Plant root growth against a mechanical obstacle: the early growth response of a maize root facing an axial resistance is consistent with the Lockhart model

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Plant root growth is dramatically reduced in compacted soils, affecting the growth of the whole plant. Through a model experiment coupling force and kinematics measurements, we probed the force–growth relationship of a primary root contacting a stiff resisting obstacle, which mimics the strongest soil impedance variation encountered by a growing root. The growth of maize roots just emerging from a corseting agarose gel and contacting a force sensor (acting as an obstacle) was monitored by time-lapse imaging simultaneously to the force. The evolution of the velocity field along the root was obtained from kinematics analysis of the root texture with a particle image velocimetry derived technique. A triangular fit was introduced to retrieve the elemental elongation rate or strain rate. A parameter-free model based on the Lockhart law quantitatively predicts how the force at the obstacle modifies several features of the growth distribution (length of the growth zone, maximal elemental elongation rate and velocity) during the first 10 min. These results suggest a strong similarity of the early growth responses elicited either by a directional stress (contact) or by an isotropic perturbation (hyperosmotic bath).

1. Introduction

1.1. Background

Plant roots take up the water and nutrients required to satisfy the shoot demand. They also ensure the mechanical anchorage of the plant in the soil to provide a stable basis for the shoot emergence and for its resistance to external loads due for example to wind blowing, soil erosion or shallow landslides [1,2].

The root system architecture, that is the three-dimensional spatial arrangement of the different root types, derives from branching and growth of individual roots, that continuously sense and adjust their growth according to their local environment [3]. In addition to environmental cues like water or nutrient availability, changes in branching, growth rate or growth direction depend on the mechanical stresses experienced by the growing roots [4]. In particular, the root growth velocity decays with increasing soil strength, resulting in a reduced total root length and poor above-ground development [5]. Typically for maize, the root growth velocity was reduced by 50% when the soil resistance measured with a mini-penetrometer increased from 0.2 to 2 MPa [6]. For all species, root elongation stops when soil strength is too large.

In the current context of climate change, the frequency of extreme wet/dry and freeze/thaw cycles can exacerbate the compaction of soils and increase...
their strength, thereby limiting crop yield [7]. As a consequence, breeding programmes for plant species of agronomic interest such as wheat, soybean, rice or maize have been developed in soil science and ecophysiology communities to identify which root traits give the better plant fitness in large strength soils [8,9]. In particular, some works focused on macroscopic traits such as the number of root axes or the root tortuosity in relation with soil strengths [10–12]. However, the underlying mechanisms at the root apex scale when the root tip encounters a hard pan, a compacted soil horizon or simply a rigid stone and experiences a huge resisting force, are still poorly understood. One of the main difficulty arises from getting reliable spatio-temporal information of root growth in opaque soils.

From a more fundamental point of view, the question arose as to quantify the maximum growth pressure, i.e. the maximum axial stress a root is capable to exert on a resisting obstacle in different species. The assumption behind these studies was that roots having greater growth pressure might more easily penetrate stronger soil layers and access water and nutrient pools. Interestingly, the first measurements of axial force generated by growing roots were done by Pfeffer as earlier as 1893 [13] and reproduced long after by Gill et al. [14] and Souty [15]. More recently, different techniques such as calibrated spring system, elastic beams, or digital balances have been used (cited in Clark et al. [16]) to measure root axial pushing forces in model experimental systems. By dividing the maximum force value by the root cross section, growth pressures \( \sigma_{\text{max}} \) of the order of 0.1 to 1 MPa have been obtained and were always in the range of the turgor pressure, that is the inner hydrostatic pressure inside the plant cells [5,17,18]. However, the precise values of \( \sigma_{\text{max}} \) appeared to vary with the species, but also with the protocol for root growth and measurements. In particular, \( \sigma_{\text{max}} \) depended on the way the root tip was anchored or on the location and the time of root diameter measurements [19]. Indeed, feedback processes due to active root responses to confining geometries could occur within different time scales [20].

In this context, real-time information on the growth processes is needed, but until now only very few studies recorded both force and strain-rate field [21] and had high temporal resolution to detect potential rapid biological responses.

### 1.2. Root growth

Primary root growth, that is root elongation, occurs in a so-called extended ‘growth zone’ located behind the root tip. This growth zone includes a meristematic zone where cells proliferate and an elongation zone where cells rapidly expand [22]. The cells reaching the transition zone between the meristem and the elongation zone, leave their meristem active state and expand rapidly, increasing their volume by up to 200 times. In addition, cell expansion is strongly anisotropic, leading to the characteristic cylindrical shape of the root. The plant cell is delimited by an semi-permeable membrane surrounded by a rigid cell wall. The imbalance of osmotic pressures between the inside and the outside of the cell, on either side of the semi-permeable membrane, results in an internal hydrostatic pressure, coined turgor pressure (or simply turgor). This pressure puts the cell wall under tension which might positively regulate cell expansion.

