Spatial organization of the gravitropic response in plants: applicability of the revised local curvature distribution model to *Triticum aestivum* coleoptiles

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SUMMARY

The revised local curvature distribution model, which provides accurate computer simulations of the gravitropic response of mushroom stems, was found to produce accurate simulations of the gravitropic reaction of wheat (*Triticum aestivum*) coleoptiles. The key feature of the mathematical model that enables it to approach universality of application is the assumption that the stem has an autonomic straightening reaction (curvature compensation or 'autotropism'). In the model, the local bending rate for any segment of the organ is determined by the difference between the 'bending signal' (generated by the gravitropic signal perception system) and a 'straightening signal' (which is proportional to the local curvature of the segment). The model reveals three major differences between the gravitropic reactions of wheat coleoptiles and *Coprinus* mushroom stems. First, in *Coprinus*, the capacity for autonomic straightening is much more concentrated in the apical region of the stem. Second, local perception of the gravitropic signal, which is necessary for exact simulation in *Coprinus*, is not needed in wheat coleoptiles (the corresponding constant in the model can be set to zero). Third, the transmission rate of the gravitropic signal is about seven times faster in wheat coleoptiles than in the mushroom stem. Thus, we demonstrate that a single model, depending on the values given to its parameters, is able to simulate the spatial organization of the gravitropic reaction of wheat coleoptiles and *Coprinus* mushroom stems. The model promises to be a valuable predictive tool in guiding future research into the gravitropic reaction of axial organs of all types.

Key words: mathematical models, computer simulation, signal transmission, curvature compensation.

INTRODUCTION

Mathematical modelling of plant gravitropic response has a long history. Some of the currently available models of plant gravitropic reaction simulate changes in space and time and have the potential to reproduce the whole shape (i.e. the local curvature distribution) of the bending organ (Johnson & Israelsson, 1968; Barlow *et al.*, 1991; Stočkus, 1994c). However, as a rule these distributions were not compared with experimental data; rather, attention tends to have been restricted to the apex angle. Analysis of the spatial organization of the bending process is a severe test of any model because it increases by about two orders of magnitude the number of experimental data points being compared

with data generated by the model. Such a more critical analysis can be useful in choosing between alternative hypotheses that might otherwise result in similar or even identical predictions of apex angle.

An observation that is important in modelling the gravitropic reaction in some plant organs is that the bending rate approaches zero before the apex reaches the vertical position. Some such organs are diagravitropic and the model can be successfully modified by adequate adjustment of the gravitropic signal perception function in the model (Zimmerman, 1924a; Metzner, 1929; Stočkus, 1994a). In such cases, the perception function from the angle α is usually written as $k_1 \cdot \sin\alpha + k_2 \cdot \cos\alpha$. This is the same as $s \cdot \sin(\alpha + \beta)$, where $k_1 = s \cdot \cos\beta$ and $k_2 = s \cdot \sin\beta$. Hence such models assume that the stationary position (in which no perception signal is generated) differs from the vertical position by the angle β.

When only the gravitropic reaction from the

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horizontal position is taken into consideration, such models demonstrate a high ability to fit to experimental data. However, it is not always true that such plant organs also bend from the *vertical* position towards this hypothetical diagravitropic angle. For example, after *Avena sativa* coleoptiles have been turned to the horizontal, the apex angle is not always restored to the vertical. However, vertical coleoptiles do not bend from the vertical position (Blaauw-Jansen & Blaauw, 1968). To explain such 'incomplete' gravitropic reaction for maize roots that do not adjust from the vertical to their diagravitropic position, Stočkus (1994b) proposed a modified perception function in which the angle β of the diagravitropic reaction is initially zero but increases during the bending process. The model was successfully fitted to experimental data.

Here we show that a model with the curvature compensation ('autotropism' or autonomic straightening) component, which was originally created to explain the spatial organization of strictly negative gravitropic response in mushroom stems (Meškauskas et al., 1999), can also be fitted to data describing the gravitropic reaction of wheat coleoptiles in which the final apex angle after 24 h of bending was about 40°. In this model it is assumed that the local bending rate is determined by two opposite processes: the bending signal (proportional to the signal about the gravitropic irritation) and the compensation signal, which arises in the place where the bending process occurs and is proportional to local curvature. As the apex angle changes towards the vertical position, the signal about the reorientation reduces. At some critical angle it becomes equal to the local compensation signal and the bending process in this subsection stops. If the apex angle continues to reduce (e.g. by bending of more basal subsections, where 'autotropism' might be less expressed), straightening begins.

