9.3 %	p = 0.035 *
Highest value day 3 *Day*	39	-7.3 %	p = 0.062	39	- 5.8 %	p = 0.11
Lowest value day 3 *Night*	47	3.1 %	p = 0.17	43	7.3 %	p = 0.033 *
End of the experiment	63			63		

Asterisks indicate significant difference (∆) between lowest and highest values of the day according to t-test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

To understand water dynamics of the tubers, it is important to also consider changes in tuber volume over time. In well-watered plants, tuber volume increased by 11.6 % over 68 hours (ANOVA, $p = 0.0011$) and tubers were significantly bigger than drought stressed tubers from 3 HAS until 47 HAS (individual ANOVAs per time point, $p < 0.05$, Figure 17). Drying soil paused tuber volume growth (Tukey HSD, $p > 0.05$ for all individual comparisons until 43 HAS). Re-watering of the drought stressed plants substantially increased tuber volume, resulting in values that did not significantly differ from well-watered plants from 51 HAS onwards (individual ANOVAs per time point, $p > 0.05$). Thus, sufficient water supply is necessary to facilitate tuber growth. However, if plants are re-watered after a drought period as short as two days at soil water potentials ≥ -0.2 MPa, compensatory growth by tubers of drought stressed and re-watered plants can recover volume to the level of well-watered plants.

Total tuber water content is the product of the relative water content and the tuber volume. Hence, in tubers of well-watered plants, the water influx overnight (Figure 17A) together with the tendency of increased volume overnight (Figure 17B) leads to an average increase of 14 % more water being stored in the tubers during the whole experiment (ANOVA, $p = 0.012$; Figure 17). In drought stressed plants the decrease in relative tuber water content combined with no change in tuber volume before re-watering lead to a 11.5 % decrease in total tuber water content over the drought stress period (ANOVA, $p = 0.0095$; Figure 17C) and no significant change in total tuber water content between the beginning (-5 HAS) to the end of the experiment (63 HAS) (ANOVA, $p = 0.20$).

Diurnal rhythm

In addition to the changes in tuber water content and tuber volume over a drought stress period of two days and subsequent watering, there are diurnal patterns in relative and total tuber water content that suggest water (re-)distribution within the potato plant. Relative and total tuber water content decreased in both treatments throughout the day and increased overnight (Table 1, Figure 17A and C). For well-watered plants, this increase started after re-watering the plants every afternoon (Figure 17A and C). For drought stressed plants, the water loss continued until the end of the light period and relative tuber water content increased in the dark period without additional water (Figure 17A and C). Note that the rate of relative water loss from the tubers throughout the day is similar in well-watered and drought stressed tubers (Figure 17A, Day 2: -0.011 and -0.010 units per hour in well-watered and drought stressed plants respectively, $p = 0.91$) as is the rate of water uptake throughout the night (Figure 17A, Night1: 0.0031 and 0.0036 units per hour in well-watered and drought stressed plants respectively, $p = 0.85$; Night 2: 0.0044 and 0.0040 units per hour in well-watered and drought stressed plants respectively, $p = 0.87$; Night 3: 0.0018 and 0.0007 units per hour in well-watered and drought stressed plants respectively, $p = 0.73$). Thus, the rate of water uptake and loss per unit volume was similar in well-watered and drought stressed plants. However, drought stressed tubers lost water for a longer period and took up water for a shorter period, which lead to an overall decrease in relative and total tuber water content compared to well-watered plants.

Although drought stressed plants had lower relative and total tuber water content, their water influx and efflux rates were similar. Nevertheless, the diurnal pattern of tuber volume differed between the treatments (Figure 17B, repeated measures ANOVA, $p = 0.02$). In well-watered plants, tuber volume tended to increase overnight and decrease throughout the day, while these changes were not present in drought stressed plants. However, when comparing the highest and lowest values of a 24h period (as done for tuber volume in Table), no significant differences occurred within a single treatment (ANOVA, $p > 0.05$). This indicates for example, that in well-watered plants the highest water content at night was not significantly different from the lowest water content in the day before or after that night. Thus, the number of replicates is too low to discriminate diurnal patterns in sequential comparisons, but the repeated measures ANOVA is powerful enough to detect differences in the patterns between the treatments.

Despite their lack of formal statistical significance, tuber volume changes affected total tuber water content. More pronounced changes in water content occurred in well-watered plants, whereas these were smaller in drought stressed plants (Figure 17C). In summary, potato tubers under well-watered conditions increased their water content overnight, while this response was attenuated or did not occur in tubers of drought stressed plants. Furthermore, tubers of drought stressed plants lost water throughout the day.

