A functional–structural model of elongation of the grass leaf and its relationships with the phyllochron

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Summary

• The emergence of a regular phyllochron from the dynamic processes of leaf initiation, leaf elongation and whorl construction suggests causal relationships between leaf elongation and leaf emergence. This paper presents a hypothesis as to how the ontogeny of the growth zone of leaves is triggered by emergence events, and implements it in a dynamic model of leaf elongation.

• Two different experiments, presenting two contrasted cases of relationships between leaf emergence and kinetics of leaf elongation, were analysed and interpreted with the model in terms of the functioning of the growth zone.

• Analysis of elongation kinetics revealed that the hypothesis allows for several contrasted elongation patterns that were observed, and for a regular phyllochron emerging from the variable dynamic of elongation. The model was able to simulate these patterns, and helped to identify the mechanisms underlying the key points of the analysis.

• The hypothesis is not demonstrated, but its coherence and robustness are established, which should inform a renewal of the modelling of leaf elongation in architectural models.

Key words: emergence, grasses, growth zone, leaf elongation, phases of elongation, phyllochron, sheath, whorl.


Introduction

In grasses the phyllochron is defined as the time interval between the emergence of successive leaves above the pseudostem cylinder (whorl) made by sheaths of previously emerged leaves (Klepper et al., 1982). As reviewed by Skinner & Nelson (1995), the phyllochron depends on the simultaneous processes of leaf initiation, leaf elongation and construction of the dimension of the whorl. Despite this, for most grasses the phyllochron, expressed in thermal time, is conservative for long periods of plant development (Rickman & Klepper, 1995). It has not yet been elucidated how this regularity emerges from a complex dynamic system. According to Lafarge & Tardieu (2002), the regularity of the phyllochron arises from a genetically defined regularity in the timing of leaf elongation, when expressed in thermal time. Erickson & Michelini (1957); Maksymowych (1973); Hay & Kemp (1990) proposed that regularities in the leaf elongation scheme occurred because the development of all leaves is controlled by the same physiological clock: the rate of initiation of the primordia at the apex. Other authors (Sharman, 1942; Etter, 1951; Gallagher, 1979; Malvoisin, 1984; Wilson & Laidlaw, 1985; Durand et al., 1999; Fournier & Andrieu, 2000a) defend the idea of a self-regulating dynamic system in which the emergence of a leaf controls the elongation and thus the timing of emergence of the younger ones. In the context of architectural modelling, the later hypothesis is particularly appealing as it points to a constructor, rather than a descriptor, of plant form. Here we provide more details on the relationships between leaf emergence and leaf elongation, and elaborate a dynamic model.

The phases of grass leaf elongation have been related to the ontogeny of the growing zone by several authors (Williams, 1975; Martin, 1988; Skinner & Nelson, 1995; Durand et al., 1999; Fournier & Andrieu, 2000a; Muller et al., 2001) as
follows (Fig. 1). In a first stage, cell division and elongation are coordinated so that mean cell length remains constant. During this stage the entire primordium is a homogeneous division zone, and might be associated with the first, exponential phase of elongation. In a second stage, cells stop dividing at the top of the division zone, giving rise to the ‘elongation only’ zone. In the later zone, cells elongate more rapidly than in the division zone. This produces the first phase change, which is an abrupt acceleration of the relative elongation rate (RER). Third, cells stop elongating at the end of the elongation zone, and enter the mature zone, marking the second phase change: the beginning of the quasi-linear phase of elongation. During this phase a quasi-steady influx of cells into the mature zone is established, which explains the relative stability of the leaf elongation rate. Ultimately, the growth zone regresses and gives rise to the fade-out phase of elongation.

This scheme applies to the ontogeny of the growth zone of the whole leaf (blade + sheath), as the position of the blade–sheath boundary inside the growth zone does not disturb its functioning (Schnyder et al., 1990; Ben Haj Salah, 1996). That is, this boundary is displaced passively within the growth zone as if it was stuck to a transverse cell wall. The characterization of blade and sheath elongation therefore consists only of being able to position the blade–sheath boundary at any arbitrary time of leaf ontogeny.

There is no definitive evidence that the emergence of leaf tips and leaf collars influences the ontogeny of the growth zone and the timing of phases of leaf elongation of that leaf.
or younger leaves, but two lines of circumstantial strongly support this hypothesis.