From a theoretical point of view, growth is usually modelled by extending the so-called Lockhart laws [23] established for the expansion of a plant cell, in which the hydraulic conductivity of the cell membrane and the biomechanical properties of the cell wall control the cell expansion rate [24]. One of the Lockhart laws relates the strain rate of a plant cell and the turgor pressure \( P \) that puts the rigid cell wall under tension. Indeed, the cell wall expansion regulated by turgor follows the same numerical law as a Bingham fluid which deforms inversely above a yield stress: when turgor pressure \( P \) exceeds a given threshold \( Y_P \), in pressure, cell wall flows and expands. Even if the pressure \( P \) is isotropic, the cell wall of an expanding cell is mechanically anisotropic so that the expansion mainly occurs in the axial direction. Thus, the simplest formulation of the Lockhart law can be stated in one dimension and involves the axial strain rate or elementary elongation rate (EER) at the cell scale with

\[
EER = c_P \left( P - Y_P \right)_+ ,
\]  

where \( c_P \) is an extensibility parameter. The sign ‘+’ means that \( EER \neq 0 \) (growing cell) if the pressure \( P \) is larger than the threshold \( Y_P \) and that \( EER = 0 \) for a non-growing cell.

These Lockhart laws established for a single plant cell have been extended to plant tissues. In roots, the membrane hydraulic conductivity appeared not to be limiting cell expansion, and thus the growth rate appears to mostly depend on the cell wall properties [25]. Experimentally root growth can be quantitatively described by the evolution of the axial strain rate \( EER \) along the root apex by use of kinematics [26,27]. Kinematics analysis provides the velocity field and its spatial derivative gives the strain-rate field [26]. This is a non-destructive way to obtain the local growth distribution, including the growth zone length (from the location where the local strain rate is non-zero) as well as the maximum strain rate. For root elongation, the experimental strain rate shows a bell-shaped curve along the growth zone, but turgor pressure is observed to be constant, meaning that the parameters involved in equation (1.1) depend on the location along the root apex.

### 1.3. Root growth in impeding soil

In the presence of an impeding soil, root growth decreases with increasing soil strength. To include the effects of soil strength on root growth velocity, soil scientists proposed to consider the external resisting pressure of the soil as simply increasing the yield threshold for growth [28]. If the derived phenomenological laws give the trends for growth velocity decaying with soil strength, the underlying assumptions are questionable and the force balance equations need to be rationalized. In particular, it is not clear what soil stress should be incorporated inside the derived Lockhart equations, as \textit{in situ} measurements probe the soil strength resisting the penetration of a penetrometer and do not probe the external resisting stress really experienced by the root tip during root growth. Moreover, the parameters such as turgor pressure, cell wall extensibility or yield threshold usually involved in Lockhart models are physiological quantities that are regulated over time by the root. They were shown to depend on the mechanical stress history of the root loading [20].

In this work, we address the question of how an external mechanical stress impacts the root growth rate by the use of a model experiment. We want to establish the force–velocity
relationship to identify clearly how the growth process is modified with time when the root encounters a stiff obstacle and pushes against it. To answer this question, we built a new experimental set-up combining force and kinematics measurements with a spatio-temporal analysis of the root growing against a force sensor acting as an obstacle. We chose a model species, maize, whose radicle has a typical diameter in the millimetre range and whose growth in the absence of mechanical stresses has been well characterized. Our analysis not only provides the root growth velocity but also fine kinematic parameters such as the growth zone extent and the local strain rate. These kinematic parameters are essential for characterizing the growth process and confronting them to growth models such as the Lockhart law [23].

2. Material and methods

2.1. Experimental set-up

The set-up is made of a growth chamber mounted on a support with a movable part allowing the vertical adjustment of the force sensor just underneath the channel the root grew in. The growth chamber was made of two parallel glass plates (height 50 mm, width 70 mm) placed vertically and held together at a distance of 10 mm by an assembly of laser-cut Plexiglass walls (figure 1a).

Agarose powder (2% w/v, SeaKem LE Agarose) was dissolved in deionized water at a temperature of 90°, until becoming transparent. A small amount of the solution was then poured in the chamber, allowed to cool in order to seal the bottom edges, then the rest was poured in until the chamber was full. A graphite rod of calibrated diameter (0.7 mm), slightly smaller than the root diameter (approx. 0.9 mm), was inserted and maintained vertically in the middle of the cell until the agarose gel solidified. Afterwards, the graphite rod was removed, forming a vertical channel for the root to grow in (figure 1b). The channel was made in order to guide the root vertically. In this way, no bending due to circumnutation could occur, the root grew straight along the gravity and arrived perpendicular to the horizontal surface of the force sensor.