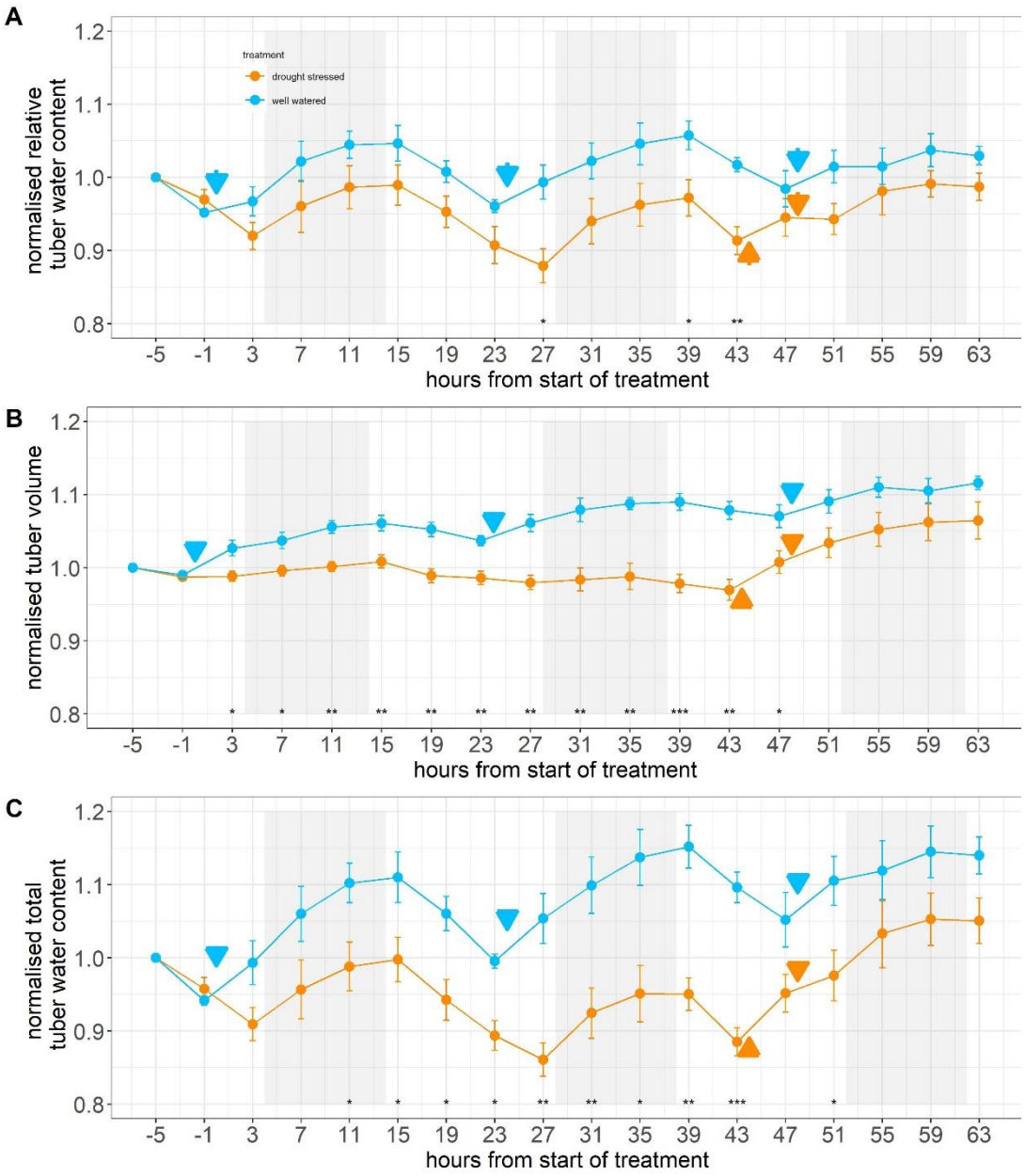

*Figure 17: Normalised tuber water content (A) normalised tuber volume (B) and (C) normalised total tuber water content of well watered (blue circles) and drought stressed (orange triangles) plants over the time course of three days (white background) and nights (light grey bars). Means ± SE of 4 plants per treatment and time point. Asterisks indicate significant differences between treatments at that time point according to t-test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

4.3. Soil compaction in controlled environment

Soil compaction had no significant effects on stomatal conductance, leaf and root water potential, shoot length and shoot fresh mass (Table 2). However, soil compaction significantly ($p < 0.01$) decreased leaf area by 30 % (Table 2, Figure 18).

Table 2: Gas exchange, water potentials and plant growth under soil compaction. Values are means of 6 plants per treatment

Treatment	g_s $\left(\frac{mmol}{m^2 \cdot s}\right)$	Ψ_{leaf} (*MPa*)	Ψ_{root} (*MPa*)	Shoot length (*cm*)	Leaf area (cm^2)	Shoot fresh mass (*g*)
Uncompacted (1.4 g / cm^3)	76.9	-0.70	-0.26	38.5	1031.8 a	68.9
Low compaction (1.5 g / cm^3)	65.2	-0.77	-0.33	37.4	1123.5 a	67.5
High compaction (1.7 g / cm^3)	57.5	-0.94	-0.40	36.2	627.5 b	53.0
	n.s.	n.s.	n.s.	n.s.	***	n.s.

Different letters indicate significant differences according to Tukey's HSD test. n.s. = no significant differences, * = significantly different to $p < 0.05$, ** = significantly different to $p < 0.01$, *** = significantly different to $p < 0.001$.

Figure 18: Potato plants, variety Maris Piper, grown in loose soil (left) and in compacted soil (right)

5. DISCUSSION

5.1. Field experiment

This is the first study to measure canopy cover and leaf gas exchange of a field grown crop weekly under a factorial combination of drought and soil compaction. Ground cover quickly increased under optimal conditions but was limited by deficit irrigation and especially soil compaction (Figure 5), whereas leaf gas exchange was similar in all treatments throughout the growing season. Slower leaf growth rate and leaf initiation in the beginning of the season reduced leaf area and thus light interception, thereby diminishing whole plant carbon gain and therefore total yield. In potato, the duration for which full ground cover is maintained explains a high percentage of yield differences (74 – 87 %) among cultivars and treatments (Boyd *et al.*, 2002). Since there were only small differences in late-season senescence between treatments (Figure 5), the time to reach full ground cover seems more important. Indeed, early-season biomass explained 71 % of the variation in final yield, with soil compaction, deficit irrigation and their combination decreasing yields similarly by 31 % (Figure 9). It has been established that the duration of light interception determines final yield (Haverkort and Struik, 2015) and full irrigation is necessary until after tuber initiation phase to ensure high yield (Jensen *et al.*, 2010). While this indicates the importance of early season shoot development in yield formation, to our knowledge the correlation between biomass at full ground cover and yield has not been reported before in potato. This finding could have an important impact on irrigation scheduling and crop management throughout the season to save water resources and maintain or increase yield per hectare. Therefore, it is important to understand the physiological mechanisms regulating canopy expansion and leaf gas exchange.

Decreased canopy growth can result from fewer leaves or reduced leaf expansion or both. Drought decreased leaf number in potato (Fasan & Haverkort, 1991;Figure 6C) while compaction inhibited tillering and thus leaf initiation in wheat (Jin *et al.*, 2015). Moreover, leaf expansion was inhibited by drought in potato (Obidiegwu *et al.*, 2015) and by soil compaction in sunflower (Andrade et al. 1993). Before full ground cover was reached, leaf expansion rate decreased in the order: control > the two single stress treatments > the combined soil compaction and drought stress treatment (Figure 6A). This reflects ground cover measurements over the same period (calendar week 24 to 29). However, ground cover curves for the different treatments diverge further after irrigation treatments were imposed in week 24 (Figure 5). This is probably because the deficit-irrigated treatments produced fewer new leaves after week 26. Differences in ground cover development before week 31 (when maximum ground cover was reached) resulted from differences in leaf expansion and number of leaves; thereafter leaf expansion rates did not differ between treatments (Figure 6B). Leaf number increased until week 33 and then remained constant (Figure 6C). After reaching maximum ground cover, the canopy continues to develop, which may enhance light interception slightly, but not considerably.