First, the length of the whorl has a strong influence on the elongation of the leaves that grow within. In pasture grasses, it is well known that cutting the whorl (by grazing) results in plants producing shorter leaves. More recent experimental studies (Davies et al., 1983; Wilson & Laidlaw, 1985; Casey et al., 1999), in which the length of the whorl has been manipulated (by means of sheath cutting, sheath incision and artificial sheath elongation with foil), demonstrated a direct effect of whorl length on both the blade and sheath length of leaves that grow within. More indirectly, Yu et al. (1975); Fournier (2000) and Ljutovac (2002) found that the relationships between leaf length and the length of the whorl they grew in were more stable in contrasted environmental conditions than classical relationships between leaf length and leaf position. Besides mature leaf length, the leaf elongation rate, the length of the growth zone, and cell flux toward the mature zone are also generally correlated with the length of the whorl the leaf grew in. Such a set of correlations was observed for leaves of different genotypes (Robson, 1974; Kemp, 1980; Volenc & Nelson, 1983; Durand et al., 1995; Beemster et al., 1996), and for leaves of contrasting lengths from different genotypes (Fiorani et al., 2000; Arredondo & Schnyder, 2003). To summarize, it appears that longer whorls are generally associated with longer leaves, which grow more quickly primarily because they have a longer elongation zone that produces a higher cell flux.

A second line of evidence is that several synchronies exist between emergence events at the top of the whorl and the major phase changes in the kinetics of elongation of leaves (Fig. 1). The first phase of leaf elongation (exponential phase) was found to end nearly synchronously with the emergence of the collar of the leaf two ranks below (Ljutovac, 2002). The end of the transition from the exponential phase to the rapid elongation phase (quasi-linear phase) was found to coincide with the time of tip emergence of the previous leaf (Williams, 1975; Gallagher, 1979; Malvoisin, 1984; Tesarová et al., 1992; Skinner & Nelson, 1995; Lafarge & Tardieu, 2002; Ljutovac, 2002). The differentiation of the ligule at the base of the growth zone was reported to occur synchronously with emergence of the tip of the same leaf (Sharman, 1942; Tesarová et al., 1992; Skinner & Nelson, 1995). Finally, the end of rapid elongation phase, after which the elongation rate rapidly decays, was found to occur around the time of collar emergence (Williams, 1975; Girardin et al., 1986; Skinner & Nelson, 1995).

The existence of such synchronies suggests that the mode of action of emergence events on leaf elongation is via a triggering of their phase changes. The hypothesis of a triggering of the end of leaf elongation by collar emergence has long been proposed to explain why cutting the whorl results in shorter leaves (Sharman, 1942; Etter, 1951; Dobrynin, 1969). More recently, Durand et al. (1998) implemented triggering of the transition from blade to sheath elongation by the emergence of leaf tips in a model of leaf elongation, and were able to predict changes in mature length and of the blade–sheath ratio. Fournier & Andrieu (2000a, 2000b) suggested that triggering of early phase changes could control the length of the growing zone of maize internodes and explain subsequent effects on the elongation rate.

The objectives of this study are to model the relationships between the ontogeny of the growth zone and the phase changes of kinetics of elongation, and to analyse how this can help in interpreting the role of emergence events on leaf elongation in two published data sets.

Materials and Methods

The model

We derived our model from that of Durand et al. (1999), which simulates leaf elongation from the functioning of the growth zone. The model simulates the change in leaf length over time, as composed of the division zone (DZ); the elongation-only zone (EOZ); and the mature zone (MZ), considering elongation of tissues in each compartment, and tissue fluxes between compartments. The original model allows simulating the sigmoidal pattern of leaf extension and realistic time courses of the changes in length of DZ and EOZ over time, except during the first phases of leaf extension (Durand et al., 1999).

We propose here a simple solution to overcome this limitation, and complemented the original model so that it could simulate blade and sheath elongation separately.

The model consists of a set of differential equations:

\[
\begin{align*}
    \frac{dT_z}{dt} &= k_1(1 - a)D_z \\
    \frac{dE_{oz}}{dt} &= k_2(1 - b)E_{oz} + ak_2D_z \\
    \frac{dM_z}{dt} &= k_3bE_{oz}
\end{align*}
\]

(Eqn 1)

Where \(D_z\), \(E_{oz}\) and \(M_z\) [L] are the lengths of DZ, EOZ and MZ, respectively; \(k_1\) and \(k_2\) [T\(^{-1}\)] are the RER of tissues in DZ and EOZ, respectively; and \(a\) and \(b\) (unitless) are the proportion of tissues expected to pass from DZ to EOZ, and from EOZ to MZ, respectively.

The system is initialized at an arbitrary time, \(t_0\), of the period during which the leaf is purely meristematic, using:

\[
\begin{align*}
    D_z &= D_0 \\
    E_{oz} &= 0 \\
    M_z &= 0
\end{align*}
\]

(Eqn 2)

Where \(D_0\) [L] is the length of the primordium at \(t_0\).