Figure 1. Experimental set-up. (a) Schematic view: the agarose-filled cell (blue) is fixed on a support (grey). The vertical position of the force sensor (red) can be adjusted just at the outlet of the vertical channel guiding the root growth. A three-dimensional-printed support (yellow) holds all the electrical wires. The input wires are connected externally (not shown) to a stabilized power supply and the output wires are connected to a LabView interface. A CCD camera saves the images of the root illuminated by infrared lighting. (b) Global view of the growth chamber with the transparent agarose gel filling the chamber and the maize root inside the vertical channel perpendicular to the horizontal surface of the force sensor.

Maize seeds (variety P9874, Pioneer) were soaked in deionized water for 24 h and held upright, root tip ionized water, aerated thanks to a bubbler for 24 h, then were transferred to humid paper for 24 h and held upright, root tip facing down in the direction of gravity. Seedlings with a 0.5 to 1 cm long radicle were selected for the experiments. The radicle was carefully inserted in the channel at the top of the chamber, and the seed was maintained in place with a parafilm sheet. Vaseline was spread on the bottom-exposed areas of the agarose gel to avoid water losses. Then the agarose-filled chamber was fixed on the support just over the force sensor.

The set-up allowed the simultaneous monitoring of the root growth (through the agarose gel) and of the root force when the radicle emerged from the gel and pushed against the force sensor. The root grew in the vertical channel for around 19 h without experiencing any mechanical resistance at its root tip. Then the root tip contacted the force sensor acting as an obstacle. Temperature was continuously monitored and experiments were conducted with temperatures in the range of \( \theta_0 \in [21^\circ\mathrm{C} - 28^\circ\mathrm{C}] \). For each experiment with a given \( \theta_0 \) the standard deviation on \( \theta_0 \) did not exceed 0.5°C during the time course of kinematics measurements. In this range, the 2% w/v agarose gel behaves as a stiff elastic material with an elastic modulus of around 22.5 ± 0.5 kPa (measured with a HAAKE Rheometer, Thermofisher).

2.2. Force measurements

The force was measured thanks to a Futek (LSB200) force sensor, with a maximum range of 100 g and a stiffness of 4828 ± 5 N m\(^{-1}\). The signal was acquired at a frequency of 1000 Hz, then averaged every second during the whole experiment duration. In order to avoid the root tip slipping when contacting the force sensor, we fixed a rough sand-covered plexiglass rectangle on the top face of the force sensor. The sand particles (diameter ≤ 350 μm) were painted black to avoid any unwanted light reflections. As said before, the root growth was guided by the vertical channel, so that the root axis was perpendicular to the top face of the force sensor. In this way, the whole pushing force of the root was measured and not only a projected component, which could be the case if the root apex arrived at an oblique angle to the vertical.

2.3. Kinematics of root growth

2.3.1. Experimental observation and image processing

Root growth was monitored by time-lapse photography. The whole set-up was illuminated with low angled infrared (IR) lighting (wavelength \( \lambda = 850 \) nm) (figure 1a). This IR lighting has two...
advantages. First, IR lighting does not stimulate photoreceptor systems, does not affect root growth nor generates tropism [29,30]. Second, IR lighting gives a texture to the root surface, that is, a pattern of bright points that can be followed along time to get the displacement field along the root [22]. For this kinematics study of the root growth, we used a high-resolution CCD camera (Nikon D5200, 4000 x 6000 pixels) whose IR absorbing filter was removed, with a macro lens (Nikkor 60 mm). The observation field was 25 mm in height with a typical resolution of 4.7 μm per pixel. When the root tip was entering the observation field and approaching the contact, the images were taken every minute during 15 h.

Then, images were processed with Kymorod, a MATLAB app developed by Bastien et al. [31], that performed particle image velocimetry (PIV) on the texture of elongating organs. Kymorod was run with a time lapse of 4 min between images. We recovered the raw displacement fields as a function of the curvilinear abscissa along the root skeleton (figure 2) and used a fitting procedure to determine the local velocity \( v_l \) and the strain rate (noted EER for elementary elongation rate) profiles.

2.3.2. Velocity, strain-rate profiles and fitting procedures

Numerical derivation can be done in numerous ways by deriving local fits (splines) or global fits. To minimize the number of parameters and make the EER estimation the more robust possible, we chose the simplest global fit with the following constraints: the fit function for the strain rate profile EER(s) must have a finite support (for modelling the limited extent of the growth zone and the absence of growth elsewhere) and once spatially integrated, it should give a sigmoid shape similar to velocity profile. These conditions required that the fit function for EER(s) had to be an isosceles triangle

\[
s \rightarrow 2a(s-b) \mathcal{Y}_{[b+c,s+c]} + (2ac^2 - a(s-b-2c)^2) \mathcal{Y}_{[b+c,s+c]} + \mathcal{Y}_{[s+c,c]} \quad (2.1)
\]

where \( \mathcal{Y}_{[x,y]} \) is the function that is 1 in between \( x \) and \( y \) 0 elsewhere and with \( a, b, c \) the three fit parameters describing the triangular shape. The function family (2.1) stands for triangle shape displacement profile whose height is the maximum strain rate \( EER_{max} = 2ac \) and whose base is the length of the growth zone \( L_{GZ} = 2c \) (see the triangular sketch of figure 8).