Restricted shoot growth under drought stress and soil compaction has often been associated with increased ABA levels (Mulholland *et al.*, 1996; Sharp, 2002), but ABA-deficient mutants grew less especially under these conditions (Hussain *et al.*, 2000; Aroca *et al.*, 2008). Deficit irrigation only once increased potato leaf tissue ABA levels (week 26, Figure 7C), thus an impact of ABA levels on plant growth in this experiment is unlikely. Furthermore, ABA limits synthesis of another growth inhibitor, ethylene (Hussain *et al.*, 2000; Sharp, 2002). Subjecting part of the root system to either soil drying (Sobeih *et al.*, 2004) or soil compaction (Hussain *et al.*, 2000) increased ethylene biosynthesis and limited leaf growth rates in tomato, but not in a transgenic tomato ($ACO1_{AS}$) with low stress-induced ethylene biosynthesis, but similar ABA levels as wildtype plants. While these observations indicate the importance of ethylene, reduced gibberellin (GA) biosynthesis limits shoot growth under soil compaction (Coelho Filho *et al.*, 2013) and drought stress decreases expression of GA biosynthesis genes while increasing expression of GA deactivation genes (Colebrook *et al.*, 2014). Hence, it is possible that ABA, ethylene and GA all interact to regulate shoot growth when plants grow in dry and/or compact soil.

Together with light interception, plant gas exchange is important for total plant carbon gain and therefore the plant's capacity to grow tubers. Deficit irrigation, but not soil compaction, decreased stomatal conductance (g_s) in factorial experiments with wheat (Whalley *et al.*, 2006) and maize (Tardieu *et al.*, 1992). Potato responded similarly, with deficit irrigation only significantly decreasing g_s and assimilation rate (both $p < 0.05$, F-Test, calendar week 29) after 17 days without irrigation (Figure 7B), confirming previous experiments (Liu *et al.*, 2006; Ahmadi *et al.*, 2010). Overall, leaf gas exchange was similar between treatments throughout most of the season and leaf-level carbon gain was thus similar in all treatments. Nevertheless, g_s was relatively low (generally < 0.4 mol m^{-2} s^{-1} (Fig. 5B) compared to other studies that report values above 0.5 mol m^{-2} s^{-1} for well-watered plants (Liu *et al.*, 2006; Ahmadi *et al.*, 2010; Puértolas *et al.*, 2014). When vapour pressure deficit (VPD) increased from 0.7 to 1.5 kPa around potato leaves (McAdam *et al.*, 2016), g_s rapidly declined to approximately the same values reported here. In the present study, although VPD was high (0.9 – 1.3 kPa) on most measurement days, it only explained 2 % of the variance in g_s ($R^2 = 0.02$, $p = 0.004$). Since measurements within the same VPD range showed no effect on potato gas exchange (Ahmadi *et al.*, 2010), other factors such as plant hormones may influence g_s under low plant water availability.

Increased leaf xylem sap ABA levels correlated with decreased g_s in potato (Liu *et al.*, 2005), tomato (Thompson *et al.*, 2007) and soybean (Castro *et al.*, 2019) in controlled environments. However, no such correlation occurred in field-grown potatoes (Ahmadi *et al.*, 2010). Similarly, leaf tissue ABA levels and g_s were not correlated ($R^2 = 0.06$, $p = 0.07$), with similar ABA accumulation between treatments due to higher ABA accumulation in the well-watered plants or (more likely) limited ABA accumulation in deficit-irrigated plants. Leaf xylem sap ABA concentration correlates with soil moisture in pot-grown tomato (Dodd, 2007) and sunflower (Dodd *et al.*, 2008). However, preferential water uptake from moister parts of the soil profile attenuates any effect of localised root ABA accumulation in potato, thereby minimising or eliminating root-to-shoot ABA-signalling (Puértolas *et al.*, 2015). Here, soil moisture was measured at 25 cm depth, but the roots of field-grown potatoes can grow as deep as 80 cm to access water (Stalham & Allen, 2004; Puertolas et al. 2014). Thus, plants likely accessed water from deeper layers and therefore the measured soil moisture does not reflect total plant water availability. Alternatively, high VPD (1.5 kPa) can stimulate foliar ABA accumulation in well-watered plants (McAdam and Brodribb, 2015), but there was no correlation between VPD and leaf ABA levels in the present study ($R^2 = 0.02$, $p = 0.26$). Stability of foliar ABA levels in deficit irrigated plants suggests that (deeper) roots acquired sufficient water to prevent leaf water deficit (Fig. 6).

Leaf water potential is highest before dawn and with plants in the dark for several hours at considerably decreased transpiration rates (Ramírez *et al.*, 2018), thus pre-dawn leaf water potential measurements indicate soil water availability. In contrast to the relative stability of daytime leaf water potential (Ψ_{day}), deficit irrigation decreased pre-dawn leaf water potential ($\Psi_{pre\text{-}dawn}$) only after prolonged times without irrigation (Fig. 6) as in maize (Tardieu *et al.*, 1992). Decreasing $\Psi_{pre\text{-}dawn}$ correlates with stomatal closure in different crops. However, potato showed small changes in stomatal conductance (0.12 mol m^{-2} s^{-1} g_s difference with $\Psi_{pre\text{-}dawn}$ between -0.4 and -0.1 MPa) compared to crops such as soybean (0.44 mol m^{-2} s^{-1} g_s difference over a similar $\Psi_{pre\text{-}dawn}$ range) and sunflower (0.54 mol m^{-2} s^{-1} g_s difference with $\Psi_{pre\text{-}dawn}$ between -0.68 and -0.25 MPa) (Granier and Tardieu, 1999). Thus, decreased $\Psi_{pre\text{-}dawn}$ in potato does not necessarily result in measurable stomatal closure. Taken together, while treatment differences in leaf gas exchange were not detected, understanding the substantial growth differences requires further investigations of plant water relations and hormone signalling effects.

Under drought stress, more potatoes fell into small size grades than under well-watered conditions, as in a study of 103 potato cultivars (Aliche *et al.*, 2019). Since drought, but not soil compaction, affected tuber size distribution (Fig. 8B), tuber development seemed to respond to systemic stress signals rather than local soil conditions. However, soil resistance did not differ between treatments in the top 20 cm, where most potatoes grow (Fig. 2), the direct impact of high soil resistance on tuber growth could not be examined. When drought stress restricted canopy growth, the available assimilates were distributed between a larger number of tubers (Fig 8B), producing a skewed tuber size distribution. Some potato cultivars undergo a second

phase of tuber initiation under well-watered conditions (Walworth and Carling, 2002), which might have occurred in the second half of the season, when soil moisture increased following rainfall (week 33 and thereafter). The time until harvest would have been shorter for these tubers, hence the final tuber size of potatoes initiated in the second wave was smaller, leading to many small tubers in deficit-irrigated plants. In addition, an interaction between ABA and GA has been suggested to regulate tuberization and therefore tuber size distribution under drought stress (Jensen *et al.*, 2010). Further research is needed to understand how tuber size is regulated.