Finally, the model assumes that \(a\) and \(b\) are linear functions of time that start from zero at \(t_0\). Such an initialization is responsible for the unrealistic simulation of the first phases of elongation of the original model, as all compartments are created during the first step of the simulation which, in turns, does not allow for the simulation of an abrupt increase of the RER at the end of the exponential phase of leaf elongation.
Here we kept the idea of a linear increase of \( a \) and \( b \) with time, but delayed the time at which this linear increase occurs by introducing two new parameters, \( t_a \) and \( t_b \), corresponding to the time at which, respectively, a flux is initiated from DZ towards EOZ, and from EOZ toward MZ. This reads:

\[
\begin{align*}
\dot{t} < t_a, & \quad a = 0 \\
t \geq t_a, & \quad a = a_{de}(t - t_a) \\
t < t_b, & \quad b = 0 \\
t \geq t_b, & \quad b = b_{oe}(t - t_b)
\end{align*}
\]

Eqn 3

where \( a_{de} \) and \( b_{oe} \) [T\(^{-1}\)] are the rates of increase with time of \( a \) and \( b \), respectively.

This modification introduces a purely exponential phase for the simulation of leaf length before \( t_a \) and a control of the duration of the transition phase with \( t_b \).

Equations 1–3 allow the simulation of the whole leaf (blade + sheath). Here we introduce the simulation of the passive displacement of the blade–sheath boundary in the growing zone, and predict blade and sheath elongation separately. Following data of Sylvester et al. (1990), we hypothesize that the boundary already exists in the meristematic stage, and try to model its positioning. This was done by dividing each compartment of the model into two parts, and accordingly splitting equation 1 into two subsets of equations:

\[
\begin{align*}
\frac{dD_i}{dt} & = k_i(1 - a_i)D_i^z \\
\frac{dE_i}{dt} & = k_i(1 - b_i)E_i + a_i k_i D_i^z \\
\frac{dM_i}{dt} & = k_i b_i E_i
\end{align*}
\]

where the index \( i \) denotes either blade or sheath compartment. To ensure that the sum of blade and sheath subsystems are not in conflict with equation 1, the fluxes (still calculated at leaf level according to equation 3) are attributed either to blade or sheath compartment, according to:

\[
\begin{align*}
\text{if} \quad D_i^z & > 0, \quad a_{\text{blade}} = a \quad \text{and} \quad a_{\text{sheath}} = 0 \\
\text{if} \quad D_i^z & \leq 0, \quad a_{\text{blade}} = 0 \quad \text{and} \quad a_{\text{sheath}} = a \\
\text{if} \quad E_i & > 0, \quad b_{\text{blade}} = b \quad \text{and} \quad b_{\text{sheath}} = 0 \\
\text{if} \quad E_i & \leq 0, \quad b_{\text{blade}} = 0 \quad \text{and} \quad b_{\text{sheath}} = b
\end{align*}
\]

Eqn 5

Defining the initial repartition between sheath and blade at \( t_0 \) requires a new parameter \( (R_{\text{sheath}}) \) representing the ratio between sheath and leaf length within the primordium:

\[
\begin{align*}
D_i^0_{\text{blade}} & = (1 - R_{\text{sheath}})D_i^0 \\
D_i^0_{\text{sheath}} & = R_{\text{sheath}}D_i^0
\end{align*}
\]

Eqn 6

The data sets

The first data set is a nearly complete record of the kinetics of elongation of successive blades and sheaths (from 0.1 mm to maturity) of the main stem of a wheat plant, obtained by Ljutovac (2002). We were interested as to whether the model could help in understanding the coordination between leaves, together with the observed variation in the elongation kinetics (Fig. 2a). The second data set (Durand et al., 1999) deals with the effect of a cold-temperature treatment on the elongation of a tall fescue leaf (Fig. 2b). The treatment simultaneously changed the phyllochron (reduced by 40% in thermal time units), the mature length of sheath and blade (reduced by 30%) and the thermal time scheduling of organ elongation, but not the elongation rates of leaves (expressed in thermal time units). The question here was to test the ability of our model to explain such complex behaviour.

In the wheat experiment, winter wheat (cv. Soisson) was sown at 250 plants m\(^{-2}\) on 15 October 1998, at INRA Grignon (near Paris, France). Measurements started on 7 December, when plants had three fully developed leaves and lasted until 6 June 1999, when plants had reached their mature size. Plants were grown under nonlimiting conditions of water and nutrients, and were kept free of diseases and weeds by appropriate fungicide and herbicide applications. Soil and organ temperatures were monitored with thermocouples inserted in the soil and in plant organs, to allow computation of the thermal time at each sampling date. Every 2 or 3 d a sample of 60 plants was collected, and the median values for leaf stage and size of last ligulated leaves were calculated. The median value was used to identify 10 ‘median plants’, which were dissected to measure the length of individual leaves of the main stem. The kinetics of leaf elongation for each phytomer, and the rates of appearance of leaf tip and collar, were calculated.