Hence the velocity fit function family is obtained by spatial integration over \( s \) of the function (2.1)

\[
s \rightarrow a(s-b)^2 \mathcal{Y}_{[b,c]} + (2ac^2 - a(s-b-2c)^2) \mathcal{Y}_{[b+c,c]} + \mathcal{Y}_{[b+2c,c]} 2ac^2 + d, \quad (2.2)
\]

with four parameters only, one of which \( d \) being due to integration. Other velocity fit functions were proposed in the literature [32]; this one was retained for having a finite support of the growth zone and only four parameters. In this way, growth velocity \( v \) of the root is given by the maximum of the fit function (2.2).

3. Experimental results

3.1. Growth before contact

The roots grew along the vertical channel inside the agarose gel. The water required for root growth was supplied by the gel surrounding the root and a drop of water probably released by the compressed gel was observed just at the root cap, preventing any dehydration of it. The root diameter being slightly larger than the channel, there was no air interface between the root and the gel, allowing a good IR specs visualization and PIV processing. After a transient regime of 2 h during which the root growth velocity increased, a stationary regime was reached with a typical velocity of around 4 cm d⁻¹.
The imaging of the root with IR lighting and the subsequent analysis with the PIV analysis of Kymorod allowed to obtain the root skeleton (yellow line in figure 2) and the local velocity profile \( v_l \) as a function of the curvilinear abscissa \( s \) along the root length. A typical example of this profile before the root contacts the obstacle is given in figure 2b. The reference \( s = 0 \) was arbitrarily set at the extreme point of the observation frame towards the seed. The velocity \( v_l \) increased from the curvilinear abscissa \( s = 14 \) mm until the root tip at around \( s = 24 \) mm (purple curve of figure 2b). The extent of this zone where \( v_l \) departs from zero corresponds to the growth zone. The raw velocity field was fitted by equation (2.2). The fit function (black curve) nicely reproduced the experimental data. The maximum of this fit gave the root growth velocity \( V_0 = 28.9 \) μm min\(^{-1}\). From this fitting procedure, we got the three fit parameters of equation (2.1) necessary to properly define the strain-rate profile (figure 2c) and the growth parameters. Hence for this example before contact, \( L_{cz} = 8.64 \) mm and \( EER_{\text{max}} = 6.18 \times 10^{-3} \) min\(^{-1}\).

We proceeded in the same way for all the roots investigated. The average growth velocity before contact \( (V_0) \) was \( 28.5 \pm 2.5 \) μm min\(^{-1}\). The average growth zone length \( (L_{cz}) \) was \( 8.9 \pm 0.6 \) mm and the average maximum strain rate \( (EER_{\text{max}}) \) was \( 5.9 \pm 0.6 \times 10^{-3} \) min\(^{-1}\). Root growth velocity, growth zone length and maximum strain rates were similar to those already described in previous maize studies [33,34], indicating that our set-up with the root inserted in gel did not induce any oxygen deficiency nor affect root growth.

### 3.2. Contact

After a typical duration of 19 h inside the gel, the root tip reached the gel bottom and touched the force sensor with a normal incidence. The precise determination of the contact time was challenging since the zone of root-sensor contact was blurred, due to the gel bottom-air interface, preventing a visual determination of the contact occurrence. Considering that the contact time did not necessarily coincide with the force increase, we used a method based on the spatio-temporal analysis of the displacements of the bright points of the root texture.

We draw a vertical line of 1 pixel width along the root on each IR image and analysed the resulting spatio-temporal pattern. In figure 3, the image of the root has been rotated by 90°. In (b,c), each horizontal line corresponds to one slice along the root at a given time, and the vertical from top to bottom corresponds to increasing times, each slice being separated by 1 min. On the right part of the slices, the growth zone can be identified by the location of oblique lines (see the right zoom of the spatio-temporal in figure 3): the progressive shift of the bright spots shows the displacement of cells along the root axis. The slope of these oblique lines increases from left to right due to the cumulative effect of local growth on the more apical cells of the root tip. In the mature zone (left zoom of figure 3) there is no growth, the bright points of the texture stay in place along time before contact, resulting in vertical lines in the spatio-temporal image. At time \( T_C \), a slope break is observed in the vertical lines, the spots appear displaced backward towards the seed. As the force sensor was much more rigid than the root, the contact led to a backward movement of the IR specks that could result from a shortening of the mature zone due to compression or to a pullback of the whole seedling. We thus considered this event as due to the contact of the root tip with the force sensor. To determine \( T_C \), isointensity lines of the spatio-temporal diagram were detected using the Contour function of MATLAB. The longest ones (going from the initial to the final times of the spatio-temporal diagram) were selected; a piece-wise linear function with two pieces was fitted. Thus the time \( T_C \) corresponded to the junction between these two pieces. Note that a second noticeable slope break occurred later when the root markedly bends.