To conclude, soil compaction and drought stress applied individually decreased shoot growth and yield, but both stresses occurring simultaneously had no additional effect on yield (Fig. 8A). Shoot biomass at full ground cover adequately predicted final yield ($R^2 = 0.71$, $p < 0.001$), indicating that vegetative growth in the first half of the growing season is critical in ensuring yield. This finding can be of great importance for crop management and irrigation scheduling. Since leaf gas exchange was not correlated with yield, leaf water status or ABA status, we conclude that plants under restricted water availability grow deeper roots to access water in deeper layers. Moreover, hormonal signals from the root system are postulated to restrict shoot growth sufficiently to ensure it can be sustained according to the available water supply. Further research is needed to test these hypotheses.

5.2. Controlled environment experiments

5.2.1. Drought stress experiments

5.2.1.1. Impact of strigolactones on stomatal conductance

Strigolactones have previously been suggested to be involved in regulating stomatal conductance as the soil dries in various species (Ha *et al.*, 2014; Liu *et al.*, 2015; Visentin *et al.*, 2016; Marzec *et al.*, 2020). *Arabidopsis* mutants impaired in SL biosynthesis or signalling for example, lost water faster than the WT in excised leaf or excised plant assays (Bu *et al.*, 2014; Ha *et al.*, 2014). However, whole plants of a tomato ccd7-silenced line closed stomata more rapidly in response to drying soil than the WT, while whole plants of a *Lotus* ccd7-silenced line subjected to drying soil closed stomata at a similar rate to the WT (Liu *et al.*, 2015; Visentin *et al.*, 2016). Thus, it is not clear to what extent SL are involved in stomatal regulation under drought stress and what other factors (e.g. environment or species) might influence the effect of SL. The present study is the first to explore the effect of strigolactones on stomatal conductance in potatoes under drought stress. There was surprising consistency in the leaf water relations of three genetically modified lines (impaired in SL biosynthesis (ccd8), SL insensitive (d14) or SL hypersensitive (d53)) in response to drying soil, suggesting that SL have a limited role in regulating water status of potatoes.

The SL deficient and insensitive lines expressed the previously described bushy phenotype (Pasare et al., 2013) with increased axillary shoot outgrowth, while the SL hypersensitive line showed no axillary outgrowth (

Figure 3). These phenotypes confirmed the known genetic alterations in the SL biosynthesis or signalling pathway. In potato, stomatal response to drying soil was similar in WT plants and genotypes impaired in SL biosynthesis or hypersensitive/ insensitive to SL (Figure 12A). Soil moisture gradually decreased in all experiments throughout the measurement period (Figure 11). Thus, all genotypes were subjected to comparable levels of water availability on respective days in the experiment.

Stomatal closure in drying soil is mediated by leaf water status and signals from the root (Tardieu and Davies, 1992). In the present study, stomatal conductance decreased linearly with decreasing soil moisture, while leaf water potential is maintained between 20 – 50 % soil moisture and only decreases linearly at <20 % soil moisture (Figure 12A and B), suggesting that stomatal conductance may be regulated by leaf water status at <20 % soil moisture and < -0.4 MPa leaf water potential (Figure 12C). However, stomatal conductance ranged from 250 to 800 mmol m^{-2} s^{-1} at mild drought stress (soil moisture > 20 %, leaf water potential > -0.4 MPa) in these experiments and therefore must be regulated by a different signal. Under rapid soil

drying leaf or shoot water potential explains > 90 % of variation in stomatal conductance in ccd7 silenced lines and WT of tomato and *Lotus* respectively (Liu *et al.*, 2015; Visentin *et al.*, 2016), suggesting that stomatal conductance is regulated by leaf water relations. Under well-watered conditions (water potentials close to zero), stomatal conductance in the ccd7-silenced line (impaired SL biosynthesis) was higher than in the WT (Liu *et al.*, 2015; Visentin *et al.*, 2016). *Lotus* maintains this difference between genotypes in drying soil (Liu *et al.*, 2015). In contrast, stomata of the tomato ccd7-silenced line close rapidly as leaf water potential decreases, resulting in stomatal conductance similar to the wildtype (Visentin *et al.*, 2016). Surprisingly, these genotypic differences could not be confirmed for potato, despite being a close relative to tomato. Stomatal conductance of all three tested transgenic lines was similar to the wildtype under well-watered conditions and decreased linearly for all genotypes in drying soil without significant differences in the slope of the linear regression. (Figure 12A). This suggests that SL may not be involved in stomatal closure under drying soil in potato.

At least three possible reasons may account for the results of the present study:

i. genotypic differences in stomatal density could explain variation in stomatal conductance in other studies,
ii. SL and other signals for stomatal closure are tightly connected via feedback loops, so that variation in SL biosynthesis/ signalling alters other signals to elicit stomatal closure, which results in a similar stomatal conductance phenotype,
iii. SL are not involved in stomatal closure in potatoes under drought stress.

Stomatal density (abaxial and adaxial) was higher in SL deficient mutants than in the WT of *Arabidopsis* (Ha *et al.*, 2014) and stomatal conductance in the same mutants was higher than in the WT (Kalliola *et al.*, 2020), suggesting that the increased stomatal conductance of SL-deficient plants at least partly results from higher stomatal density. In contrast, SL-deficient/-insensitive potato had lower abaxial stomatal density than the WT, with adaxial stomatal density higher in the SL-hypersensitive genotype than in the WT (Figure 13). Hence, different species seem to express different phenotypes in response to genetically altering the SL pathway and assumptions made from experiments in the model plant *Arabidopsis* may not be valid in other plants.

Despite the differences in stomatal density between potato genotypes, no differences in stomatal conductance were observed (Figure 12). Thus, genotypes with lower stomatal density must have had a wider stomatal aperture (and vice versa), leading to a similar transpiration rate per unit leaf area (= stomatal conductance) in all genotypes. This suggests that internal signals regulate stomatal aperture to prevent excessive water loss. ABA is a long-distance signal that decreases stomatal conductance in potato under drying soil (Liu *et al.*, 2005). Similarly, stomatal conductance was inversely related to root ABA concentration in *Lotus*, with higher stomatal conductance in the ccd7-silenced line compared to the WT at the same root ABA concentration (Liu *et al.*, 2015), indicating that stomata of the SL deficient line are more sensitive to endogenous ABA than the WT. Stomatal conductance in potato decreased with increasing root xylem sap ABA concentration (Figure 14B), but similarly in the ccd8-silenced line and the WT, unlike the above-mentioned response of *Lotus*. The speed of soil drying and consequently the level of drought stress experienced by the plant differed between the studies with rapid soil drying and severe stress in *Lotus* (Liu *et al.*, 2015) and slow soil drying and initially mild drought stress in potato (Figure 11), which could explain different responses of SL-deficient genotypes. Moreover, SL-deficient genotypes in different species seem to respond differently to drying soil as previously discussed. Note that different carotenoid cleavage deoxygenase genes were silenced in the compared studies (CCD7 in *Lotus*, CCD8 in potato). In SL biosynthesis, these enzymes are both involved in the step of forming carlactone from 9-cis-ß-carotene (Ruyter-Spira *et al.*, 2013). However, silencing CCD7 or CCD8 could have different effects on plant responses. For example, CCD7 may be essential in SL biosynthesis while CCD8 may be substituted by another enzyme with a similar function. Alternatively, the by-products of one cleavage step may have an impact on stomatal closure or stomatal sensitivity to ABA, in which case SL as an end-product may be of minor importance in drought stress signalling.