The straightening signal is normally negative and would cause straightening whenever any tropic bending signal was close to zero. Such a situation can be created in horizontal organs by placing the specimen on a clinostat. Gravitropically bent cress (*Lepidium sativum*) roots placed on a clinostat do, indeed, straighten (Stanković et al., 1997).

Incorporating the 'autotropism' parameter permitted the creation of a successful mathematical model of the gravitropic reaction of mushroom stems, the model having the further advantage of fitting data (such as the effects of metabolic inhibitors on the gravitropic reaction) that were not part of its design envelope. Because this model does not contain functions or parameters that might be specifically fungal, it might have universal applicability. In this analysis we show that the revised local curvature distribution model (Meškauskas et al., 1999) can indeed be applied to data describing the gravitropic reaction of *T. aestivum* coleoptiles.

MATERIALS AND METHODS

Experimental material

Three-d-old etiolated spring wheat (*T. aestivum* L. cv. Selpek) seedlings were grown in moistened sand in darkness at a temperature of $25 \pm 1°C$ and an rh of approx. 100%. To reduce variability, the seeds were oriented uniformly in the sand. Seedlings were subsequently reoriented to the horizontal position without removing them from the growing dishes and without detaching the coleoptiles from the plants. The shapes of gravitropically bending coleoptiles were photographed every 10 min for 360 min using a 20 s exposure to weak red light (shown to have no significant effect on the gravitropic reaction in preparative experiments). The lengths of the coleoptiles varied between 7–20 mm at the start of observation but they were all the same age. Each data set comprised 14–16 coleoptiles.

Image capture, analysis and mathematical model fitting

Images were scanned from photo tapes with Image Assistant® version 1.10 peripherals and software at a resolution of 230×155 dots per image (approx. 4 dots mm^{-1}). For each coleoptile 37 images were produced. Images were manipulated using Image Assistant version 1.10. Graphic images were digitized into *x*–*y* coordinates with UnGraph® version 3.0. Images were rotated before regression analysis (to avoid coordinates with shared values (Meškauskas et al., 1998)). The distribution of local curvatures was calculated using Maple® V, version 4.00b after polynomial regression, as described by Meškauskas et al. (1998). The growth of the upper and lower sides of coleoptiles was measured by hand after projecting the image at a magnification of $\times 50$.

The final model was written in Borland C++ version 5.02 with the use of object-oriented programming. The fitting procedure aimed at minimizing the lack-of-fit mean square value in tests for goodness of fit between the curves (composed of up to 200 coordinates) representing observed and modelled stem shapes. Fitting was done by dichotomy (i.e. a bisection or dichotomous line search), an iterative method in which the interval between values is halved at each iteration by testing two new functions that bracket the midpoint of the interval.

DESCRIPTION OF THE MODEL

The revised local curvature distribution model is described explicitly in the accompanying paper (Meškauskas et al., 1999). It is based on the the following assumptions:

● the bending process is influenced by the 'auto-tropic' (that is, the curvature compensation or straightening) signal, which causes straightening if the gravitropic signal is reduced below a critical level;

● the local bending rate is proportional to the sum of three signals: the gravitropic signal from the apex, which is being transmitted in the basipetal direction, the gravitropic signal from the local signal perception system and the negative curvature compensation signal from the local curvature perception system;

● the level of the 'autotropic' signal at any point in the organ is proportional to the local curvature at that point;

● the gravitropic signal perception system in the coleoptile apex generates a signal proportional to the cosine of the apex angle;

● this signal is transmitted away from the apex, weakening as it progresses.

FITTING THE MODEL TO EXPERIMENTAL DATA

The development of local curvature is a 1 + 1 (space and time) dimensional process, and two projections of the data can be considered. It is possible to present a set of curves, each of which describes how the local curvature at a certain point of the organ changes with time (Fig. 1a). Alternatively, it is possible to present multiple curves, each describing the distribution of the curvature over the length of the organ at a particular time (Fig. 1b).