### 3.3. Force build-up

Once the contact time was determined, we could precisely follow the evolution of force \( F \) as a function of the rescaled time \( t = T - T_C \), for which we set \( F(t = 0) = 0 \). A typical evolution of the force \( F \) exerted by the root on the force sensor is plotted as a function of the rescaled time \( t \) (figure 4) with insets corresponding to the images of the root and the computed skeletons (in yellow) at different characteristic times.

The force was observed to increase with time, as the root continued to grow and thus to push against the force sensor. From time \( t_I \) which denotes the time where the force started to increase noticeably, the signal evolved in a linear way until time \( t_C \). The end of this linear regime was obtained mathematically following the successive steps below:

- calculating linear fits for the portions of the \( F \) versus \( t \) curve beginning at \( t_I \) and ending at the successive times \( t \).
- calculating the successive quadratic distances between the portions of curve \( F \) versus \( t \) and the corresponding fits. Each quadratic distance is normalized by the corresponding \( t \).
- plotting the histogram of quadratic distances and identifying its first peak. This first peak corresponds to all the data where the linear fit was very close to the experimental force-time data. The location and width of this first peak are calculated by the MATLAB function findpeaks.
With this method, we determined for the typical example of figure 4, a time \( t_L = 23.4 \) min for a time \( t_I = 2.5 \) min and a typical slope \( \Delta F/\Delta t = 3.9 \) mN min\(^{-1}\). When normalized by the initial root growth velocity \( V_0 = 28.9 \) \( \mu \)m min\(^{-1}\) we obtained a value of \( \Delta F/\Delta t \times V_0 = k_{\text{eff}} = 135 \) N m\(^{-1}\), which has the dimension of an effective root stiffness \( k_{\text{eff}} \).

Then after time \( t_L \), the signal of force versus time rounded off until a maximum force value of around \( F_{\text{max}} = 11 \) N was reached for the root. After this maximum force \( F_{\text{max}} \) was reached, there was a marked and spatially extended bending of the root; the root axis appeared curved along a typical length of \( 7.5 \pm 0.7 \) mm (right inset of figure 4). This event was thus associated with a macroscopic buckling of the root inside the gel.

The existence of a linear regime of force increase and then a rounding off of the force signal until a maximum force value were observed for all investigated roots. When averaged over \( n = 7 \) roots, the duration of the linear regime was \( t_L - t_I = 15 \pm 4 \) min and the characteristic slope was \( 4.5 \pm 0.7 \) N min\(^{-1}\) corresponding to an effective stiffness \( k_{\text{eff}} = 159 \pm 32 \) N m\(^{-1}\) (the value following \( \pm \) is the standard deviation over \( n = 7 \) roots).

3.4. Growth response

From the successive images and Kymorod analysis, we could follow the kinematics of root growth before and during the contact with the force sensor. The velocity profiles varying with time are represented with a three-dimensional map in figure 5 for the same root as in figure 4. The white line is the velocity profile at the rescaled time \( t = 0 \) corresponding to the contact with the force sensor \((T_C)\). Before contact, that is for times \( t < 0 \), the successive velocity profiles were similar, with a growth zone starting at around \( s = 14 \) mm and extending until \( s = 24 \) mm. Small variations of the maximum velocity (that is the root growth velocity \( V \)) are visible during the 30 min preceding the contact with a value of \( V \) in between 0.0278 and 0.031 mm min\(^{-1}\). Once the root tip contacted the force sensor, we observed a drastic change of behaviour. The maximum velocity decayed rapidly over 15 min and then more gradually over the next 15 min.

Growth monitoring by kinematics allowed to highlight that the root continued to grow although its tip was blocked by the rigid force sensor. This could not have been shown by a more classical method such as root tip displacement monitoring. This also implies that the increase in root length was ‘dispersed’ by either seed pullback, mature tissue compression or micro-bendings.

3.5. Coupling force and growth

The fitting procedures were applied to all the velocity profiles before and after the contact and gave the growth parameters

\( \text{MinPeakHeight} = 40 \) and \( \text{MinPeakProminence} = 4 \). \( t_L \) is given by the maximal time whose quadratic distance lays in the first peak of the histogram.

\[ F_{\text{max}} \]
shown in figure 6. In particular, the growth velocity $V$ was plotted as a function of time (figure 6a). In the typical example, we could observe a quasi-linear decay of the growth velocity over a duration of around 10 min after contact. Then the decay was slower and the growth did not stop in the represented time range. Note that we stopped the kinematic fitting procedure when the root clearly bent at time $t_B = 40$ min. This bending did not necessarily occur in the observation plane, which does not allow to use the kinematics analysis beyond this time. The simultaneous acquisitions of force and IR images also allowed to plot the growth velocity as a function of force (figure 6b). After a marked decay of velocity with increasing force until an amplitude of 0.04 N, the growth velocity seemed to decay much slower. Growth persisted even if the resisting force was still increasing.