In summary, strigolactones do not seem to affect stomatal closure when potato plants are grown in drying soil, despite previous reports in related species (Liu *et al.*, 2015; Visentin *et al.*, 2016). Further research is needed to reconcile this discrepancy.

5.2.1.2. Diurnal tuber volume and water relations

Potato tubers account for around 80 % of the total plant biomass (Fasan and Haverkort, 1991) and therefore tuber responses to drought stress play an integral part in understanding whole plant water relations. However, they are difficult to study *in vivo* and previous studies could only measure water potential of different tubers at the beginning and the end of the night period (Gandar and Tanner, 1976) or continuously measure the same tuber growing in an artificial (non-soil) environment (Baker and Moorby, 1969). More recent studies in potato plants employed MRI technology (Aliche *et al.*, 2020*a*) and investigated the impact of drought on below ground organs (Lahlou and Ledent, 2005), but surprisingly do not consider tuber water relations. This study frequently (every 4 hours) measured all tubers of multiple plants per treatment in intact soil columns using magnetic resonance imaging to understand diurnal tuber volume growth and water relations in response to drying soil. Upon soil drying, changes in tuber water uptake and tuber volume were detected hours before shoot physiological responses were measured, indicating that the tuber is tightly connected to the water flow from the soil.

Physiological measurements indicated typical plant drought stress responses, despite precautions to limit foliar damage through robotic handling (Figure 4). Soil drying decreased leaf gas exchange and increased foliar ABA concentration from day 2 (Figure 15), with these variables recovering after re-watering. Since the roots were distributed throughout the soil column (Figure 16B), all soil layers dried. However, basal re-watering of the drought stressed plants lead to increased moisture values at the top and the bottom of the pot, but not in the middle (Figure 16A, 47 HAS). This is most likely a redistribution of water from the soil at the bottom of the pot into the tubers. If capillary rise of water in the soil was responsible for increased soil moisture values at the top of the pot, the values in the middle of the pot would necessarily increase as well. Hence, the soil dries out evenly, but the distribution of moisture in the pot upon re-watering suggests water is relocated via the roots to the top of the pot, probably into the tubers. After re-watering of the drought stressed plants, relative tuber water content and tuber volume increase significantly (Figure 17,Table 1).The increase in tuber volume magnifies the effect of water influx into the tubers so that the total moisture measured in the top of the pot after re-watering accounts for bigger tubers with a higher water content per volume unit compared to pre-re-watering. Compared to well-watered plants, the significantly higher moisture values in the top of the pots of drought stressed plants after re-watering can be explained by water influx into the tubers.

Mild drought stress impaired tuber volume growth, but subsequent re-watering substantially increased tuber growth rate such that both treatments had similar tuber volumes at the end of the experiment (Figure 17B). Cell and plant organ growth are a function of turgor:

$$G = m(P - Y)$$

with G = cell elongation rate, m = yielding coefficient, P = cell turgor, Y = yield threshold turgor (turgor over which irreversible cell extension occurs) (Lockhart, 1965; Passioura and Fry, 1992). Thus, tuber growth directly depends on water influx to increase turgor to P > Y to allow growth. Overnight, relative tuber water content increases in well-watered plants, while tuber volume shows positive growth rates in this time (Figure 17). Hence, we can assume that the water influx in tubers of well-watered plants after re-watering and overnight was high enough to increase the turgor to levels that facilitate growth (P > Y). In drought stressed plants, the water efflux from the tuber in the day exceeded the influx into the tuber at night (Figure 17) and therefore nocturnal turgor levels did not reach the yield threshold turgor (0 < P < Y), even though the tuber water content increased (Figure 17, Table 1, Hohl and Schopfer, 1992). Thus, tuber growth in drought stressed plants paused under mild or moderate drought stress conditions. However, re-watering drought stressed plants initially substantially increased tuber growth rates, which later returned to similar growth rates as in well-watered plants (Figure 17). This behaviour reflects the underlying interdependence of P, m and Y (Hohl and Schopfer, 1992; Passioura and Fry, 1992)

and shows that short-term, mild drought stress may not impair cell wall extensibility (Durand *et al.*, 1995), but that turgor is indeed the main driving force for tuber growth.

To maximise tuber yield, water influx into the tuber overnight should not be restricted and daytime water efflux should be minimal. In the absence of other considerations, irrigation in the late afternoon or evening may be a practical solution to adjust soil moisture dynamics to the periodicity of tuber growth. During times of low transpiration losses (evening, night), water is directed into the tubers allowing cell extension (Figure 17). This process is irreversible and tuber shrinkage due to the subsequent loss of water in the daytime is unlikely (Green *et al.*, 1971; Passioura and Fry, 1992). However, these conclusions are based on mild to moderate drought stress and re-watering of the plants after a short stress period. Prolonged and/or more severe drought stress may irreversibly constrain tuber volume growth or cause tuber shrinkage, so that re-watering would not allow full recovery of tuber volume. Further research is needed to assess the effects of severe or prolonged drought stress on tuber growth *in vivo.*

While the timing of irrigation will affect the influx of water into tubers, restricting daytime water efflux from the tuber is more difficult because it is not fully understood where the water is lost to. Direct water loss to surrounding soil is unlikely, as the tuber periderm is an effective water barrier under drying conditions (Vogt *et al.*, 1983) and tubers took up minimal amounts of water soluble Cadmium from the surrounding soil through the periderm (Reid *et al.*, 2003). However, xylem and phloem vessels within the stolons may act as conduits for water exchanges between the tuber and the rest of the plant, driven by water potential or osmotic gradients. Diurnal fluctuations in tuber water content may thus be determined by processes in other plant organs, rather than local soil moisture availability. In the phloem, soluble sugars are transported from the leaves (source) to the tubers (sink) (Aliche *et al.*, 2020*b*), while xylem flow is mainly driven by differences in water potential (Holbrook and Zwienicki, 2005). The water potential gradient between tubers and leaves suggests daytime water flow from the tuber to the leaves, while the water potential difference is close to zero during the night (Gandar and Tanner, 1976), which is reflected in xylem flow rates in potato stems under well-watered conditions (Aliche *et al.*, 2020*a*). However, acropetal xylem flow in the lower stem of potato under drought stress decreased significantly in a cultivar that hardly produced any tubers under drought stress, while xylem flow rates were maintained in a cultivar that did produce tubers under drought stress (Aliche *et al.*, 2020*a*,*b*). This means the tuber could hypothetically supply water to the shoot during the daytime, when the roots cannot extract sufficient water from the soil to support shoot demands. During the night, with a minimal water potential gradient between tuber and leaf, the tuber takes up water extracted by the roots. Under water limiting conditions, the water flow to the leaves may be reduced because the water would be shared between tubers and leaves at night. Studying these hypotheses requires the use of deuterium labelled water (Hafner *et al.*, 2017) to understand the pathway of water through a potato plant under restricted soil water availability. Screening different genotypes could give insights into whether tuber number, stolon size or osmotic potential of the tuber affect water efflux in the daytime and possibly point out future markers for drought tolerance breeding.

