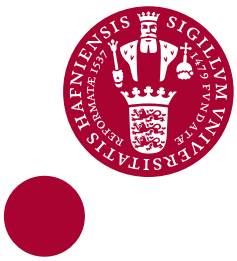

Timelapse scanning reveals spatial variation in tomato (Solanum lycopersicum L.) root elongation rates during partial waterlogging

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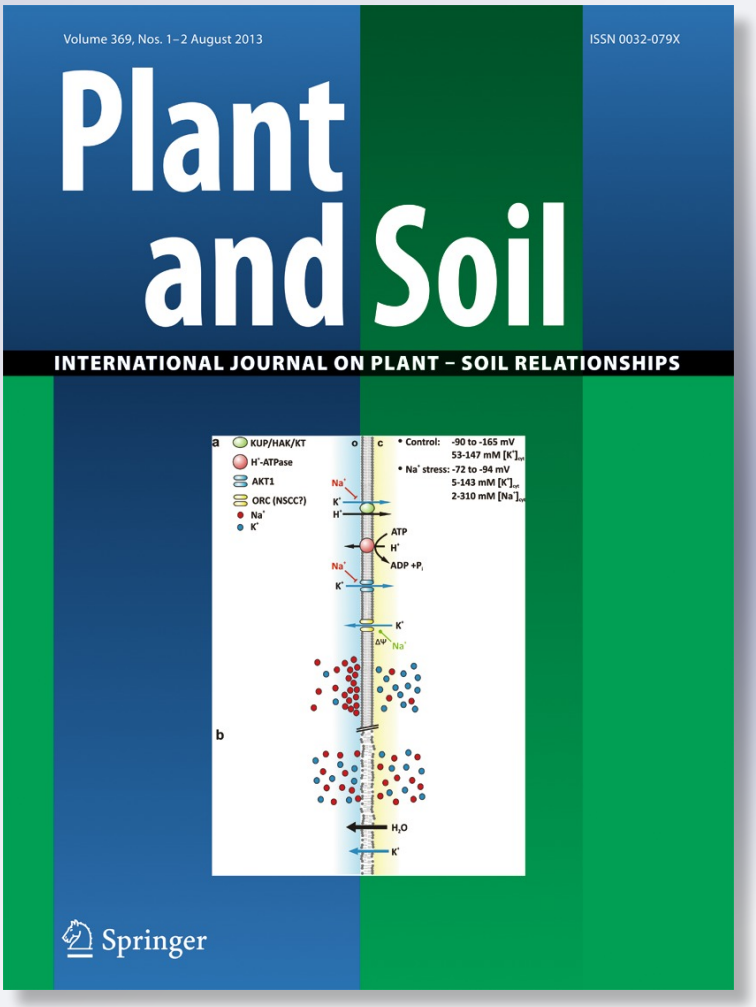

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REGULAR ARTICLE

Timelapse scanning reveals spatial variation in tomato (*Solanum lycopersicum* L.) root elongation rates during partial waterlogging

Dorte Bodin Dresbøll · Kristian Thorup-Kristensen · Blair M. McKenzie · Lionel Xavier Dupuy · A. Glyn Bengough

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Abstract
Background and aims Root systems show considerable plasticity in their morphology and physiology in response to variability within their environment. Root elongation below a water-table was expected to slow due to hypoxia, whilst roots above the waterlogged zone were expected to compensate by increasing elongation rates.
Methods Tomato plants (*Solanum lycopersicum* L.) were grown in peat in root chambers (300×215×6 mm) with a transparent front. Root chambers were maintained in flatbed scanners tilted at 30° to vertical and scanned every 3 h before, during and after waterlogging the lower layer for 24 h or 5 days. Root elongation rates were calculated from the displacement of randomly selected root tips between successive scans. Oxygen content was determined in the waterlogged layer and plant and root parameters were determined at cessation of the experiment.
Results Root elongation rates decreased rapidly when waterlogged. Growth rates of the waterlogged roots decreased, while growth rates of roots above the waterlogged zone increased. In 24 h waterlogged roots new lateral root growth occurred in the lower layer of the root chamber when water was drained while after 5 day waterlogging new root growth had to be initiated from roots above the waterlogged zone.
Conclusions Plants increased growth rates in roots above the waterlogged zone probably as compensation for the suboptimal conditions in the waterlogged zone which eventually led to roots dying.

Keywords Oxygen · Anoxia · Root tips · Peat · Growing media · Automated imaging

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Introduction

Abiotic stresses, such as salinity, drought or waterlogging, in the rhizosphere, are detrimental to root growth and plant health (e.g. Morard et al. 2000). Although waterlogging, like many other stresses, is often local, the functioning of the entire plant can be affected. A striking example of systemic response to abiotic stress is the considerable plasticity in both root morphology and physiology, which are observed in response to heterogeneity within the soil environment. When roots of a starved plant encounter a nutrient rich patch, root growth in this area increases and is often accompanied by a decrease in root growth in nutrient poorer areas (Hodge 2009). Hence roots tend to allocate biomass in areas with higher return, and divert resources from less favourable regions of soil. The ability to invest in root growth in beneficial volumes of the soil can be vital for plants, even when grown in small pots with restricted space. Variation in rhizosphere properties occurs within short distances (millimetres), and since exploitation of root-free volumes is often not possible, investment in root growth where conditions are superior, may be important to maintain overall plant growth.