As shown in Fig. 1, significant oscillations were observed during wheat coleoptile bending and are clearly seen even in the averaged data. Nevertheless, it is evident that the local curvature simply increases, approaching a final value that for most of the coleoptile does not exceed 60° (except for regions very close to the tip). Hence, the final apex angle does not reach the vertical, and the general picture of the spatial development of the gravitropic reaction is quite similar to that reported for *Avena* coleoptiles in darkness (Blaauw-Jansen & Blaauw, 1968).

Fitting the model to the gravitropic reaction of *T. aestivum* coleoptiles is shown in Fig. 2. The values of the parameters, established by using the method of dichotomy, are given in Table 1. Statistical analysis of the goodness of fit between model and experiment effectively compared the polynomial regressions represented by the modelled and observed stem shapes. Table 2 shows that the model fits the experimental data; deviations were not statistically significant. The 'autotropism' component of the equations accounts for the cessation of gravitropic
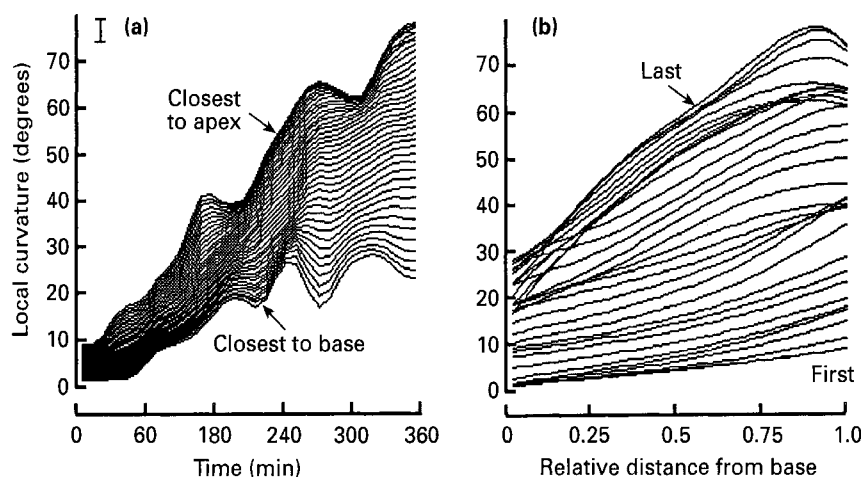

Fig. 1. The distribution of local curvature over the length of the coleoptile for base-pinned *Triticum aestivum* coleoptiles. The data are shown in two ways: (a) a time-telescoped projection in which each curve represents the changing curvature of a different subsection of the stem during the time course of the experiment, and (b) a space-telescoped projection in which the curves show the distribution of curvature over the whole length of the stem at 10 min time intervals. The distance from the base is given in arbitrary units of stem length. The error bar at the top left represents the maximal SD for both projections.
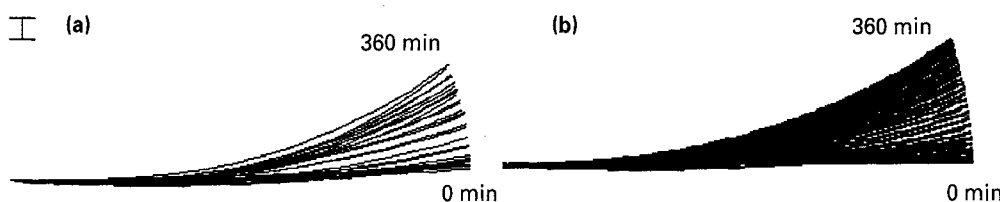

Fig. 2. Comparison of averaged bending of base-pinned *Triticum aestivum* coleoptiles (a) with the images generated by the mathematical model (b); values of parameters are given in Table 1. In (b), computer-generated images (thick lines) are plotted over the experimentally observed images (regular lines). Times of subsequent images differ by 10 min. The error bar at the top left defines the maximal SD of the mean for experimentally observed shapes. The goodness of fit was tested by the lack-of-fit test (Table 2).