To conclude, magnetic resonance imaging allowed repeated measurements of water content and volume of all tubers of a potato plant. Tubers show a similar water content pattern to the shoot (Gandar and Tanner, 1976), but in contrast to the root or shoot they do not directly exchange water with their environment through pores (Vogt *et al.*, 1983; Taiz and Zeiger, 2010). However, since tubers form a large percentage of the total plant biomass and contain around 75 % water at harvest, they may influence plant responses to drying soil. While further experiments with dyes or tracer molecules are desirable, tubers seem to provide water to the shoot as the soil dries out.

6. CONCLUSIONS

Reduced plant water availability affects potato crops differently at individual developmental stages (Jensen *et al.*, 2010). In the first month after emergence, reduced water availability restricts potato shoot growth (Huntenburg *et al.*, 2021), but not root growth (Stalham and Allen, 2004). After tuber initiation, irrigation may be reduced by 30% until canopy senescence without yield penalties (Jensen *et al.*, 2010). This study set out to understand potato plant physiology and signalling under drought stress by investigating the role of ABA and strigolactones on stomatal closure under drought stress and by using magnetic resonance imaging to reveal diurnal changes in tuber volume growth and tuber water content.

Sufficient soil water availability was important in the vegetative growth stage to ensure maximum canopy growth rates and therefore maximum light interception as shown in field experiments (Huntenburg *et al.*, 2021; Figure 5). Avoiding drought stress during tuber volume growth (tuber bulking stage) was also important, although short periods of decreased soil water availability can be tolerated with subsequent irrigation recovering tuber volume. Although potatoes show an isohydric response to mild drought stress, root-derived strigolactones do not seem to be involved in regulating this response.

In conclusion, irrigation scheduling needs to be tailored to different developmental stages to achieve maximum yield. A possible irrigation scheme has been outlined here, based on crop phenological stages and evaporative demand, which can be adapted to different varieties and environmental conditions (Figure 19). This makes the suggestions made here more universally applicable and more flexible than fractions of full irrigation given elsewhere without reporting the actual levels of evapotranspiration (Jensen *et al.*, 2010).

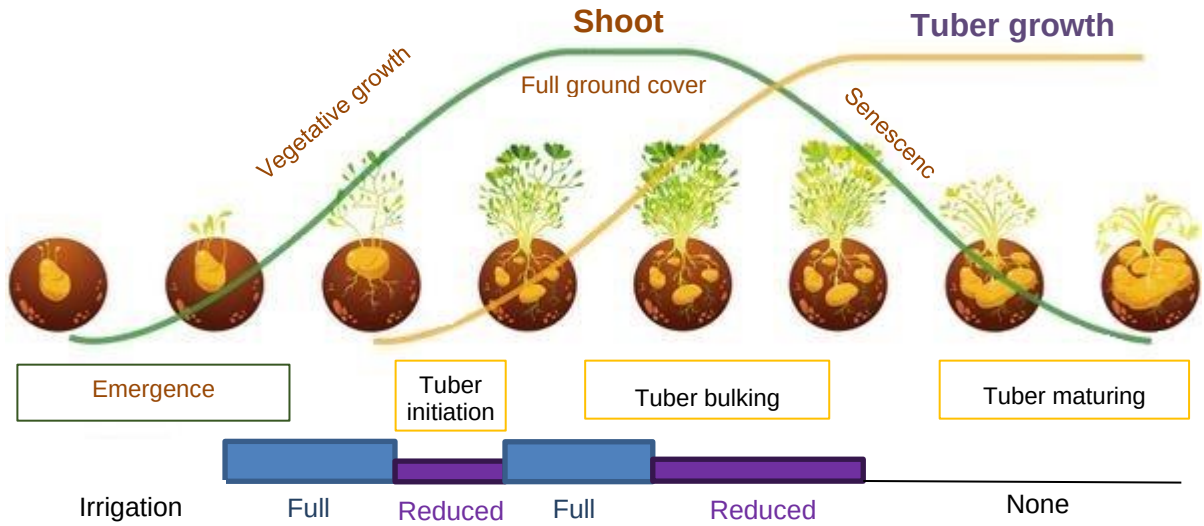

Figure 19: Growth cycle of potato crops with a possible irrigation scheme for water saving crop management. This schematic is only approximate and needs to be adjusted according to evaporative demand in the specific season.
(Adapted from: Castlecor Potatoes)

It is particularly important to compare different genotypes in future studies, as there is wide cultivar variation in potato drought stress responses (Aliche *et al.*, 2018) and national organisations such as the UK Agriculture & Horticulture Development Board's (AHDB) Potato Variety Database (https://varieties.ahdb.org.uk/). Institutional networks such as the International Plant Phenotyping Platform (IPPN) can allow access to novel technologies such as MRI and be of great value to compare genotypes in the future. Understanding the mechanisms of water fluxes in the potato plant *in situ* will be valuable to compare varieties of differing drought tolerance. However, these experiments should always be accompanied by larger field experiments to determine if the effect seen in controlled environment also occurs in the field.

Furthermore, it is important to conduct controlled environment experiments in conditions that resemble the field as closely as possible to mitigate unwanted side effects and genotype x environment interactions (Köhl *et al.*, 2021).

This study measured plant biochemical / hormonal processes to reveal mechanisms of water use in the potato plant, based on results of previous field experiments. This approach ensures that the results from model systems are relevant to the reality of potato farming by enhancing our physiological understanding of this crop, leading to practical suggestions for potato farming and further research.

7. REFERENCES

Ahmadi SH, Andersen MN, Plauborg F, Poulsen RT, Jensen CR, Sepaskhah AR, Hansen S. 2010. Effects of irrigation strategies and soils on field-grown potatoes: Gas exchange and xylem [ABA]. Agricultural Water Management **97**, 1486–1494.