Plants, like all aerobic organisms, require oxygen as electron acceptors for respiratory energy metabolism. When soil or other growing media are waterlogged, partial oxygen availability will decrease leading to hypoxia, or total lack of oxygen (anoxia) (Licausi 2011). Thus, waterlogging directly affects plant metabolism and root growth. This, in turn disrupts water and nutrient uptake and consequently overall plant growth and survival (Drew 1997; Dat et al. 2004). The manner in which waterlogging affects root growth is dependent on duration and severity of waterlogging as well as the physical properties of the surrounding environment. During short-term waterlogging (less than 24 h) roots will primarily be exposed to hypoxic conditions. Cells will still be able to produce ATP via oxidative phosphorylation, but the rate of oxygen consumption in cells and energy usage is decreased (Licausi 2011). Long-term waterlogging (more than 24 h), will result in prolonged anoxic conditions, and lead to fermentative ATP production, that will decrease growth and eventually lead to cell death (Morard et al. 2000).

Although a plant may trigger localised growth in response to waterlogging, most previous studies have focused on response to drought and nutrient stresses (Hodge et al. 1999; McKenzie et al. 2009). The *in situ* root growth of potted plants grown in peat or other growing media has received little attention. However, with high value irrigated crops increasingly being grown in media to maximize commercial yield, there is a need to understand root responses to transient waterlogging caused by over-watering. Conventional methods to characterise root growth (e.g. washing roots and minirhizotron studies) are either destructive or not suitable for roots grown in organic media in small pots. When washing roots free from peat, the risk of loosing fine roots is considerable, due to the colouring caused by phenolic acids in the peat medium, causing roots to look like peat fibres. As a consequence, many root growth studies have used artificial systems where biotic and abiotic parameters are more easily controlled such as in hydroponics or agar media (Bengough et al. 2004; Kläring and Zude 2009). These media allow detailed information on root growth, architecture and functioning to be measured easily. However, root growth in these artificial conditions may differ greatly from that observed in soil environments. Physical structure is usually homogeneous and unrelated to the soil complex tri-phasic structure. In addition, it is usually difficult to create heterogeneous environments with such systems. In two studies comparing root lengths from agar media and soil, significant effect were found for barley and wheat genotypes, indicating significant soil environment-genotype interactions (Gregory et al. 2009; Hargreaves et al. 2009; Wojciechowski et al. 2009). New methods for observing root growth in soil are essential to better understand the effect of abiotic stress on root growth. Simple visual non-destructive techniques such as time-lapse scanning of roots are expected to take root-substrate interactions into account, and will provide more detailed knowledge on root growth under heterogeneous conditions.

The objective of this study was to elucidate the effect of transient partial waterlogging of the root system, and in particular to study changes in root growth rates within and above a waterlogged zone, before, during and after waterlogging. This was achieved using a novel experimental setup where root chambers were fixed rigidly to flatbed scanners during

the experiment. Time lapse images of the root system were obtained, and the elongation of individual roots followed through time in the peat growth medium. We tested the hypothesis that root system growth is affected by transient partial waterlogging; that roots stressed directly by waterlogging slow their elongation rates, whereas roots above the waterlogged zone increase elongation rates to compensate. Responses to short (1 day) and long (5 day) periods of waterlogging stress were investigated.

Materials and methods

Root chambers and growing media

Root chambers were constructed of two plates: a transparent Perspex front and a black polyvinylchloride back each with the dimensions of 215×300×3 mm (described in Bengough et al. 2004). The front had 6 mm deep Perspex strips on the base and sides giving a volume of approximately 365 cm^3 when the plates were kept together by plastic clips. The chambers were open at the top to allow gas exchange with the surrounding atmosphere and unimpeded shoot extension (Fig. 1).

The growing medium was based on a blonde peat moss, 65 % Baltic peat and 35 % Danish peat with a particle size distribution of <10 mm. Limestone, wetting agent (Fiba-Zorb, Turftech International, UK) and fertilizer (78 gm^{-3}N, 48 gm^{-3} P and 130 gm^{-3}K) were mixed into the medium. This medium has previously been used for potted tomato plants (Dresbøll and Thorup-Kristensen 2012). The experiments were conducted in peat based media in order to resemble growth conditions in pots. Before use the medium was distributed evenly in six 2 L containers, wetted from below and left submerged for 24 h. Subsequently, the water was drained from the containers and these were moved to a sandbox (08.01 Sandbox, Eijkelkamp Agrisearch Equipment, NL) and left for 48 h with a tension of −5 kPa in order to ensure even water distribution in the medium. Five replicate samples were oven dried at 70 °C for 24 h to determine the peat dry weight. Root chambers were filled with 290 g peat corresponding to 86 g dry weight, and two tomato seeds (*Solanum lycopersicum* L.) were sown 10 mm from the upper edge of the chamber. If both seeds germinated one was removed within 24 h, leaving one plant per chamber.