Besides growth velocity, the fitting procedures also gave the growth zone length $L_G$ (figure 6c,d). Starting from $L_G = 9$ mm well before contact, the growth zone length shrank rapidly to 6.5 mm after around 10 min, then decayed more slowly. In a similar manner as the growth velocity, $L_G$ also decayed with force, seemed to stabilize at 6.5 mm for a force level of 0.03–0.04 N, before a small rise and a further decrease down to 5.5 mm.

We proceeded to the same analysis for force and growth for the different roots and summarized it in figure 7. The growth velocity was normalized by its value $V_0$ just before contact and the force $F$ was divided by a constant force level of $F_N = 0.04$ N corresponding to the levelling off of the velocity in the typical example. Despite the inherent biological variability of the seeds, the curves of the rescaled velocity $V/V_0$ versus rescaled force $F/F_N$ collapse rather well for the $n = 7$ roots we measured.

4. Lockhart’s law dictates root–obstacle interaction at short time scale

4.1. Model

We propose to interpret our experimental results in the framework of a Lockhart law [23]. In this model, the cell wall is under the tension produced by the turgor pressure and deforms irreversibly when the tension exceeds a yield
threshold. Conversely, the growth velocity is proportional to the turgor pressure in excess of a critical value.

We have adapted this approach to the case of a root encountering an obstacle for modelling the growth–force relationship. Notations and analysis are inspired by the work of Dyson et al. [35]. $F$, the force exerted at the root tip by the obstacle is balanced by the contributions of $P$, the turgor pressure, and $T$, the cell wall tension. Other environmental forces such as the frictional forces on the root flanks are neglected. The force balance applied to the root part laying between the $s$ cross section and the tip (figure 8) gives

$$- F - \int_{\text{wall}} T \cdot dA_{\text{wall}} + \int_{\text{cytoplasm}} P \cdot dA_c = 0.$$

(4.1)

The area of the $s$ cross section $A(s)$ being $\pi [R(s)^2]$ can be decomposed in its cytoplasmic part $A_{\text{cytoplasm}}(s)$ (liquid part under turgor pressure $P$) in blue in the left panel of figure 8 and its cell-wall part $A_{\text{wall}}(s)$ (solid part under tension $T$ in green in figure 8, left):

$$A(s) = A_{\text{wall}}(s) + A_{\text{cytoplasm}}(s).$$

Using $\bar{T}$, the tension averaged on the cell wall of the $s$ cross section, and $\bar{P}$, the turgor averaged on the cytoplasmic area of the $s$ cross section, equation (4.1) rewrites

$$- A_{\text{wall}} \bar{T} + A_{\text{cytoplasm}} \bar{P} - F = 0.$$

$T$ is a simple function of $P$ and $F$

$$\bar{T} = \frac{A_{\text{cytoplasm}} \bar{P} - F}{A_{\text{wall}}}.$$

(4.2)

Neglecting the tension variations over the $s$ cross section, the Lockhart equation expressing the strain rate EER($s, F$) for an applied force $F$ reads

$$\text{EER}(s, F) = e_T(s) \left( \bar{T} - Y_T(s) \right).$$

(4.3)

e_T (respectively $Y_T$) being the local extensibility (respectively the local threshold) expressed in tension. The subscript ‘+’ indicates that the formula is valid if $(\bar{T} - Y_T(s)) > 0$ and EER($s, F$) = 0 otherwise. Substituting (4.2) in (4.3) gives

$$\text{EER}(s, F) = e_T(s) \left( \frac{A_{\text{cytoplasm}} \bar{P} - Y_T(s) - \frac{F}{A_{\text{wall}}}}{A_{\text{wall}}} \right).$$

(4.4)

The strain-rate profile before contact EER($s, 0$) is given by the same equation (4.4) by setting $F = 0$. Then, it is possible to rewrite the strain rate during contact EER($s, F$) in the following way:

$$\text{EER}(s, F) = \left( \text{EER}(s, 0) - \frac{e_T(s) F}{A_{\text{wall}}} \right).$$

(4.5)

The extensibility $e_T$ has been estimated indirectly by retrieving data points from Frensch & Hsiao [36] who were studying the maize root growth submitted to water stress. Their data in fig. 7d of [36] showed the extensibility to decrease slowly from tip to base: the extensibility expressed in turgor was $e_T = 2.36 \pm 0.7 \text{MPa}^{-1} \text{h}^{-1}$ with a relative standard deviation 0.0545 ($N = 3$), whereas the threshold profile followed a bell shape inversely correlated with the EER profile (relative standard deviation 0.14 ($N = 3$)). These observations led us to suppose $e_T$ to be constant along the growth zone and to explain the strain-rate variation solely by the threshold variation

$$\text{EER}(s, F) = \left( \text{EER}(s, 0) - \frac{e_T F}{A_{\text{cytoplasm}}} \right).$$