Aliche EB, Oortwijn M, Theeuwen TPJM, Bachem CWB, van Eck HJ, Visser RGF, van der Linden CG. 2019. Genetic mapping of tuber size distribution and marketable tuber yield under drought stress in potatoes. Euphytica **215**, 1–19.

Aliche EB, Oortwijn M, Theeuwen TPJM, Bachem CWB, Visser RGF, van der Linden CG. 2018. Drought response in field grown potatoes and the interactions between canopy growth and yield. Agricultural Water Management **206**, 20–30.

Aliche EB, Prusova-Bourke A, Ruiz-Sanchez M, Oortwijn M, Gerkema E, Van As H, Visser RGF, van der Linden CG. 2020*a*. Morphological and physiological responses of the potato stem transport tissues to dehydration stress. Planta **251**, 1–15.

Aliche EB, Theeuwen TPJM, Oortwijn M, Visser RGF, van der Linden CG. 2020*b*. Carbon partitioning mechanisms in POTATO under drought stress. Plant Physiology and Biochemistry **146**, 211–219.

Aroca R, Del Mar Alguacil M, Vernieri P, Ruiz-Lozano JM. 2008. Plant responses to drought stress and exogenous ABA application are modulated differently by mycorrhization in tomato and an ABA-deficient mutant (Sitiens). Microbial Ecology **56**, 704–719.

Baker DA, Moorby J. 1969. The transport of sugar, water, and ions into developing potato tubers. Annals of Botany **33**, 729–741.

Boyd NS, Gordon R, Martin RC. 2002. Relationship between leaf area index and ground cover in potato under different management conditions. Potato Research **45**, 117–129.

Bu Q, Lv T, Shen H, *et al.* 2014. Regulation of drought tolerance by the F-box protein MAX2 in Arabidopsis. Plant Physiology **164**, 424–439.

Castro P, Puertolas J, Dodd IC. 2019. Stem girdling uncouples soybean stomatal conductance from leaf water potential by enhancing leaf xylem ABA concentration. Environmental and Experimental Botany **159**, 149–156.

Coelho Filho MA, Colebrook EH, Lloyd DPA, Webster CP, Mooney SJ, Phillips AL, Hedden P, Whalley WR. 2013. The involvement of gibberellin signalling in the effect of soil resistance to root penetration on leaf elongation and tiller number in wheat. Plant and Soil **371**, 81–94.

Colebrook EH, Thomas SG, Phillips AL, Hedden P. 2014. The role of gibberellin signalling in plant responses to abiotic stress. Journal of Experimental Biology **217**, 67–75.

Dodd IC. 2007. Soil moisture heterogeneity during deficit irrigation alters root-to-shoot signalling of abscisic acid. Functional Plant Biology **34**, 439–448.

Dodd IC, Egea G, Davies WJ. 2008. Abscisic acid signalling when soil moisture is heterogeneous: Decreased photoperiod sap flow from drying roots limits abscisic acid export to the shoots. Plant, Cell and Environment **31**, 1263–1274.

Durand JL, Onillon B, Schnyder H, Rademacher I. 1995. Drought effects on cellular and spatial parameters of leaf growth in tall fescue. Journal of Experimental Botany **46**, 1147–1155.

van Dusschoten D, Kochs J, Kuppe CW, Sydoruk VA, Couvreur V, Pflugfelder D, Postma JA. 2020. Spatially resolved root water uptake determination using a precise soil water sensor. Plant Physiology **184**, 1221–1235.

van Dusschoten D, Metzner R, Kochs J, Postma JA, Pflugfelder D, Bühler J, Schurr U, Jahnke S. 2016. Quantitative 3D analysis of plant roots growing in soil using magnetic resonance imaging. Plant Physiology **170**, 1176–1188.

Fasan T, Haverkort AJ. 1991. The influence of cyst nematodes and drought on potato growth. 1. Effects on plant growth under semi-controlled conditions. Netherlands Journal of Plant Pathology **97**, 151–161.

Gandar PW, Tanner CB. 1976. Potato leaf and tuber water potential measurements with a pressure chamber. American Potato Journal **53**, 1–14.

Gomez-Roldan V, Fermas S, Brewer PB, *et al.* 2008. Strigolactone inhibition of shoot branching. Nature **455**, 189–194.

Granier C, Tardieu F. 1999. Water deficit and spatial pattern of leaf development. Variability in responses can be simulated using a simple model of leaf development. Plant Physiology **119**, 609–619.

Green PB, Erickson RO, Buggy J. 1971. Metabolic and Physical Control of Cell Elongation Rate. Plant Physiology **47**, 423–430.

Ha C Van, Leyva-Gonzalez MA, Osakabe Y, *et al.* 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. Proceedings of the National Academy of Sciences of the United States of America **111**, 851–856.

Hafner BD, Tomasella M, Häberle KH, Goebel M, Matyssek R, Grams TEE. 2017. Hydraulic redistribution under moderate drought among English oak, European beech and Norway spruce determined by deuterium isotope labeling in a split-root experiment. Tree Physiology **37**, 950–960.

Haverkort AJ, Struik PC. 2015. Yield levels of potato crops: Recent achievements and future prospects. Field Crops Research **182**, 76–85.

Hohl M, Schopfer P. 1992. Growth at reduced turgor : irreversible and reversible cell-wall extension of maize coleoptiles and its implications for the theory of cell growth. Planta **187**, 209–217.

Holbrook NM, Zwienicki MA (Eds.). 2005. *Vascular Transport in Plants*. Elsevier.

Huntenburg K, Dodd IC, Stalham M. 2021. Agronomic and physiological responses of potato subjected to soil compaction and/or drying. Annals of Applied Biology, 1–13.

Hussain A, Black CR, Taylor IB, Roberts JA. 2000. Does an antagonistic relationship between ABA and ethylene mediate shoot growth when tomato (Lycopersicon esculentum Mill.) plants encounter compacted soil? Plant Cell and Environment **23**, 1217–1226.

Jensen CR, Battilani A, Plauborg F, *et al.* 2010. Deficit irrigation based on drought tolerance and root signalling in potatoes and tomatoes. Agricultural Water Management **98**, 403–413.

Jin K, Shen J, Ashton RW, White RP, Dodd IC, Phillips AL, Parry MAJ, Whalley WR. 2015. The effect of impedance to root growth on plant architecture in wheat. Plant and Soil **392**, 323–332.

Kalliola M, Jakobson L, Davidsson P, *et al.* 2020. Differential role of MAX2 and strigolactones in pathogen, ozone, and stomatal responses. Plant Direct **4**, 1–14.

Köhl KI, Mulugeta Aneley G, Haas M, Peters R. 2021. Confounding Factors in Container-Based Drought Tolerance Assessments in Solanum tuberosum. Agronomy **11**, 865.