Experimental setup

Two experiments were conducted; a short-term waterlogging experiment where the lower part of the root chambers was waterlogged for 24 h and a long-term waterlogging experiment where roots were waterlogged for 5 day (Table 1). In both experiments the waterlogged plants were compared to a control irrigated regularly (but not waterlogged).

The root chambers were placed with the transparent side kept dark and facing down at an angle of 30° from

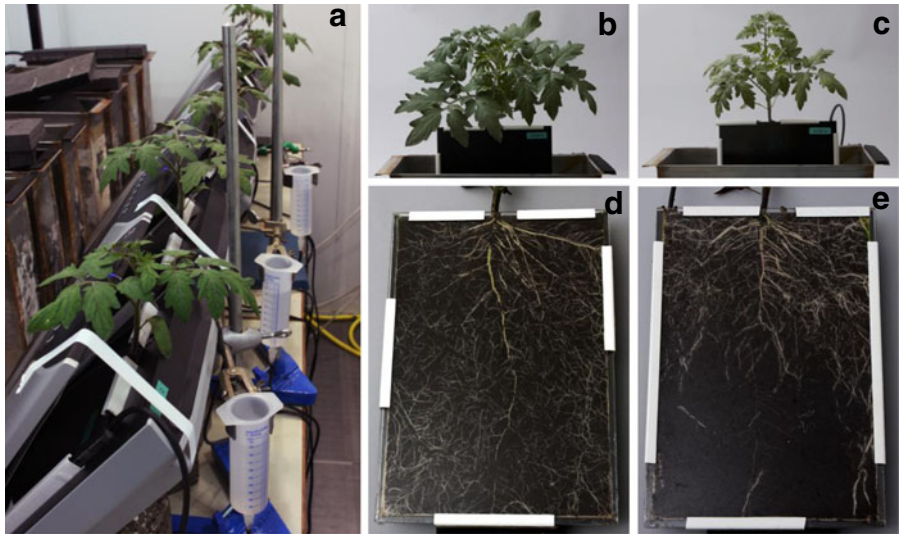

Fig. 1 Experimental setup with vertical scanners containing the root chambers and shoot/root appearance in waterlogging conditions. **a** a general view of the system used; **b**–**d** shoot and root of a tomato control plant; **c**–**e** shoot and root appearance at cessation of the 5 day waterlogging experiment

Table 1 Time table for the short-term (24 h) and long-term (5 day) waterlogging experiments

	24 h waterlogging	5 day waterlogging
Sowing	Day 1	Day 1
Initiation of timelapse scanning	Day 14	Day 14
Waterlogging	Day 16	Day 16
Draining	Day 17	Day 21
End of timelapse scanning	Day 22	Day 26

the vertical to assure root growth towards the transparent plate (Fig. 1). The experiments were conducted in a climate chamber with a temperature of 22 °C, relative humidity of 70 %, and 18 h light with a photosynthetic photon flux of approximately 450 $\mu molm^{-2}s^{-1}$.

Scanning of the root systems was initiated when the tomato seedlings were 2 weeks old. At that time, the main root reached the bottom of the chambers, and primary laterals were present in both the upper and lower layers. The root chambers were scanned for 2 day, before the chambers were removed from the scanners and sealed for 24 h (see below). The chambers were then waterlogged, and placed in the scanners again for either 24 h or 5 day. Root chambers were removed from the scanners after the waterlogging period, the silicon sealant was peeled off and the chambers were drained. The drained root chambers were placed in the scanners again and scanned for additional 5 day to follow recovery after waterlogging.

In each experiment, three root chambers were used as control treatments and three root chambers were either short-term (24 h) or long-term (5 days) waterlogged. Before waterlogging, a tube (3 mm diameter) was placed inside the chambers from the bottom to the top along the side. Chambers were sealed at the bottom and 200 mm up the side by aquarium silicon sealant and left for 24 h to harden. Six short strips (approximately 40 mm) of silicon sealant were applied to the sides of the control chambers to ensure that possible differences in root elongation rates was not a result of application of silicon sealant. In the waterlogged treatments, demineralised water was applied through the tube, waterlogging the chambers from beneath. The tube was connected to a 100 ml syringe functioning as a reservoir for water, which was placed at a height corresponding to the water level of the chambers. The lower layer (defined as the bottom 150 mm) of the chambers were waterlogged, while the upper layer (defined as the top 150 mm) were kept moist but not waterlogged. Whenever necessary during the waterlogging treatment (when water level dropped >5 ml), water was applied to the reservoir in order to keep the water level constant.

Six flatbed scanners (Canon, Canoscan 5600 F) placed at an angle of 30° from the vertical (the same as when the plants had been growing) were used for the experiment (Fig. 1a). Software enabling all scanners to scan automatically every 3 h with a resolution of 2552× 3508 pixels was developed. Two weeks after sowing, the root chambers were placed in the scanners and only removed occasionally from scanners for short durations, when sides had to be sealed after 2 days of scanning, when drained after waterlogging, or for irrigation. Root chambers were irrigated from beneath by placing the chambers in a container with the bottom 100 mm filled with fertilizer solution for 0.25 h allowing the solution to enter in the gaps between the front and back plates, and distributed by the capillarity of the growing medium. This was done before waterlogging, after cessation of the long-term waterlogging experiment, and once during the scanning period after waterlogging. The fertilizer solution contained 0.5 M $Ca(NO_3)_2$, 0.5 M KNO_3, 0.2 M $MgSO_4$, 0.1 M KH_2PO_4 as well as micronutrients. In addition, irrigation was applied from above whenever necessary without removing the chambers from the scanners.