The fescue experiment is described in detail by Durand et al. (1999), and only a few details are given here. Plants were grown for 6 wk in a glasshouse, then divided into two sets and grown for 5 wk in two growth cabinets at a constant temperature of 24\(^\circ\)C. During these 5 wk, plants were cut twice to a height of 7 cm, to impose homogeneity of whorl length for all tillers. At the end of 5 wk, plants were cut one more time to a height of 7 cm, and the temperature of one of the growth cabinets was set to 14\(^\circ\)C. Serial dissections, pinning techniques (Schneider et al., 1987), and observations of mitoses at the base of leaves allowed establishment of the time course of the length of the division zone; the length of the elongation zone; and the total length of the fourth leaf that appeared after the temperature change.

Fitting procedure and statistics

Overview In the first stage we attempted to fit the eight parameters from the whole data set simultaneously, using a weighted nonlinear regression algorithm (Jhaka & Gentleman, 1996). This procedure failed, for both wheat and fescue, even when initializing the process with realistic parameter values. We thus used analytical solutions or accurate analytical approximations of the model, valid in either the exponential or the quasi-linear stage, to estimate five parameters with their confidence intervals; and estimated the three others fitting the complete model to the full data set, using the weighted nonlinear regression procedure. Finally, a global test was
performed by analysing the residuals of the model prediction and observations.

**Estimation of $D_z$, $R_{sheath}$ and $k_1$**  
$D_z$, $R_{sheath}$, and $k_1$ were estimated fitting an exponential model, which is an analytical solution of the $D_z$ and $D_{sheath}$ time courses during the first stages of elongation. The three parameters were fitted simultaneously with a linear model, using the time course of the logarithm of leaf length and of sheath length during their exponential stage. $k_1$ is the common slope of the regression; the intercept relative to leaf data is the logarithm of $D_z^0$; the intercept relative to sheath data is the logarithm of $D_{sheath}^0$; and $R_{sheath}$ is the ratio between the two (equation 6).

**Estimation of the product $k_2 \cdot b_{eoz}$**  
The product $k_2 \cdot b_{eoz}$ was estimated from the leaf elongation rate (LER) time course, calculated from experimental data during the linear stage, using an analytical approximation of the model valid during this stage. The exact equation for the elongation rate is:

$$LER = k_2 E_{eoz} + k_1 D_z$$  \hspace{1cm} \text{Eqn 7}

An approximation of which yields:

$$LER = L_{eoz_{max}} \exp\left[-(k_2 b_{eoz}/2)(t - t_{max})^2\right]$$  \hspace{1cm} \text{Eqn 8}

Where $L_{eoz_{max}}$ is the maximum of the LER time course, and $t_{max}$ the time at which the leaf elongation rate is maximum.
Equation 8 was obtained by neglecting, during the linear stage, the contribution of the division zone to LER and to the dynamic of EOZ, that is assuming \( k_1 D_z \ll k_2 E_{ac} \) in equation 7, and that \( ak_2 D_z \ll k_2 E_{ac} \) in equation 1. A test of the validity of these assumptions was performed using the estimate of \( k_1 \) obtained in the previous step, and measurements of \( E_{ac} \) and \( D_z \) during the linear stage from Beemster et al. (1996) on wheat, and from Durand et al. (1999) on fescue. We calculated that, during the linear stage, \( k_1 D_z \) was about 1% of \( k_2 E_{ac} \) in either species. Simulation with the complete model showed that \( a \) remained between 0 and 5 during a run, therefore \( ak_2 D_z \) is also expected to be a few per cent of \( k_2 E_{ac} \) during the linear stage.

LER data were obtained from the kinetics of leaf length during the linear stage, using the cubic spline smoother method described by Shipley & Hunt (1996).