Lahlou O, Ledent JF. 2005. Root mass and depth, stolons and roots formed on stolons in four cultivars of potato under water stress. European Journal of Agronomy **22**, 159–173.

Liu J, He H, Vitali M, *et al.* 2015. Osmotic stress represses strigolactone biosynthesis in Lotus japonicus roots: exploring the interaction between strigolactones and ABA under abiotic stress. Planta **241**, 1435–1451.

Liu F, Jensen CR, Shahanzari A, Andersen MN, Jacobsen SE. 2005. ABA regulated stomatal control and photosynthetic water use efficiency of potato (Solanum tuberosum L.) during progressive soil drying. Plant Science **168**, 831–836.

Liu F, Shahnazari A, Andersen MN, Jacobsen SE, Jensen CR. 2006. Physiological responses of potato (Solanum tuberosum L.) to partial root-zone drying: ABA signalling, leaf gas exchange, and water use efficiency. Journal of Experimental Botany **57**, 3727–3735.

Lockhart JA. 1965. An analysis of irreversible plant cell elongation. Journal of Theoretical Biology **8**, 264–275.

Marzec M, Daszkowska-Golec A, Collin A, Melzer M, Eggert K, Szarejko I. 2020. Barley strigolactone signalling mutant hvd14.d reveals the role of strigolactones in abscisic acid-dependent response to drought. Plant Cell and Environment **43**, 2239–2253.

McAdam SAM, Brodribb TJ. 2015. The evolution of mechanisms driving the stomatal response to vapor pressure deficit. Plant Physiology **167**, 833–843.

McAdam SAM, Sussmilch FC, Brodribb TJ. 2016. Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms. Plant Cell and Environment **39**, 485–491.

Mulholland BJ, Black CR, Taylor IB, Roberts J a, Lenton JR. 1996. Effect of soil compaction on barley (Hordeum vulgare l) growth .1. possible role for ABA as a root-sourced chemical signal. Journal of Experimental Botany **47**, 539–549.

Obidiegwu JE, Bryan GJ, Jones HG, Prashar A. 2015. Coping with drought: stress and adaptive responses in potato and perspectives for improvement. Frontiers in Plant Science **6**, 1–23.

Pasare SA, Ducreux LJM, Morris WL, *et al.* 2013. The role of the potato (Solanum tuberosum) CCD8 gene in stolon and tuber development. New Phytologist **198**, 1108–1120.

Passioura J, Fry S. 1992. Turgor and Cell Expansion: Beyond the Lockhart Equation. Functional Plant Biology **19**, 565.

Puértolas J, Ballester C, Elphinstone ED, Dodd IC. 2014. Two potato (Solanum tuberosum) varieties differ in drought tolerance due to differences in root growth at depth. Functional Plant Biology **41**, 1107–1118.

Puértolas J, Conesa MR, Ballester C, Dodd IC. 2015. Local root abscisic acid (ABA) accumulation depends on the spatial distribution of soil moisture in potato: Implications for ABA signalling under heterogeneous soil drying. Journal of Experimental Botany **66**, 2325–2334.

Quarrie SA, Whitford PN, Appleford NEJ, Wang TL, Cook SK, Henson IE, Loveys BR. 1988. A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. Planta **173**, 330–339.

Ramírez DA, Yactayo W, Rolando JL, Quiroz R. 2018. Preliminary Evidence of Nocturnal Transpiration and Stomatal Conductance in Potato and their Interaction with Drought and Yield. American Journal of Potato Research **95**, 139–143.

Raymundo R, Asseng S, Robertson R, Petsakos A, Hoogenboom G, Quiroz R, Hareau G, Wolf J. 2018. Climate change impact on global potato production. European Journal of Agronomy **100**, 87–98.

Reid RJ, Dunbar KR, McLaughlin MJ. 2003. Cadmium loading into potato tubers: The roles of the periderm, xylem and phloem. Plant, Cell and Environment **26**, 201–206.

Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H. 2013. The biology of strigolactones. Trends in Plant Science **18**, 72–83.

Saeed W, Naseem S, Ali Z. 2017. Strigolactones biosynthesis and their role in abiotic stress resilience in plants: A critical review. Frontiers in Plant Science **8**, 1–13.

Sharp R. 2002. Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. Plant, Cell & Environment **25**, 211–222.

Sobeih WY, Dodd IC, Bacon MA, Grierson D, Davies WJ. 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (Lycopersicon esculentum) plants subjected to partial root-zone drying. Journal of Experimental Botany **55**, 2353–2363.

Stalham MA, Allen EJ. 2004. Water uptake in the potato (Solanum tuberosum) crop. The Journal of Agricultural Science **142**, 373–393.

Taiz L, Zeiger E. 2010. *Plant Physiology*. Sinauer Associates.

Tardieu F, Davies WJ. 1992. Stomatal Response to Abscisic Acid Is a Function of Current Plant Water Status. **98**, 540–545.

Tardieu F, Zhang J, Katerij N, Bethenod O, Palmer S, Davies WJ. 1992. Xylem ABA controls the stomatal conductance of field-grown maize subjected to soil compaction or soil drying. Plant, Cell & Environment **15**, 193–197.

Thompson AJ, Mulholland BJ, Jackson AC, McKee JMT, Hilton HW, Symonds RC, Sonneveld T, Burbidge A, Stevenson P, Taylor IB. 2007. Regulation and manipulation of ABA biosynthesis in roots. Plant, Cell and Environment **30**, 67–78.

Umehara M, Hanada A, Magome H, Takeda-Kamiya N, Yamaguchi S. 2010. Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice. Plant and Cell Physiology **51**, 1118–1126.

Visentin I, Vitali M, Ferrero M, Zhang Y, Ruyter-Spira C, Novák O, Strnad M, Lovisolo C, Schubert A, Cardinale F. 2016. Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. New Phytologist **212**, 954–963.

Vogt E, Schönherr J, Schmidt HW. 1983. Water permeability of periderm membranes isolated enzymatically from potato tubers (Solanum tuberosum L.). Planta **158**, 294–301.

Walworth JL, Carling DE. 2002. Tuber initiation and development in irrigated and non-irrigated potatoes. American Journal of Potato Research **79**, 387–395.

Weyers JDB, Johansen LG. 1985. Accurate Estimation of Stomatal Aperture From Silicone Rubber Impressions. New Phytologist **101**, 109–115.

Whalley WR, Clark LJ, Gowing DJG, Cope RE, Lodge RJ, Leeds-Harrison PB. 2006. Does soil strength play a role in wheat yield losses caused by soil drying? Plant and Soil **280**, 279–290.

8. ACKNOWLEDGEMENTS

We are grateful for the AHDB funding for this PhD, the EPPN2020 funding to conduct the MRI experiments and NIAB CUF for providing the field experiments.