Oxygen

Oxygen content was determined in the lower waterlogged layer of the growing medium by oxygen sensors (TNO optical oxygen sensor, Zeist, The Netherlands) based on measurements of fluorescence created by an oxygen sensitive fluorescent dye at the tip of the sensor. The sensor consisted of a fibre optic connection ending in a stainless steel probe with the oxygen sensitive fluorescent dye on the tip. The sensors were 150 mm long with a 6 mm diameter, and were inserted into the waterlogged layer of the chambers through a hole in the side.

Shoot and root parameters

At termination of the experiments the shoot was cut just above the uppermost root, and fresh weight was

determined as well as leaf area on a LI-3100 Area meter (LI-COR, inc. Lincoln, Nebraska, USA). The plant material was oven dried at 70 °C for 24 h, and dry weight was determined. The medium of the six root chambers in the long-term waterlogging experiment was divided in an upper and lower layer, and roots were carefully washed free of peat. The roots were colored by neutral red (3-Amino-7-dimethylamino-2-methylphenazine hydrochloride), distributed in a water filled tray and analyzed for total root length and diameters by the software WinRhizo (Regent Instruments, Quebec, Canada). Roots were dried at 70 °C for 24 h, and dry weight was determined.

Root elongation rate per 3 h time interval were calculated from the displacement of each root tip, in both *x* and *y* directions at successive scans by the use of image analysis software (Image J, http://rsbweb.nih.gov/ij/), assuming linear growth within the 3 h intervals. The x coordinates of the root tip of two successive scans were subtracted from each other, as were the y coordinates, and the root elongation was calculated as the hypotenuse of a right-angled triangle. Root tip coordinates were determined on 16 single roots selected at random from within each root chamber; eight roots were selected from the upper layer, and eight roots from the lower layer. The same root tip was followed during subsequent scans for as long as possible. Whenever a root ceased growth or grew away from the observation surface it was replaced by another root.

Statistical analysis

Root elongation rates, plant parameters and root length and dry weight were averaged over 3 plants and statistical analysis of differences were tested by analysis of variance (F-test). Multiple comparisons were based on values of least significant difference (LSD) derived from analyses of variance (Proc GLM, SAS Institute Inc., Cary, NC). Statistical tests with probability $p<0.05$ were considered significant (rejection of null hypothesis).

Results

Root elongation rates

Root elongation rates varied between 0.1 and 1.1 mm h^{-1} $root^{-1}$ in the control treatments with the slowest elongation rates observed at initiation of the scanning period as well as towards the end of the long-term waterlogging experiment (Fig. 2a). Root elongation rates were fastest (up to 1.3 mmh^{-1} $root^{-1}$), during the waterlogged period (19–23 days old tomato plants) in the upper layer of the long-term waterlogged treatments, and in the lower layer of the waterlogged treatments (up to 1.4 mmh^{-1} $root^{-1}$) at the end of the experiment.

Waterlogging the lower layer of the root chambers for either 24 h or 5 day immediately decreased the root elongation rate. In the short-term waterlogging experiment root elongation decreased significantly after 3 h, and most roots had ceased elongation 6 h after initiation of waterlogging. After 9–12 h and for the remaining waterlogged period root elongation stopped (Fig. 2b). When the water was drained and air re-

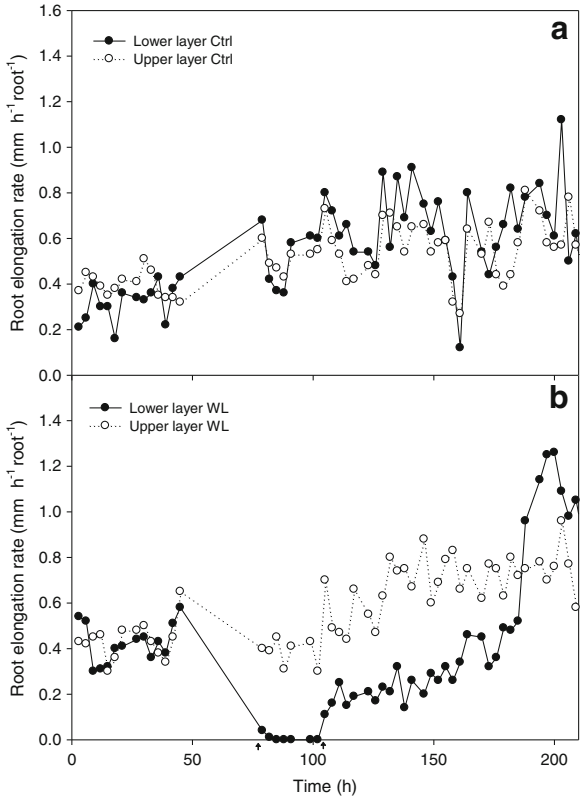

Fig. 2 Root elongation rates for short-term waterlogging determined as mm h^{-1} on 3 h intervals. **a** Root elongation rates in the upper (*hollow circles*) and lower (*filled circles*) layer of the control treatment irrigated regularly and **b** root elongation rates in the upper and lower layer of root chambers waterlogged for 24 h. *Arrows* at the x-axis indicate the initiation and cessation of the waterlogging period. Results are means of three replicates