Table 1. *Comparison of model parameters obtained after fitting the revised local curvature distribution model to the gavitropic reaction of* Triticum aestivum *coleoptiles*

Parameter	Model feature defined by this parameter	Units	Calculated value for *Triticum aestivum* coleoptiles
K_S	Perception in the apex	Relative	0.675 ± 0.32
A_U	Distribution of ability for autotropism	Relative	$0.015 - 0.015$ to $0.015 + 0.055$*
A_D (exponent of A_U)	Distribution of ability for autotropism	Relative	1.8 ± 1.8
S_{DE}	Signal decrement during transmission	Relative	$1.5 - 0.6, +0.35$
V_T	Signal transmission speed	h^{-1}	1.78 ± 0.78
K_W	Local bending rate dependence on local signal level	degrees h^{-1}	$50 - 30$ to $50 + 40$*

*The optimal value differs from the average of minimal and maximal values. The minimal and maximal values are the boundary values, within which the model still satisfies the lack of fit test.

bending before the apex reaches the vertical position and significantly increases the accuracy of the model. The best fit can be obtained if we suppose a little lower capacity for autotropism in more basal sub-sections of the coleoptile.

The model suggests that the signal transmission speed in wheat coleoptiles is quite high, reaching *c.* 50 mm h^{-1} (it takes approx. 30 min for the signal to travel from the tip to the base). Such a rapid transmission also can be suspected from earlier models proposed by Stočkus (1994a), in which a very low constant of initial signal delay before bending was indicated for *Triticum* coleoptiles (approx. 20 min). Indeed, after this time the more basal part of the coleoptile had already started to bend. The signal decrement during transmission results in the signal's losing approx. 80% of its level while passing through the full length of the coleoptile.

According to this model, the main reason why the final apex angle of the wheat coleoptile often does not exceed 50° is the distribution of the 'autotropism'. The wheat coleoptile is 'autotropic' over most of its length, and straightening of the more basal parts prevents the apex reaching the vertical. Fig. 3 reveals a significant negative correlation between coleoptile length and the maximal tip angle of the gravitropic response from the horizontal position. Very short coleoptiles can bend up to the vertical position, whereas longer coleoptiles respond in smaller angles. Such a correlation could be caused by the 'food factor' postulated by Dolk (1936) (namely, the longer

Table 2. *Results of the lack-of-fit test performed on the revised local curvature distribution model applied to* Triticum aestivum *coleoptiles*

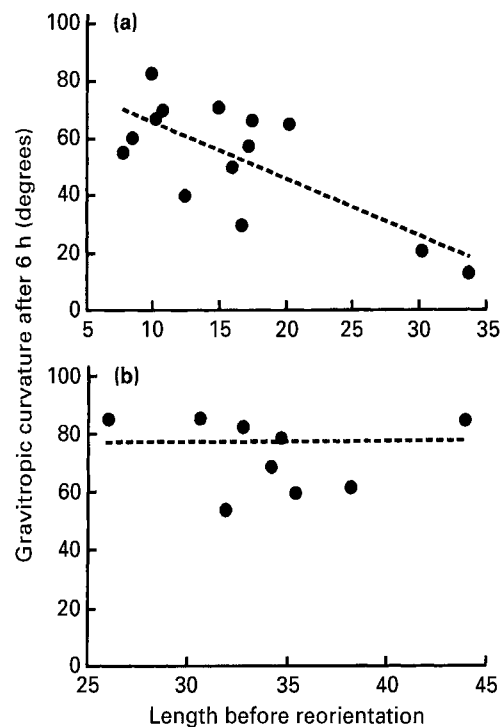|	
Number of stems	14
Lack-of-fit mean square	0.0012
Pure error mean square	0.001
df	1020, 14336
Ratio	0.15353
Value of z for which the distribution function $F(z) = 0.95$	1.077
Conclusion	Differences not significant

Fig. 3. Correlation between the initial length of the organ and the gravitropic curvature 6 h after reorientation to the horizontal. (a) Data for 16 *Triticum aestivum* coleoptiles. The ages of the organs are the same; the data set represents the natural length variation of coleoptiles. The slope of the line is -1.96 ± 0.073. (b) Equivalent plot for the mushroom stems of *Coprinus cinereus*, included to show that the coleoptile behaviour is not shared by all axial organs that react tropically. The slope of the line is 0.006 ± 0.086.

the coleoptile is, the less potential it has for differential growth and hence for gravitropic response), or even a purely mechanical effect arising from the tensile strength of the tissues. However, the 'autotropic' mechanism remains obscure.

