An Empirical Function for the Description of Root Growth

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ABSTRACT

Vertical growth of young cress roots (Lepidium sativum L.) which have not yet attained their steady state is analyzed in terms of the relative elemental rate of elongation (RELEL). To this end, an empirical model function is suggested which describes, by means of six parameters, the movement of marker particles on the root surface. These parameters are interpreted as morphological and physiological quantities. Their determination by independent measurements is in good agreement with that obtained by fitting the model function to the experimental data. The RELEL is then evaluated analytically, so that numerical smoothing and differentiating algorithms with all their problems are avoided. The result demonstrates that—apart from the root cap—nearly the whole root is elongating, including the root hair region and part of the basal portion beyond it. This explains the basal curvature component that is observed during the graviresponse of cress roots.

Young roots are favorable objects for the analysis of growth processes, as their growth is mainly confined to the longitudinal direction. The RERG,2 which has been the basic quantity for the description of growth in three dimensions since its introduction by Richards and Kavanagh (13), thus reduces to its one-dimensional equivalent, the RELEL. Fundamental in this field is the investigation pursued by Erickson and Sax (7) who registered the trajectories of marker particles on the maize root surface and then evaluated the RELEL pattern by means of numerical differentiation of their data.

The knowledge of the RELEL distribution during normal (vertical) root growth is not only interesting in itself, but also a prerequisite for understanding gravitropism, which results from differential growth of the "upper" and "lower" root flank. Some efforts have been made to analyze the RELELs during gravitropism (3), but, often, the RELEL pattern is taken to be constant during normal growth. This approximation is, however, only valid in favorable cases (14). In the case of primary cress roots, for example, very early developmental stages (3–10 mm of length) are normally used for gravitropism experiments. As this is only shortly after the onset of cell division activity in the meristem (at about 3 mm of length) (11), it must be doubted whether steady state conditions have already established.

For these reasons, we intend to analyze vertical growth of cress roots, paying special attention to the time-dependent development of the young root. To avoid the problems which arise when numerical differentiation algorithms are applied to experimental data, an empirical model function will be developed which describes the trajectories of marker particles on the root surface.

The paper is organized as follows. After a short description of the experimental setup we present some theoretical considerations where the fundamental formula of the RELEL function in terms of the position function of marker particles is derived. Then, guided by experimental results, we derive boundary conditions for an analytical approach that severely restrict the possible model functions. The final ansatz is compared with experimental data by means of a parametric fit which afterward allows the analytical calculation of the growth rate. Finally, the results are discussed and compared with published ones.

MATERIALS AND METHODS

Seeds of Lepidium sativum L. were soaked in tap water for 30 min and fixed on moist filter paper covering a ledge in a humid Plexiglas chamber. Their micropyles were oriented downward, so the roots could grow out freely. After a germination period of 17 to 19 h at a temperature of 23 ± 1°C under weak illumination, the roots had attained a length of 2 to 3 mm and were then marked by immersing them shortly into a suspension of carbon particles in tap water. During the following 6 h, their growth was recorded by taking photographs in 10-min intervals with a Zeiss Luminar (1:4.5/40 mm) mounted via a bellows to a reflex camera (Canon F1-system), where a circular flash served as illumination source. Seedlings were not moved during the whole period including germination and experiment. Under these conditions, the mutations performed by the growing roots are reduced to a minimum. For the evaluations, three specimens were chosen whose oscillatory movements were negligible (±2°).

The photographs from an evaluation period of 170 to 200 min (beginning 2 to 3 h after marking; see Fig. 1) were enlarged, and, on each of them, the position of about 20 particles on either root flank was digitized with the aid of an inversely driven plotter (HP 7221A). Errors due to optical bias were estimated by photographing a grid, and turned out to be negligible (0.12% on an average at the margins of the enlarged pictures). Errors due to digitizing will be discussed later. In control experiments, the

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2 Abbreviations: RERG, relative elemental rate of growth; RELEL, relative elemental rate of elongation.
growth of unmarked roots was registered from 15 to 24 h of root age, omitting the flashlight. A comparison of the growth kinetics of marked and control roots showed that the experimental conditions (marking, illumination) did not alter growth velocity (5).

THEORETICAL CONSIDERATIONS

For the description of longitudinal root growth, it is convenient to choose a one-dimensional coordinate system originating at the root tip, whose x axis corresponds to the symmetry axis of the organ, the positive direction pointing toward the root basis.