entered (time 100 h), root elongation rates increased again although with slower elongation rates than roots in the upper layer initially (day 5–7) ($p<0.001$). At the end of the short-term experiment root elongation rates in the lower layer accelerated steeply (peaking at 1.2 mm h^{-1} $root^{-1}$ at time 200 h) exceeding the elongation rates of the upper layer and both layers in the control treatment ($p<0.001$). The root elongation in the upper layer of the short-term waterlogged treatment increased slightly, on average to 0.63 mm h^{-1} $root^{-1}$ during and after the waterlogging period compared to an average of 0.55 mm h^{-1} $root^{-1}$ in the upper layers of the control.

In the long-term (5 day) waterlogging treatment root elongation was not determined 3 h after the lower layer was waterlogged due to a technical problem with the scanners. Thus, the first measurement was made after 6 h when root elongation had ceased (Fig. 3b). During the period of waterlogging no root elongation was observed in the lower layer. Roots that had been waterlogged for 5 days did not resume growth, making the plants dependent on new roots growing into the previous waterlogged layer from above. A steep acceleration in growth rate was seen when the new roots grew into that layer (Fig. 4b). Root elongation rates in the upper layer above the waterlogged zone (open triangle symbols at time 5–8 day) accelerated significantly and their elongation rate was on average 40 % higher than that of the upper layer in the control treatment during and after the waterlogging period ($p<0.001$). There were few differences in elongation rates of roots in the upper and lower layers of the control treatments (Figs. 2a and 3a). In the short-term waterlogging experiment, elongation rates were not significantly different ($p<0.05$) at day 1 where elongation rates were faster in the upper layer (Fig. 4a). In the long-term waterlogging treatment significantly ($p<0.001$) faster elongation rates were observed in the lower layer of the control treatment in the range 7–9 days from initiation of the time-lapse scanning (21–23 days old tomato plants) (Fig. 4b).

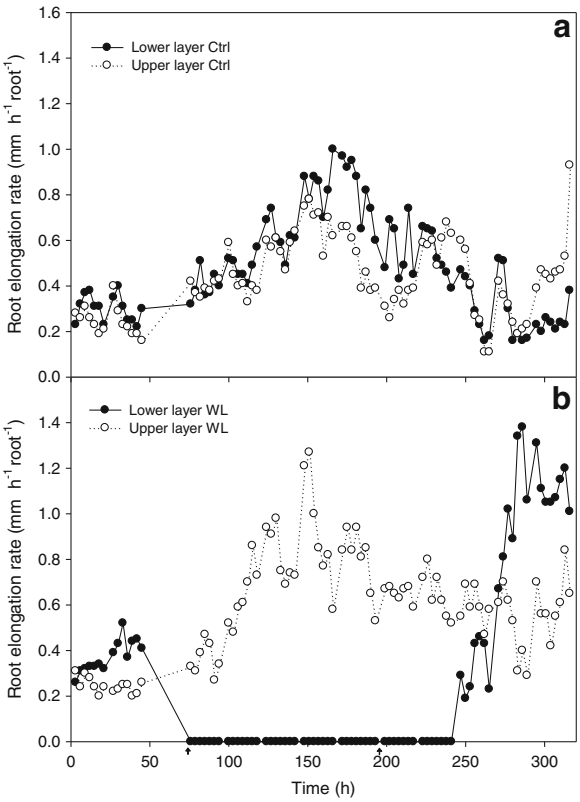

Fig. 3 Root elongation rates for long-term waterlogging determined as mm h^{-1} on 3 h intervals. **a** Root elongation rates in the upper and lower layer of the control (ctrl) treatment irrigated regularly and **b** root elongation rates in the upper and lower layer of root chambers waterlogged (WL) for 5 day. *Arrows* at the x-axis indicate the initiation and cessation of the waterlogging period. Results are means of three replicates

A tendency towards a diurnal pattern in root elongation rates was observed during the 9–13 days of root observation in the two experiments. During most days root elongation seemed to increase during the light period, peaking around 6 h after initiation of the light period. Hereafter, root growth rates decreased again until initiation of the dark period. This was the general trend although not all cycles followed this pattern strictly.

Oxygen content

Oxygen concentration corresponded to ambient concentrations before and immediately after initiation of the waterlogging. However, during the first 20 h of waterlogging the oxygen concentration gradually decreased resulting in anoxic conditions (Fig. 5). The anoxic conditions continued as long as the medium was waterlogged. As soon as the medium was drained oxygen re-entered, and within 6 h oxygen concentrations reached ambient levels again (Fig. 5).