**Estimation of \( k_2 \)** Equation 7 provides a direct way to estimate \( k_2 \) from the estimate of \( k_1 \) and LER, \( D_z \) and \( E_{ac} \) time-course data. However, these data were available only for fescue, and were noisy. We thus estimated \( k_2 \) using an approximation based on an interpretation of the RER time course with the model, and checked for the validity of this approximation using the fescue data. The RER is given by:

\[
\text{RER} = \frac{(k_2 E_{ac} + k_1 D_z)/(D_z + E_{ac} + M_z)}{E_{ac}}
\]

Eqn 9

During leaf elongation, the RER is first constant, then increases abruptly during the transition phase, and decreases during the linear phase. During the exponential phase, \( E_{ac} = M_z = 0 \), so that the RER is constant and equal to \( k_1 \). During the transition phase, \( M_z \) is zero or just starts to increase, and \( D_z \) becomes smaller and smaller compared with \( E_{ac} \) so that the RER is changing from \( k_1 \) towards \( k_2 \). During the linear stage, \( E_{ac} \) and \( D_z \) both become small compared with \( M_z \), and the RER tends towards zero. A plausible estimation of \( k_2 \) is thus the maximum value of the RER time course. On fescue we checked for \( E_{ac} \) time-course data that this method yields a very realistic estimate of \( k_2 \) (Fig. 3).

**Estimation of \( t_b \), \( t_g \) and \( a_{de} \)** The last three parameters of the model were estimated by fitting the complete model. The nonlinear least-squares procedure was used to perform an optimization, but aborted before satisfying the convergence criterion in all cases. That is, at the end of a run we obtained parameter estimates that minimize the weighted sum of square, but no estimates of their confidence intervals.

All three parameters are related to characteristic times of the leaf length time course. By construction, \( t_b \) is close to the time of the end of the exponential phase, and \( t_g \) close to the end of the transition phase. \( t_b \) is also related to \( t_{max} \), as could be shown using equation 8:

\[
t_b = t_{max} - \left(\frac{1}{b_{res}}\right)
\]

Eqn 10

An exact analytical solution for the \( D_z \) and \( D_z \) time courses allows us to relate \( a_{de} \) to a third characteristic time, namely \( t_g \), the time at which the blade–sheath boundary leaves the division zone:

\[
a_{de} = -2 \cdot \log(R_{sheath})/k_1(t_g - t_d)^2
\]

Eqn 11

The final fitting procedure thus mostly consisted of searching for the timing of the transition from the exponential to linear phase of leaf elongation, and for the timing of the end of the exponential phase of sheath elongation, yielding a good estimation of the timing of leaf and sheath elongation together with a good estimation of the amplitude of the LER time course.

![Fig. 3](image-url) Time course (phytolochronic units) of the elongation rate (cm·°Cd⁻¹) of the fourth to emerge from fescue plants grown at 24°C (△) or transferred at 14°C at the beginning of the experiment (○). Diamonds are for the length of the elongation zone of the same leaves multiplied by \( k_2 (°Cd⁻¹) \) which is an equivalent expression of the elongation rate in the model. The parameter \( k_2 \) was estimated as the maximum of the RER time course for each temperature.
Results

Elongation of wheat leaves

The wheat data set is characterized by the regularity of the timing of emergence of leaf tips and leaf collars (Ljutovac, 2002) – a conservative phyllochron expressed in thermal time – together with a continuous change of length of sheaths and leaves and of leaf elongation rate with leaf rank (Fig. 2a).

The use of a phytomer-based time reference, having as its origin the emergence of the leaf, and dividing lengths by mature lengths, reveals that the time course of the normalized leaf length was nearly identical for all leaves after leaf emergence (Fig. 4b), and for all leaves but the last two before leaf emergence (Fig. 4a). That is, the time course of the RER has been identical at nearly all leaf positions. This provides two pieces of evidence for the hypothesis of a triggering of phase change by emergence events. First, this similarity suggests that all major phase changes in the elongation curves occurred at a similar phyllochronic time (Fig. 4). Second, it indicates that almost all the variability of elongation kinetics could be attributed to differences in the length of leaves at an early stage of leaf development, probably related (as the differences in RER between leaves were small) to differences in the thermal duration of their exponential phase. Our hypothesis is able to predict and explain this thermal time variability simply: the end of the

![Figure 4](image-url)
exponential phase is triggered by the same emergence events at all leaf positions. The time course of sheath length divided by mature leaf length was also very similar at all leaf positions (except the flag leaf), although the ratio between mature sheath length and mature leaf length increased with leaf position.

The model confirms and details this analysis. For leaves 5–9, no significant difference was found in normalized leaf length at any time of the entire time course of leaf elongation, so that these data were pooled before fitting the model. For the same reasons, normalized sheath length data were pooled for leaves 7–9. The model reproduced satisfactorily the observed kinetics and the variations between ranks (Fig. 5): before emergence normalized leaf length is greater for leaves 10 and 11, but all leaves synchronized after emergence; and mature normalized sheath length increased with leaf position, which was associated with a higher normalized sheath length during exponential stage. For all leaves but one (leaf 4), \( r^2 > 0.99 \) between observed and predicted leaf length, and \( > 0.98 \) between observed and predicted sheath length. There was, however, a significant autocorrelation of the residuals (Durbin–Watson quotient near zero) at all leaf positions, mostly because the model, because of its dynamic nature, propagates small errors of timing, which results in a systematic under- or overestimation of data during long phases of leaf elongation.