Consider a particle on the root surface which is at position xo at time t0 (Fig. 2). The position x of this particle at time t ≥ t0 may be described by

\[ x = f(x_0, t). \]  

(1)

For fixed t, f is a strictly increasing function of x0, because cells do not shrink during vertical growth. So the starting point of a particle may be evaluated if its position x at some later instant t is known:

\[ x_0 = \xi(x, t), \]  

(2)

\( \xi \) being the inverse function of f with respect to the first variable.

The velocity v of a particle is, of course,

\[ v(x_0, t) = f_t(x_0, t) = \frac{\partial}{\partial t} f(x_0, t), \]  

(3)

whereas the spatial velocity u (the velocity at a fixed coordinate x) results from v by inserting the starting point given by Eq. 2,

\[ u(x, t) = v(x_0, t)_{|x_0=f(x,t)}. \]  

(4)

The relative elemental rate of elongation G is then defined as the partial derivative of the spatial velocity u with respect to x:

\[ G(x, t) = u_x(x, t). \]  

(5)

G has the dimension [time]−1 and is the local and instantaneous production rate for root material (‘length elements’).

It is important to note that G is the one-dimensional equivalent of the RERG as originally defined by Richards and Kavanagh (13) but does not coincide with the RELEL of Erickson and Sax (7) (the latter is defined as the total derivative of the special velocity with respect to x).

The definition of G by Eq. 5 implies the following continuity equation:

\[ \int_{x_0}^{x_0+\Delta x} G(x, t) \, dx = v_{rot}(t), \]  

(6)

where x0 is the beginning of the meristem (i.e. the beginning of the elongating portion), x is the end of the elongation zone at time t0 and vrot is the growth velocity of the whole root (i.e. the velocity of the root tip with respect to the laboratory frame). It is evident from Eq. 6 that the steady state approximation

\[ G(x, t) = G(x), \]  

(7)

which is conventionally used for the RELEL evaluation (7, 8), may not be applied if \( v_{rot} \) depends on time. Growth kinetics of cress roots in the interval from 15 to 24 h of root age (5) undoubtedly show that in this case, \( v_{rot} \) is a linear function of t:

\[ v_{rot} = b + c \cdot (t - t_0), \]  

(8)

with the coefficients b and c such that growth velocity roughly doubles during the observation period. So the steady state approximation must not be applied here, and G will be evaluated as a function of x and t in the following. To this end, some simple transformations of Eq. 5 are performed:

\[ G(x, t) = u_x(x, t) = \frac{\partial}{\partial x} v(\xi(x, t), t) \]  

(9)

\[ = v_{x_0}(x_0, t)_{|x_0=f(x,t)} \cdot \xi_x(x, t). \]  

(10)

If f and \( \xi \) are known, the evaluation of \( f_{x_0}(x_0, t)_{|x_0=f(x,t)} \) is straightforward. \( \xi_x \) may be come to by considering an implicit representation of Eq. 1:

\[ f(\xi(x, t), t) - x = 0. \]  

(11)

As cells do not shrink during normal root growth, \( f_{x_0}(x_0, t) = \xi_x(x_0, t, t) \) is nowhere less than one. On this condition, application of the implicit function theorem to Eq. 10 yields

\[ \xi_x(x, t) = \frac{1}{f_x(\xi(x, t), t)}. \]  

(12)

Hence, combining Eqs. 9 and 11, G may be expressed in terms of partial derivatives of f:

\[ G(x, t) = \frac{f_x(\xi(x, t), t)}{f_x(\xi(x, t), t)} \]  

(13)

\[ = \frac{f_{x_0}(x_0, t)_{|x_0=f(x,t)}}{f_{x_0}(x_0, t)_{|x_0=f(x,t)}} \]  

which is the basic formula for the following analysis, where we will always assume that \( f_{x_0}(x_0, t) = f_x, \) (xo, t), i.e. that f is twice continuously differentiable.

DATA FOR f(x0,t) AND PRECONSIDERATIONS FOR THEIR EVALUATION

The transformation of the digitized data into the one-dimen-

sional comoving coordinate system mentioned above yields a data matrix \( f(x_0,t) \) which may be presented as a surface in three-
dimensional space (Fig. 3).