Shoot and root properties

The duration of the short-term waterlogging (24 h) was too short to cause significant effects on the shoot

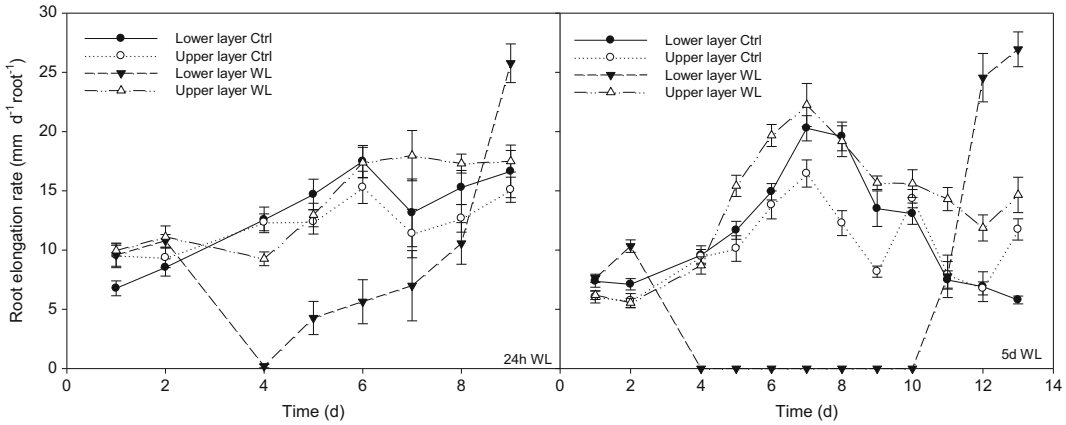

Fig. 4 Root elongation rates determined on 3 h intervals and averaged over a day. **a** Root elongation rates in the control and 24 h waterlogged treatments, and **b** root elongation rates in the control and 5 day waterlogged treatments. Results are means of three replicates and bars indicate standard error

parameters determined (Table 2). However after 5 days of waterlogging the shoot growth was significantly reduced. Fresh weight was lower than in the control treatment ($p<0.05$), whereas differences observed in the dry weight of the shoots were smaller and not significant. This was due to significantly greater dry matter content in the long-term waterlogged plants. The total fresh weight, stem fresh weight and leaf area of the long-term waterlogged plants was significantly lower ($p<0.05$) than these properties in the control treatment. No significant differences in total fresh weight, stem fresh weight, and leaf area was found between the control and waterlogged plants of the short-term waterlogging experiment.

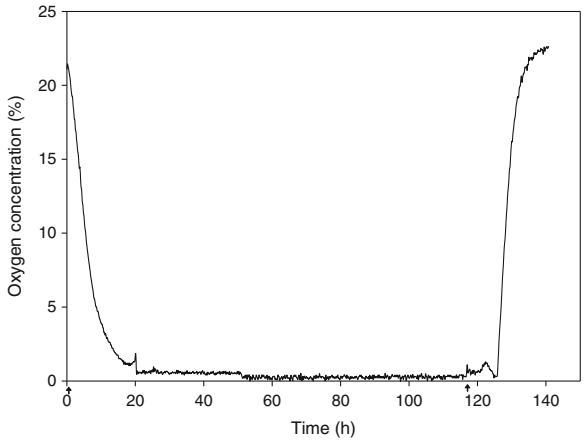

Fig. 5 Oxygen concentration in the 5 day waterlogged treatments in lower layer of root chambers during waterlogging and after water was drained. *Arrows* indicate initiation and cessation of waterlogging. Results are means of three replicates. (SE=1.8)

Long-term waterlogging decreased the total root length but increased the average root diameter at the end of the experiment compared with the control (Table 3). Despite the increased growth rate during the waterlogged period in the upper layer the total root length was not higher than in the control. In the lower layer of the waterlogged chambers the root length was significantly decreased ($p<0.05$) after 5 days of waterlogging. Root dry weight differed between the upper and lower layers of the root chambers with less dry weight in the lower layers ($p<0.05$). No significant differences in total root dry weight were observed between the waterlogged treatment and the control (Table 3).

Discussion

Oxygen in the growth medium

Waterlogging part of the root system altered elongation throughout the entire root system. Roots responded fast by ceasing growth in the waterlogged layer after just few hours of waterlogging. Waterlogging decreases oxygen diffusion into the medium very strongly. The rate of transition to hypoxia and anoxia can be explained by the depletion of oxygen around the root (presumably due to root respiration). The change to hypoxic or anoxic conditions has an immediate effect on plant metabolism (Licausi 2011). Despite oxygen remaining in the medium up to 20 h after waterlogging, especially the plants in the long-term waterlogged

Table 2 Shoot parameters determined at harvest after short-term waterlogging (24 h) or long-term waterlogging (5 day) compared to a regularly irrigated control. Results with different letters within each column are significantly different ($P<0.05$), ($n=3$, ±SE)