The examination of model parameters reveals that the similarity observed in the normalized length time course originated from similarities in the functioning of the growth zone (Table 1). First, no significant differences were found between

\[ \text{Fig. 5 Time course of simulated normalized length of leaves (black) and sheath (grey) of rank 4–11 of the main stem of field-grown wheat. (a) Semi-logarithmic scale; (b) normal scale. Phyllochronic time is obtained by dividing thermal time by the phyllochron. The zero point on the x axis represents leaf-tip emergence.} \]
ranks for $k_1$ and $k_2$, which revealed a constancy of RER (°Cd$^{-1}$) within each zone. Second, $R_{sheath}$ was also very stable with leaf position, except at rank 10. Third, analysis of the LER time-course data (Fig. 6) leads to the conclusion that $t_b$ and $b_{eoz}$ were identical at all leaf positions. This result was not obtained by comparing directly the parameter estimates from individual fits, but by fitting the LER model (equation 8) on pooled data and testing its ability to describe individual data. Finally, $t_a$ was found nearly synchronous to emergence of the leaf two ranks below ($n-2$) for leaves 6–9, which might indicate a triggering of the end of the exponential phase by tip emergence.

Differences between leaves therefore resulted mainly from differences in $D_z$ at $t_a$, hence, as $k_1$ is constant, from differences in the duration of the exponential stage. Differences concerning sheath elongation resulted from differences in $t_s$, the time at which the blade–sheath boundary leaves the division zone (Table 1), caused by the rank-to-rank variation of $a_{zd}$ and $t_d$.

Such a system establishes a regular phyllochron thanks to a triggering of the linear phase of elongation by an emergence event, and thanks to its ability to maintain a systematic correlation between mature length and leaf elongation rate, which results in a thermal time constancy of the interval between the beginning of the linear phase and leaf emergence.

**Analysis of effect of temperature on leaf elongation of fescue**

In the fescue data set the phyllochron (expressed in thermal time units), although constant within each treatment, varied between treatments. Therefore phyllochronic time and thermal time were not equivalent across treatments, and were allowed to discriminate if a process depended on emergence events or thermal time.

Concerning the timing of the phases of leaf elongation elongation, the results clearly reveal that dissimilarities exist.

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**Table 1** Values of model parameters (first eight rows) and characteristic times (last two rows) resulting from a fit of the model on individual kinetics of elongation of wheat leaves 4–11, with the emergence of leaf n-4 as the time origin

<table>
<thead>
<tr>
<th>Leaf number</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_z$ (cm) ± 5.1%</td>
<td>0.0029</td>
<td>0.0034</td>
<td>0.0044</td>
<td>0.0057</td>
<td>0.0064</td>
<td>0.0076</td>
<td>0.0150</td>
<td>0.0226</td>
</tr>
<tr>
<td>$k_1$ (phyllochron$^{-1}$)</td>
<td>1.07 ± 0.011</td>
<td>3.07 ± 0.30</td>
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<tr>
<td>$k_2$ (phyllochron$^{-1}$)</td>
<td>0.32</td>
<td>0.34</td>
<td>0.44</td>
<td>0.49</td>
<td>0.68</td>
<td>0.60</td>
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<tr>
<td>$a_{zd}$ (phyllochron$^{-1}$)</td>
<td>0.606</td>
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<tr>
<td>$b_{eoz}$ (phyllochron$^{-1}$)</td>
<td>0.279</td>
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<tr>
<td>$R_{sheath}$</td>
<td>1.85</td>
<td>1.90</td>
<td>1.98</td>
<td>2.01</td>
<td>2.35</td>
<td>2.6</td>
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<tr>
<td>$t_b$ (phyllochron)</td>
<td>3.40</td>
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<td>$t_s$ (phyllochron)</td>
<td>5.05</td>
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<tr>
<td>$t_{max}$ (phyllochron)</td>
<td>4.6</td>
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<tr>
<td>$t_a$ (phyllochron)</td>
<td>4.21</td>
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<td>$t_b$ (phyllochron)</td>
<td>4.35</td>
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<tr>
<td>$t_g$ (phyllochron)</td>
<td>4.6</td>
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**Fig. 6** Time course (phyllochronic units) of the normalized leaf elongation rate (°Cd$^{-1}$) of leaves 4–11 of the main stem of field-grown wheat. Symbols are smooth spline estimates of LER; error bars represent 95% confidence interval of estimates. The line is for the model fitted on pooled data. The time origin is the time of leaf-tip emergence.
between treatments on a degree-day time scale, even using a phytomer-based time origin. The time courses of leaf length and of sheath length are not synchronous in early phases (Fig. 7b); the end of leaf elongation occurred much sooner at 14 than at 24°C; and the linear phases of sheath extension are not synchronous (Fig. 7a). All these differences disappeared when data were plotted against the phyllochronic time scale (Fig. 7c,d). The constancy (in phyllochronic units) of the duration of elongation of the leaf in the two treatments is not, per se, clear evidence of control via emergence events, but provides another example where our hypothesis explains simply the thermal time variability: the end of leaf elongation is triggered by the same emergence event in the two treatments. In contrast, the thermal time scale reveals a similarity of the RER during the exponential phase (Fig. 7b), and of the (nonnormalized) LER during the linear phase (Fig. 7a). The reduction of leaf length in the cold treatment can therefore be attributed to the shortening of the phyllochron expressed in thermal time. The origin of this shortening is the reduction of the length of the sheath, and hence of the whorl, of the oldest growing leaf at transfer (leaf n – 4). The triggering by emergence mechanism does not explain this behaviour, but explains why it was conserved afterwards.