The task of evaluating G from such data has conventionally been solved by numerical differentiation (7, 8). But though the digitizing procedure yields rather precise data in absolute terms (\( \Delta x \approx 1\% \)), errors run up to 25% relative to the distance covered by a particle during the time interval of 10 min. As can easily be calculated by means of the standard formula for Gaussian error propagation, the uncertainty in G(x,t) due to a direct discrete evaluation of Eq. 12 without smoothing is at least of the order of magnitude of G(x,t) itself. It is obvious that, under these conditions, rigorous data smoothing is necessary. The analysis of Erickson and Sax (7), for example, includes averaging over four roots, averaging over ten instants of time, application of a smoothing formula and, in the end, numerical differentiation by an algorithm which also provides some smoothing by means of splines (see also Silk [14]). It should be noticed, however, that the local approximation due to the smoothing may yield quite unrealistic results, especially if G depends on time, as in our case.
EMPIRICAL FUNCTION FOR DESCRIPTION OF ROOT GROWTH

Fig. 3. The position of marker particles, \( x = f(x_0, t) \), as a surface in three-dimensional space (left flank of root 1).

To avoid these difficulties, an analytical approach will be pursued in the following by searching for an empirical function \( f(x_0, t) \) which describes the data globally (i.e. a so-called parametric fit), where the free parameters will correspond to biological quantities. Analytical differentiation of \( f \) will then yield \( G(x, t) \).

BOUNDARY CONDITIONS FOR AN ANALYTICAL APPROACH

A realistic model function \( f(x_0, t) \) must fulfill some elementary boundary conditions. First of all, particles in the root cap region \( (x_0 \leq x_d) \) move with zero velocity, i.e.

\[
f(x_0, t) \Big|_{x_0=x_d} = 0.
\]

Furthermore, particles beyond the elongation zone move away from the root tip with maximum velocity \( b + c \cdot (t - t_0) \), which is the velocity of the root tip with respect to the laboratory frame, \( V_{\text{rel}} \).

\[
f(x_0, t) \Big|_{x_0=x_E} = b + c \cdot (t - t_0).
\]

Additionally, the particle velocity must be an increasing function of \( x_0 \), which guarantees that particles do not overtake each other. On the other hand, \( f_{x_0}(x_0, t) \) cannot be less than one for the reason already mentioned. Finally, it must be respected that every cell will leave the elongation zone at some time which means that every particle \( (x_d < x_0 < x_E) \) is accelerated until it reaches maximum velocity. This leads to another boundary condition:

\[
\lim_{x_0 \to x_d} f(x_0, t) \big|_{x_0=x_d} = b + c \cdot (t - t_0).
\]

With these boundary conditions in mind we now turn to the construction of a suitable ansatz.

THE MODEL FUNCTION

The requirements stated above may be met in a transparent way by making an ansatz for particle velocity first:

\[
v(x_0, t) = f(x_0, t) = \text{int}(x_0, t) \cdot [b + c \cdot (t - t_0)],
\]

where \( \text{int}(x_0, t) \) must be an interpolation function with the following properties. For fixed \( t \), \( \text{int}(x_0, t) \) must be 0 for \( x_0 \leq x_d \), increase from 0 to 1 on the interval \([x_d, x_E]\) and must be 1 for \( x_0 \geq x_E \); this guarantees that Eqs. 13 and 14 and the monotonicity condition for \( v \) are fulfilled. For fixed \( x_0 > x_d \), \( \text{int}(x_0, t) \) must be an increasing function with

\[
\lim_{x_0 \to x_d} \text{int}(x_0, t) \big|_{x_0=x_d} = 1,
\]

which answers the validity of Eq. 15. Finally, \( \text{int}(x_0, t) \) should possess continuous second derivatives everywhere. A suitable function for this purpose is the Beta distribution (1) with time-dependent parameters \( \alpha \) and \( \beta \) (Fig. 4):

\[
\text{int}(x_0, t) = \begin{cases} 0, & x_0 < x_d \\ 1, & x_0 > x_E \\ \frac{1}{B_1(\alpha(t), \beta(t))} \int_0^{(x_0-x_d)/(x_E-x_d)} y^{\alpha-1}(1-y)^{\beta-1} dy, & x_d \leq x_0 \leq x_E \\ \end{cases}
\]

where

\[
\alpha(t) = \alpha + B(t - t_0), \quad \beta > 0,
\]

and \( B_1(\alpha, \beta) \) is the complete Beta function:

\[
B_1(\alpha, \beta) = \int_0^1 y^{\alpha-1}(1-y)^{\beta-1} dy.
\]

Inserting Eq. 18 into Eq. 16 yields an expression for particle velocity which can be integrated to give \( f(t) \) (this integration must in practice be carried out numerically):

\[
f(x_0, t) = x_0 + \int_0^t v(x_0, \tau) d\tau
\]