Experiment	Treatment	Fresh weight	Dry weight g	Stem fresh weight	Leaf area cm^2	Dry weight percentage %
24 h	Control	16.4±1.4^{b}	2.1±0.3^{b}	6.8±0.6^{b}	387.1±21.6^{b}	12.5±0.6^{b}
	Waterlogged	14.5±1.9^{b}	1.9±0.2^{b}	6.1±0.8^{b}	342.4±44.8^{b}	13.4±0.6^{b}
5 day	Control	25.8±1.7^{a}	3.2±0.2^{a}	11.5±0.6^{a}	552.4±35.9^{a}	12.2±0.1^{b}
	Waterlogged	15.3±1.2^{b}	2.5±0.3^{ab}	6.8±0.6^{b}	325.1±24.4^{b}	16.5±0.6^{a}

treatment responded fast by decreasing root elongation within 6 h of waterlogging. Oxygen was determined by the sensor at one point in the medium, closer to the upper layer than most roots. Hence, oxygen concentrations around the individual roots probably decreased even faster. The rate of oxygen depletion depended on the surroundings of the individual root tips as well. In a study measuring redox potentials by microelectrodes, Fischer et al. (1989) showed that redox potentials decreased steeply when a root tip crossed the microelectrodes indicating high oxygen consumption in active root tips. Thus, low oxygen concentrations in the entire medium would probably result in anoxic conditions around the root tip. The oxygen in the waterlogged layer would be used rapidly during root respiration, and with the greatly reduced diffusion of oxygen in water compared with air; diffusion into the waterlogged layer was too slow to maintain aerated conditions.

Lack of aerated conditions is detrimental to the development of root systems. Most roots depend exclusively on oxygen found in the surrounding environment for aerobic metabolism, and well-aerated tomato plants have been shown to roughly take up 0.15 $mg\,g^{-1}$root h^{-1} O_2 (Veen 1988). Thus, when kept waterlogged the oxygen concentration in the medium will decrease rapidly, and roots will cease growth, and may eventually die (Visser and Voesenek 2004). This was the case during long-term waterlogging where roots in the lower layer did not resume growth after drainage, and the subsequent root growth originated from roots above the previous waterlogged layer. However, after short-term waterlogging new lateral roots were produced within the lower layer, indicating that roots survived the short-term waterlogging in a stand-by state (Morard and Silvestre 1996). The steep increase in elongation rates towards the end of the short-term waterlogged treatment was probably caused by new roots growing into the lower layer from above. When plants are grown in small pots the shift in oxygen availability is expected to occur rapidly when waterlogged, due to the small medium volume with high density of roots, leading to ceased elongation. As potted plants normally are irrigated frequently the risk of repeated (or prolonged) waterlogging, leading to low oxygen concentrations will increase stress on roots.

Waterlogging and individual root elongation

While root elongation ceased when waterlogged, root elongation rates increased above the waterlogged layer compared to the aerated control, especially during long-term waterlogging. By increasing root elongation

Table 3 Root length, diameter and dry weight determined at harvest after long-term waterlogging (5 days) compared to a regularly irrigated control. Results with different letters are significantly different ($P<0.05$), ($n=3$, ±SE)

Treatment		Root length m	Root diameter mm	Root dry weight g
Control	Upper part	26.5±2.3^{a}	0.49±0.009^{b}	0.28±0.035^{a}
	Lower part	23.9±1.0^{a}	0.50±0.001^{b}	0.12±0.008^{b}
	Total	50.3±3.3		0.40±0.042
Waterlogged	Upper part	26.8±1.7^{a}	0.54±0.003^{a}	0.31±0.032^{a}
	Lower part	10.6±0.5^{b}	0.55±0.011^{a}	0.06±0.004^{b}
	Total	37.4±1.6		0.36±0.028

rates in the non-affected layer, plants may compensate for the lack of growth in the lower layer maintaining a constant root/shoot ratio. Generally, plants invest in roots located where the greatest return can be derived, and remove resources from areas where the returns are less (Hodge 2009). During short-term waterlogging only a slight increase in root elongation rates was observed in the upper layer, most likely because the entire root system was not detrimentally affected within this short time span. However, an increase in uptake rates in the non-affected roots might be the initial step of compensation as the N demand of the shoot remains unaltered (Dresbøll and Thorup-Kristensen 2012). In addition, plants are expected to have a certain response time to reallocate resources, and 24 h might be too short to achieve this, explaining the limited increase in root elongation rates in the upper layer of the short-term waterlogging treatment.

The total root and shoot biomass did not change significantly during short-term waterlogging even though roots were inactive in the waterlogged layer. On the contrary, during long-term waterlogging, shoot growth was impeded and hence N demand decreased. As the roots in the waterlogged layer of the long-term waterlogged plants were irreversibly damaged, significant higher root growth was observed above the waterlogged layer in order to maintain a stable root/shoot ratio. Comparable results were shown in split-root systems, where removal of half of the root system led to an increase in new primordial formation on the remaining roots. In this way, number of primordia was kept at the same level as in roots which had not been halved (Gersani and Sachs 1992). Thus, the increased root elongation in the upper layer compared to the control upper layer was probably a result of compensation for the lack of growth in the lower layer.