(4.6)

e_T being converted in $e_P$ according to

$$e_T = \left( \frac{A_{\text{wall}}}{A_{\text{cytoplasm}}} \right) e_P.$$

Thus the strain-rate profile at a given force $F$ can be simply expressed as a linear combination of the force $F$ and the strain-rate profile before contact

$$\text{EER}(s, F) = \left( \text{EER}(s, 0) - \frac{e_P F}{\pi R^2} \right).$$

(4.7)

where $A_{\text{wall}}(s)$ is neglected compared with $A_{\text{cytoplasm}}(s)$, leading to $A_{\text{cytoplasm}}(s) \approx \pi [R(s)^2]$.
Substituting the fit of $s \rightarrow \text{EER}(s, 0)$ with formula (2.1) in equation (4.7) gives
\[
\text{EER}(s, F) = \left(2a_0(s - b_0) + (2a_0(b_0 + 2c_0 - s))\right) - c_p F / (\pi R^2) + .
\]

The function $s \rightarrow \text{EER}(s, F)$ calculated with this formula is still triangle shaped with a height (figure 8, right)
\[
\text{EER}_{\text{max}} = 2a_0c_0 - c_p F / (\pi R^2).
\]

Then the growth zone length ($L_{\text{GZ}}$) corresponding to the basis of the triangle $s \rightarrow \text{EER}(s, F)$ is easily obtained by noting that $s \rightarrow \text{EER}(s, F)$ and $s \rightarrow \text{EER}(s, 0)$ are two similar triangles
\[
L_{\text{GZ}} = 2c_0 \frac{(2a_0c_0 - c_p F / (\pi R^2))}{2a_0c_0},
\]
which simplifies in
\[
L_{\text{GZ}} = 2c_0 - c_p F / (2a_0 R^2).
\]

The growth velocity is given by the area of the triangle ($s \rightarrow \text{EER}(s, F)$)
\[
v = \frac{(2a_0c_0 - c_p F / (\pi R^2))^2}{2a_0}.
\]

After substitutions with the parameters before contact $L_{\text{GZ},0}$, $\text{EER}_{\text{max},0}$, and $v_0$, the kinematic parameters after contact are
\[
L_{\text{GZ}} = L_{\text{GZ},0} \left(1 - \frac{c_p F}{\pi R^2 \text{EER}_{\text{max},0}} \right)
\]
and
\[
v = v_0 \left(1 - \frac{c_p F}{\pi R^2 \text{EER}_{\text{max},0}} \right)^2.
\]

With such a simple model based on a Lockhart law, both the velocity $v$ and the growth zone length $L_{\text{GZ}}$ can be predicted with formulae (4.14) and (4.13) from the displacement profile before contact, the root radius and the force. These predictions were compared with the experimental data of the velocity (figure 9a) and growth zone length (figure 9b) as a function of the force for all experiments. The match between prediction and experimental data is surprisingly good. Note that there is no adjustable parameter (or fitting parameter) for these plots in figure 9, as we used the value of the extensibility in pressure $c_p = 2.36$ MPa $^{-1}$ $h^{-1}$ derived from the measurements of Frensch & Hsiao [36].

Alternatively the formulae (4.14) and (4.13) can be inverted to get the force as a function of the velocity or the growth zone length and the parameter $c_p$ can be left free to vary. Indeed, the linear regression coefficient of the curve $F$ versus $(\pi R^2 \text{EER}_{\text{max},0})(1 - \sqrt{v/v_0})$, respectively $F$ versus $(\pi R^2 \text{EER}_{\text{max},0})(1 - L_{\text{GZ}/L_{\text{GZ},0}})$, leads to estimations of $c_p$ both very close to each other $(1.87 \pm 0.36$ MPa $^{-1}$ h$^{-1}$ and respectively $1.87 \pm 0.52$ MPa $^{-1}$ h$^{-1}$, $N = 7$) and close to the measurements of 2.36 MPa $^{-1}$ h$^{-1}$ of [36].

4.2. Discussion

Using our dedicated set-up, we first established experimentally how root growth velocity, growth zone length and strain rates were modified when the root pushed against a force sensor. The experimental set-up was designed so that the root did not experience any mechanical stress at its tip or water stress before reaching the force sensor: during its progression in the channel, the root was well hydrated (by the gel around), did not suffer from hypoxia while growing in the open channel (indeed the growth velocity and root diameter were similar to those measured in hydroponics or in soils), and the root grew in a channel free of obstacles before reaching the force sensor. If the root had grown directly in the gel, the root tip would have already experienced a normal stress while digging into the gel. This would have led to a possible acclimation of the root to the mechanical stress before contacting the force sensor. We chose to investigate the model situation of an abrupt change of mechanical impedance, starting from a stress-free situation at the root tip. In this way, we could measure the normal force experienced by the root tip when contacting the obstacle. Note that sidewall forces may occur due to the contact of the root surface with the vertical walls of the channel inside the gel. These forces do not act on the root tip but on the root flanks. They should be included in the force balance equation (4.1) and would give an additional correction to determine $T$ in equation (4.2). In separate experiments, we verified that the frictional components acting on the
whole growth zone were small compared with the force magnitude observed at the root tip (less than 8%), which justifies neglecting them in the current model.