As \( v \) is nonnegative, \( f_{x_0} \geq 1 \) everywhere, so all boundary conditions are obeyed. The six parameters of the model function (Eq. 21), \( x_d, x_E, b, c, \alpha \) and \( \beta \) can now be come to by fitting Eq. 21 to the data for particle position (the beginning of the evaluation period was chosen as \( t_0 \)). This was done separately for the particles on 'the right' and the 'left' flank of three roots by means of a quasi-Newton-algorithm (15). The model function turns out to agree very well with the data (Fig. 5). Furthermore, it is important to note that all parameters possess a biological meaning, and four of them may be measured independently (the outcomes of the fits and of the measurements are summarized in Table 1):

- \( x_d \) is the beginning of the meristem (length of the root cap) and can be measured in microscopic sections.
- \( x_E \) is the end of the elongation zone at time \( t_0 \) and may be estimated by plotting the distance of adjacent particles as a

Fig. 4. Time-dependent interpolation function (Eqs. 18 and 19) with \( A = 3.4, B = 0.75/h \) and \( t_0 = 19 h, 20 min \).
function of time. For roots 1 and 2, however, even the most basal particles continued to separate throughout the evaluation period, so \( x_E \) must be located beyond their starting position. For root 3, which was slightly longer, this distance became constant at \( x_E = 4 \text{ mm} \).

\( b \) is the constant and \( c \) the linear coefficient of maximum velocity. Independent estimates for \( b \) and \( c \) can be come to by fitting a parabola to root length. The linear and double quadratic coefficients of these parabolas do, however, not precisely coincide with the \( b_s \) and \( c_s \) from the model function fit, as root length (measured as the distance of root tip and micropyle) is additionally influenced by the growth movements of the hypocotyl.

\( A \) and \( B \) are the parameters of the interpolation function and determine the asymmetry of \( G \), the position of its maximum and the time-dependent change of its shape. There is, unfortunately, no simple way to measure them independently.

### EVALUATION OF G

Now that \( f \) is known, its partial derivatives defining \( G \) can be written down analytically \((x_d \leq x_0 \leq x_E)\):

\[
f_{s0}(x_0, t) = 1 + \int_0^t \int_{x_0}^{x_d} b + c \cdot (\tau - l_{d0}) \, d\tau \, dt
\]

\[
= 1 + \frac{1}{x_E - x_d} \int_{x_0}^{x_E} b + c \cdot \tau \cdot B(A, A + B \cdot \tau) \cdot (1 - (1 - y)^{\frac{b + c \cdot \tau}{A} + 1}) \, dy |_{y=(x_0-x_d)/(x_E-x_d)}
\]

and

\[
f_{s0} = \left. \frac{y^t}{1 - y} \cdot \left(1 - y\right)^{s + B \cdot \tau - 1} \cdot (b + 2c \cdot t^2) \right|_{y=(x_0-x_d)/(x_E-x_d)}
\]

The two steps which remain to be done numerically (integration of \( f_{s0} \) to give \( f_{s0} \) and determination of the starting point \( x_0 = \xi(x, t) \)) are possible to any degree of exactness. Therefore any numerical artefacts are excluded. The calculation of \( G \) according to Eq. 12 then yields \( G \) as a function of \( x \) and \( t \) which may be presented as a surface in three-dimensional space (Fig. 6). Time dependency of \( G \) is obvious from the broadening and increasing asymmetry of the surface. Correlating the pattern of \( G \) with root shape for selected instants of time (Fig. 7), reveals that apart from the root cap, almost the entire length of the root exhibits elongation, including the root hair region which, in textbooks of botany (e.g. [16]), is taken to have stopped growing.

### DISCUSSION

The evaluation of \( G \) for young developmental stages of cress roots has shown that, apart from the root cap, the whole root including the root hair region and the basal portion beyond it, should be regarded as 'extension zone.' This once more confirms the notion of Green (9) who recommends using the terminus of