Waterlogging and the consequences for the root system

Root elongation rates in the control upper and lower layer of the chambers were found to be similar. This indicated an even distribution of water and nutrients as well as evenly compacted media. Root growth and uptake rates are generally high in young roots and decrease with time (Volder et al. 2009), and the elongation rates determined here correspond to ranges previously determined during and after waterlogging (e.g. Laan et al. 1991). During the experiment new laterals were produced, eventually taking up most of the space within the root chambers. Towards the end of the experiment, the decrease in root elongation rates in the control treatments might be explained by drying of the medium—root elongation in drying soil will decrease as a combination of water stress and increased mechanical impedance (Bengough et al. 2011). The proportionally large control plants, with high water usage as a proportion of water stored in the medium volume, made it difficult to maintain sufficient water content in the medium. In addition, at this point roots were very well distributed in the entire medium, which might have led to internal competition for water and nutrients.

Uses of scanning systems to study root responses

The frequent scans revealed a tendency towards a diurnal pattern with lower root elongation during late day/early dark period and higher during the light period. However, as the experiments were not designed to take diurnal patterns into account, some 3 h scanning intervals could bridge both the dark and the light period. A diurnal pattern in root elongation peaking a few hours after the beginning of the light period was shown in *Arabidopsis thaliana* when capturing time lapse records every 30 min of seedlings grown on agar in petri dishes (Yazdanbakhsh and Fisahn 2011). Thus, if scanning more frequently, this method could reveal circadian rhythms of root elongation.

The method used, placing root chambers permanently in the scanners for time lapse scanning, provided detailed information of root elongation rates. This made it possible to follow single root growth and elongation for a relatively long period of time. We saw no evidence of root growth being affected by the short exposure (<5 s every 3 h) to light when scanned as roots remained at the transparent front. However, handling of the chambers when preparing them for waterlogging or irrigation should be done carefully to avoid exposure to light, as this would result in roots growing into the medium away from the transparent front. Root system architecture, growth, and function are difficult to study *in situ*. All root methods have their strengths and weaknesses as roots are either disturbed (excavation, minirhizotrons) when studied, grown under artificial conditions (hydroponics, gel media), or in very restricted volumes such as those used for CT scans (Zhu et al. 2011). Despite the fact that these thin

chambers also provide an artificial and restricted environment for the roots, the permanent placement in flatbed scanners proved to be efficient in showing root responses to spatial and temporal variation, as well as ensuring that substrate-root interactions were taken into account. The method is still limited to relatively young plants depending on plant species (up to 26 days old tomato plants) as the roots are distributed in the entire chamber at that time. Hammac et al. (2011) showed detailed information on root hair growth and development by placing scanners in the soil in large pots, confirming that recent developments in technology renders it possible to use these high-resolution flatbed scanners for detailed root studies.

Plants generally grew well in the flatbed scanner setup. The shoot size was smaller in long-term waterlogged plants, whereas no significant effect of short-term waterlogging was seen on the aboveground plant parts. The size of the long-term waterlogged shoots corresponded to the size of the control and waterlogged plants from the short-term experiment despite having grown for additional 4 days. This indicated strong inhibition of shoot growth during the extra days of waterlogging in the long-term experiment. Episodic waterlogging was shown to promote stomatal closure and disturb the functioning of the photosynthetic apparatus of field bean plants, and these responses were expected to contribute to the slow growth of waterlogged plants (Pociecha et al. 2008). The greater dry matter content seen after long-term waterlogging in the tomato plants was probably due to storage of starch, as the amount of soluble sugars and starch generally increases in leaves of waterlogged plants (Gonzalez et al. 2009). When root growth and activity decrease, sink capacity for sugars produced in leaves become limited, and plants will accumulate starch and soluble sugars in the leaves, leading to an increased dry matter percentage. This has previously been shown in long-term waterlogged tomato, where starch contents approximately doubled during 5 days of waterlogging compared to non-waterlogged control treatments (Dresbøll and Thorup-Kristensen 2012).

Implications of root responses to waterlogging for container grown and other crops

No differences in total root dry weight between waterlogged and control treatments were found at the end of the experiments. The time after waterlogging revealed that root elongation recovered fast when air re-entered the medium. Towards the end of the experiment the plants were large with a well distributed root system exploiting the entire area and thus not resulting in any differences in root length. However, waterlogged roots had a larger diameter than control roots, which corresponded to results showing that when oxygen partial pressures decreased, an increase in root diameter was correlated to radial cell expansion whereas root elongation decreased (Eavis 1972). The fast recovery of the roots, resulting in similar root dry weights just 5 days after half of the root system was damaged during waterlogging, revealed the plasticity of roots.

In conclusion, the method using time-lapse scanning to follow root growth in semi-natural environments was shown to be applicable, and provided detailed information of root elongation rates. The entire root system of tomato plants grown in restricted volume was shown to be affected by oxygen deficiency in part of the medium. Roots above the waterlogged layer increased elongation rates compared to regularly irrigated controls as compensation for the decrease in elongation rates of roots directly affected by waterlogging and hence oxygen shortage. This was especially the case when plants were long-term waterlogged. For high value crops grown in pots in media with optimal physical properties, compensation in the upper layer will result in less damage. However, if capillarity is disrupted, waterlogging can be even more detrimental, as elongation and nutrient uptake ceases in the wet anoxic zone in the bottom layer of the pots, while the upper layer will be dry preventing compensatory root growth in this layer.

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