Simulations with the model satisfactorily reproduced leaf and sheath elongation, together with the time course of the length of the growth zone (Fig. 8), except for a short period around leaf emergence, where there was a systematic discrepancy between leaf length data and the model predictions in both treatments. This occurred because of the existence of a discontinuity of the leaf elongation rate at the beginning of the quasi-linear phase of elongation, which was not included in the model. Examining the data reveals more clearly that the discontinuity was synchronous with the appearance of the collar of the encircling leaf, and that before this date the elongation rates of these two leaves were almost identical (Durand et al., 1999). This strongly suggests the existence of a temporary mechanical coupling between the two leaves around leaf emergence. Despite this, the model was able to explain...
most of leaf length time course \( (r^2 > 0.99) \); sheath length time course \( (r^2 > 0.98) \); \( E_{oz} \) time course \( (r^2 > 0.84) \); and \( D_z \) time course \( (r^2 > 0.86) \). Like for wheat, probably for similar reasons, a significant autocorrelation of the residuals (Durbin–Watson quotient near zero) was found for all the variables except \( D_z \) and \( E_{oz} \).

Fitted parameter values are given in Table 2, and three major points emerge from their analysis. First, the parameters defining the RER of tissues within the different compartments, \( k_1 \) and \( k_2 \), were identical when expressed in thermal time (but not in phyllochronic time); whereas parameters that estimate the timing of ontogenic changes, \( t_a \) and \( a_{oz} \), were almost conservative when expressed in phyllochronic units. \( t_a \) was very close to the emergence of the previous leaf in both cases, probably indicating a triggering of this ontogenic change by tip emergence. Second, \( t_b \) and \( b_{oz} \) have significantly different estimates between treatments in either units, whereas \( t_{max} \) and the time course of LER, which both directly depend on \( t_b \) and \( b_{oz} \) (Eqns 8 and 10), were similar between treatments in phyllochronic time (Table 2; Fig. 3). \( t_b \) and \( b_{oz} \) have therefore compensated for the variation in phyllochronic units of \( k_1 \) and \( k_2 \), so that the beginning and the end of the linear phase of leaf elongation synchronize with an emergence event. Third, \( D_z^0 \) and \( R_{sheath} \) were different, even when rescaled to...
mature leaf length, which is unexpected, as the protocol was
designed to ensure homogeneity between plants at $t_{r}$. This
might be attributed to a temporary reduction of the RER just
after transfer, because of a cold shock stress.

To summarize, the asymmetry of effects of temperature
change on the elongation of fescue leaves is because the func-
tioning of the growth zone combines thermal time constancy
of the intrinsic dynamic with control of the ontogeny by
emergence events, which leads to constancy of leaf elongation
rates in thermal time units, and conservation of the durations
of phases in phyllochronic units. The model revealed, how-
ever, that such control did not occur because of a direct trig-
gering of the end of the linear phase, but because of an early
adjustment of tissue fluxes toward MZ at the beginning of
that phase. The mechanism for such an adjustment might be
related to the conservation of the length of the elongation
zone between treatments (Fig. 3). This suggests an increase of
the physical resistance to tissue extension, which parallels the
increase of RER within each compartment.