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**Table 1. Fit Estimates of the Model Function Parameters for Left and Right Root Flank, Respectively, in Comparison with Independent Measurements**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Root 1</th>
<th></th>
<th>Root 2</th>
<th></th>
<th>Root 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Fit</td>
<td>Measurement</td>
<td>Left</td>
</tr>
<tr>
<td>( x_d ) (mm)</td>
<td>0.175</td>
<td>0.175</td>
<td>0.15-0.25</td>
<td></td>
<td>0.175</td>
</tr>
<tr>
<td>( x_e ) (mm)</td>
<td>3.86</td>
<td>4.03</td>
<td>&gt;3.6</td>
<td></td>
<td>3.54</td>
</tr>
<tr>
<td>( A )</td>
<td>3.36</td>
<td>3.19</td>
<td></td>
<td></td>
<td>3.27</td>
</tr>
<tr>
<td>( B ) (h^-1)</td>
<td>0.747</td>
<td>0.794</td>
<td></td>
<td></td>
<td>0.450</td>
</tr>
<tr>
<td>( b ) (mm/h)</td>
<td>0.507</td>
<td>0.507</td>
<td>0.587</td>
<td></td>
<td>0.733</td>
</tr>
<tr>
<td>( c/2 ) (mm/h^2)</td>
<td>0.0887</td>
<td>0.0887</td>
<td>0.0743</td>
<td></td>
<td>0.0750</td>
</tr>
<tr>
<td>No. of data points</td>
<td>504</td>
<td>483</td>
<td></td>
<td></td>
<td>525</td>
</tr>
<tr>
<td>Residual sum of squares (mm^2)</td>
<td>0.36</td>
<td>0.59</td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
</tbody>
</table>

* A discussion of errors is given in Buff (5). \( x_d \) = length of the root cap; \( x_e \) = end of the elongation zone at \( \xi \); \( A \) and \( B \) = parameters of the interpolation function; \( b/c \) = constant (linear) coefficient of maximum velocity.
'extension-only zone' instead of the conventional term of 'extension zone' in order to emphasize the fact that substantial extension growth takes place in the meristem.

The fact that the extension-only zone includes the root hair region and even the basal region beyond it, which, in textbooks of botany (e.g. [16]), are usually considered to have stopped growing, provides some insight into gravireaction. For cress roots, gravicurvature has been reported to consist of a (fast) apical and a (slow) basal component (12), which may be separated by application of external electrical fields (4). The basal component of curvature is difficult to explain within the conventional root model, as it would imply growth induction in a nongrowing region, but it provides no conceptional problems if the root hair region and the basal portion beyond it are elongating during normal growth.

A quantitative comparison of the RELELs evaluated here with the results of previous investigations (e.g. [3, 7]) is difficult, because they deal with roots growing at roughly constant velocity and determine $G$ as a function of $x$ only according to Eq. 7. There is, however, a qualitative agreement with the RELEL patterns presented for the roots of maize (3, 7), Phleum (8) and Allium (6).

As was mentioned above, the numerical methods applied in these investigations do not allow reliable statements about individual roots or single instants of time. In more recent work on maize roots (3), the attempt is made to avoid this problem by describing spatial velocity $u$ (in time-independent approximation) with a logistic equation,

$$u(x) = a + \frac{c}{1 + e^{-b(x-x_0)}}$$

$$= \left( a + \frac{c}{2} \right) + \frac{c}{2} \cdot \tanh \left( \frac{b \cdot (x - x_0)}{2} \right)$$

(24)

as translated into the notation of this paper; $b$ is the growth constant, $a + (c/2)$ is the initial velocity ($u(x_0)$) and $a + c$ is the maximum velocity

$$(u(x) \mid x \text{ beyond the elongation zone}).$$

This function may well describe the data, but there is a problem: Particles at the very beginning of the elongating region move with the initial velocity $a + (c/2) > 0$. This violation of boundary condition (Eq. 13) is not only a formal problem but implies that

$G(0, t) \neq 0$, thus leading to the separation of root cap and meristem. The logistic equation is, therefore, not adequate to the description of spatial velocity.

The position function $f$ presented in this paper is, of course, a merely empirical model function which describes particle movement realistically but does not originate from a microscopic theory. Though developed for the special example of the cress root, where a linearly increasing growth velocity is a useful approximation, it should be applicable to roots of other plants, even in the case of nonlinear $v$, if the asymptotic velocity or the interpolation function are suitably modified. The qualitative similarity of the RELELs of different plants suggests a more general relationship anyway. The reduction of root growth to the growth of single cells has been described recently (2). This line will have to be pursued further by taking into account realistic microscopic models of single cell elongation. It should, however, be mentioned here that such a task is very difficult because the present knowledge of root growth regulation is very poor (17). On the other hand, the study of RELELs might help to gain more detailed information from the conventional hormone-application experiments, as has been done by Hejnowicz (10) who examined the alteration of the RELEL pattern after external
application of IAA. With the analytical method presented here, such type of analysis should be possible with an accuracy considerably higher than that of the conventional numerical methods.

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LITERATURE CITED