Mechanical cues trigger lots of responses at different time scales [37]; the present data show that the first phase (first 10 minutes or \( F < 0.04 \) N between the blue and green vertical dotted lines on figure 4) of the root growth response to an obstacle can be described with evolution laws derived from the Lockhart model. In this model, the strain rate (EER) remained proportional to the wall tension (above a predeter\-mined threshold), while the wall tension was decreasing due to the increasing force exerted at the root tip. After this first phase, the root growth velocity decreased more slowly than expected from the model while the force kept increasing and finally reached a plateau (see figure 6 between the green and red vertical dotted lines). In a third phase (after the red line of figure 6 or after \( t_3 \), figure 4), the force was no more linear with time and the velocity–force relationship was noisy. We interpret this phase as due to a localized bending allowing the dispersion of new tissue (produced by growth) transversely to the vertical axis. Note that the macroscopic bending corresponding to a clear buckling event happened later on and kinematics analysis were stopped there.

In the past, most tests of the Lockhart growth law were non-directional (i) by varying cell internal pressure either using a cell pressure probe [38] or varying external osmolarity [36], or (ii) by applying an external pressure by use of a pressure chamber [24] while monitoring growth. The reduction of cell wall tension by hyperosmotic treatments was shown to elicit two homeostatic responses of the plant: a reduction of the growth threshold to come back to the initial growth rate coined ‘cell wall loosening’ [39] and an increase in internal osmolyte concentration to at least partially restore turgor, coined ‘osmotic adjustment’. For instance, a maize root with an initial turgor of 0.67 MPa immersed in a 0.3 MPa mannitol solution (hyperosmotic bath) ceased to grow very rapidly when the turgor decreased below 0.6 MPa [40] suggesting an initial very high turgor threshold. Turgor reached a minimum (0.34 MPa) 2 min later, after which it started to recover for 30 min by osmotic adjustment. Osmotic adjustment in response to turgor drop lower than 0.05 MPa (calculated with \( t_3 \)) could also be studied.

Directional methods to test Lockhart growth by stretching organs with small weights were first developed as an alternative to measure plastic deformation associated with growth of soybean stem [44] and proved to be numerically equivalent to non-directional methods [45]. The method was refined to estimate easily both yield threshold and extensibility of maize leaves after exposure to salinity [46]. Compression experiments of stem pieces (coined ‘External Force method’) were carried out by Cosgrove [24] to study the dynamics of the turgor–growth relationship, but ‘the pattern of force were highly variable’ and the technique was not pursued. In the light of our experiments, it was probably due to the variability of the buckling threshold. Our study is thus the first proof of the equivalence between non-directional and directional methods for plants in the case of compression: parameters estimated with a non-directional method [36], an hyperosmotic treatment, can predict quantitatively the response of the EER distribution \((L_{CZ} \text{ and } EER_{\text{max}})\) to a directional solicitation, the contact with an obstacle (figure 9). This model illustrates the power and limitations of the analogy between cell wall growth and rheology to make predictive models of plant tissues with complex growth patterns: the yield threshold distribution inferred from the pre-contact kinematics and the extensibility are sufficient parameters to describe the first 10 minutes of the interactions with an obstacle.

5. Conclusion

As a conclusion, we built a model experimental system to study how an external mechanical stress impacts the primary growth of the maize radicule. We coupled force and kinematics measurements to investigate the first stages of the root apex contacting a stiff obstacle. We established the force–velocity relationship and characterized fine kinematics parameters such as the growth zone extent and the maximum strain rate. We proposed a derived Lockhart model to take into account the compression force produced by the axial growth against the obstacle. Through this model and by using parameters of the literature for maize roots submitted to water stresses (non-directional methods), we could predict without any adjustable parameter the decrease of velocity and growth zone lengths with force, within the first 10 min of contact. These results suggest a strong similarity of the early growth responses elicited either by a directional stress (contact) or by an isotropic perturbation (hyperosmotic bath).

Data accessibility. The data are available at the Dryad Digital Repository: doi:10.5061/dryad.47d7wm3h1 [47].

A movie of the root contacting the force sensor under infrared lighting is provided in the electronic supplementary material [48].

Authors’ contributions. M.Q.: conceptualization, formal analysis, methodology, resources, visualization; M.-B.B.-T.: conceptualization, methodology, writing—original draft; E.C.: conceptualization, data curation, writing—original draft; E.K.: investigation, supervision, writing—original draft. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interest.
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