Discussion

The hypothesis of direct control by emergence events of leaf
growth zone ontogeny and elongation kinetics allows us to
explain several important aspects of leaf elongation patterns
in two contrasting cases. In the case of wheat, the hypothesis
offers a synthetic explanation for the observed variation of
elongation curves with leaf rank, together with the emergence
of a regular phyllochron. In the tall fescue example, the
hypothesis allowed us to understand better the effect of a
temperature treatment, which could not be explained by a
thermal time analysis.

The simple improvements we introduced into the model of
Durand et al. (1999) allow for a realistic simulation of the first
phases of elongation, and significantly ease the interpretation
of its parameters. Particularly, the less intuitive ones, relating
to the regulation of tissue fluxes between compartments, were
associated with characteristic times of the elongation curve.

This allows the model to infer the functioning of the growth
zone, using only data on leaf and sheath elongation. The
fitting exercise confirms almost all of the hypothesis used to
build the model, and demonstrates its ability to predict realistic
dependencies between phases, such as the differences in RER
between leaves and sheaths during the transition period.
However, the model lacks an automatic fitting procedure, and
has some structural drawbacks for direct use in a predictive
dynamic model. For example, conservation of parameter
values in phyllochronic units is of no help for simulation, as
the phyllochron is precisely dependent on the dynamic that
is being modelled. A way to address this problem in future
versions of the model might be to express more mechanistically
the dependencies of $a_{dc}$ and $b_{uc}$ on cell dynamics within the
growth zone.

The physiological mechanism that underlies the triggering
of leaf kinetics by emergence events remains unclear. The
hypothesis of a role of light quality (Begg & Wright, 1962) is
supported by experimental evidence of the direct effect of red/
far-red ratio on leaf elongation (Skinner & Simmons, 1993),
possibly acting at long distance via systemic signalling
(Thomas et al., 2003). Alternatively, enrichment in ethylene
within the whorl is realistic, and might affect leaf extension
(Fiorani et al., 2002). Finally, a signal could be induced by
direct contact of tips and collars with the ligule at the top
of the whorl, as the ligule is known to deposit a lot of chemical
compounds on growing leaves (Chaffey, 2000).

To conclude, the analysis presented here questions the
classical approach of analysing and modelling leaf length as the
product of elongation rate and duration of elongation, as both
terms were found here to be dynamically determined during
leaf growth. Understanding of how a plant regulates leaf
length and leaf elongation therefore cannot be separated
from the analysis and modelling of their relationships with leaf
emergence. The model presented here offers a framework for
such an analysis, but still needs to be adapted for use in
predictive models. Architectural models of grasses, which
consider both the elongation process at the level of individual
leaves, and the modelling of whorl geometry (Fournier &
Andrieu, 1998; Kaitaniemi et al., 1999; Werneke et al., 2000;
Fournier et al., 2003), appear particularly well suited for the
development of such applications.

Acknowledgements

We thank J. Vos and J. Hillier for helping us to improve the
English.

References

Arredondo J, Schnyder H. 2003. Components of leaf elongation rate and
their relationship to specific leaf area in contrasting grasses. New Phytologist
158: 305–314.

resistance to root penetration on leaf expansion in wheat (Triticum

Table 2 Values of parameters resulting from a fit of the model on
individual normalized kinetics of elongation of fescue leaves grown at
two temperatures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>14°C</th>
<th>24°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{z}$ (unitless)</td>
<td>6.47 ± 0.9e-5</td>
<td>1.51 ± 0.3e-5</td>
</tr>
<tr>
<td>$k_{1}$ (°Cd⁻¹)</td>
<td>0.006 ± 0.0002</td>
<td>0.006 ± 0.0002</td>
</tr>
<tr>
<td>$k_{2}$ (°Cd⁻¹)</td>
<td>0.046 ± 0.004</td>
<td>0.046 ± 0.004</td>
</tr>
<tr>
<td>$d_{dc}$ (phyllochron⁻¹)</td>
<td>1.04</td>
<td>1.06</td>
</tr>
<tr>
<td>$b_{uc}$ (phyllochron⁻¹)</td>
<td>0.687</td>
<td>0.375</td>
</tr>
<tr>
<td>$R_{sheath}$</td>
<td>0.26</td>
<td>0.09</td>
</tr>
<tr>
<td>$t_{s}$ (phyllochron)</td>
<td>2.99</td>
<td>2.98</td>
</tr>
<tr>
<td>$t_{e}$ (phyllochron)</td>
<td>3.15</td>
<td>1.97</td>
</tr>
<tr>
<td>$t_{max}$ (phyllochron)</td>
<td>4.61</td>
<td>4.63</td>
</tr>
</tbody>
</table>

The emergence of leaf n-4 is the time origin.

Effects of leaf sheath length on cellular and spatial parameters of leaf growth in tall fescue. Effects of temperature, cutting height and species? A kinematic study.


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