COMPARISON OF GROWTH IN THE UPPER AND LOWER SIDES DURING THE GRAVITROPIC BENDING OF *T. AESTIVUM*

Growth of the upper and lower sides of coleoptiles was measured separately in two subsections. The apical subsection was initially located at 3.0–6.3 mm from the apex and the more basal subsection
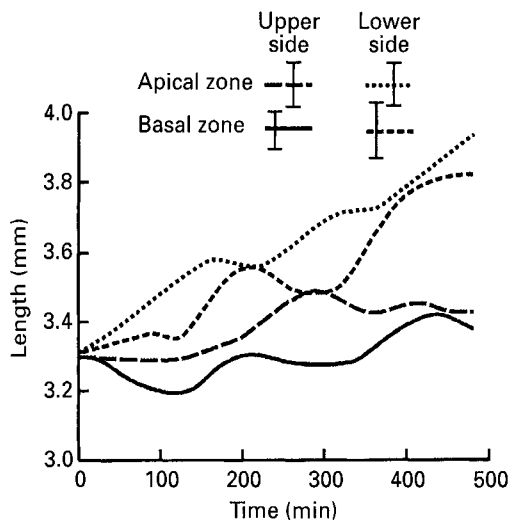
Fig. 4. Growth of the upper and lower sides of the *Triticum aestivum* coleoptiles during the gravitropic reaction. Growth was measured in two subsections; the apical zone (3.0–6.3 mm from the apex) and the more basal zone (6.3–9.6 mm from the apex). The error bars in the upper part of the figure represent the maximum SDs of the mean for each curve.

6.3–9.6 mm from the apex (Fig. 4). These results indicate that the characteristic bending oscillations of this material are caused mainly by oscillations in the growth close to the apex. The more basal subsection grows more smoothly.

It is known that the oscillation of the growth acceleration in wheat coleoptiles coincides with the oscillation of bioelectric potentials (Merkys *et al.*, 1973). This could mean that some kind of electrical signal is used to transmit the information about the gravitropic irritation. This would presumably be a rapid means of signal transmission and would be compatible with the high signal transmission rate indicated by the model. Oscillations in the more basal subsection tend to occur later and it might be that the bending process (including oscillations) in the basal subsection begin later, when it is reached by the gravitropic signal from the apex.

DISCUSSION

Experience with the revised local curvature distribution model indicates that: after turning to the horizontal position the apex angle does not reach the vertical position (at least in the first 24 h); and there is no bending from the vertical position (i.e. no diagravitropic functions need be assumed).

Application of the model suggests three major differences between the gravitropic reactions of wheat coleoptiles and the *Coprinus* mushroom stems analysed by Meškauskas *et al.* (1998, 1999). First, in *Coprinus*, the capacity for autonomic straightening is much more concentrated in the apical region of the stem. Second, local perception of the gravitropic signal, which is necessary for exact simulation in *Coprinus*, is not needed in wheat coleoptiles (the

corresponding constant in the model is set to zero for accurate simulation of coleoptile curvature). Third, the transmission rate of the gravitropic signal is approximately seven times faster in wheat coleoptiles than in the mushroom stem. At least some of these differences might reflect the major differences between gravity perception in plants and that in fungi, particularly the fact that no cells, nor even cell organelles, specialized to gravity perception have yet been identified in mushroom stems.

We are aware that gravity perception occurs throughout the elongation zone in several plant organs but in this analysis it has not been necessary to invoke the local perception function incorporated in the model (Meškauskas *et al.*, 1999). That wheat coleoptiles do not employ local perception in their gravitropic response under the conditions of these experiments might be the first discovery of the model. It is much more important that the revised local curvature distribution model, which is the first mathematical model to give an adequate explanation for the compensation process that occurs in the gravitropic reaction of the mushroom stem, can also be fitted to the gravitropic reaction of *Triticum* coleoptiles. Indeed, this model accounts for several features of plant gravitropism that earlier models have ignored. Models that suppose that the gravitropic signal is proportional to the cosine of the apex angle (α), for example those of Johnson & Israelson (1968) and Barlow *et al.* (1991), do not deal with cases in which the bending stops before α reaches 90°. Models that incorporate a diagravitropic component (β) to account for a horizontal organ's reaching a final rest angle less than 90° by supposing the gravitropic signal to be proportional to $\sin(\alpha-\beta)$ (e.g. Model 2 in Stočkus, 1994a) are unable to account for the fact that the vertical organ does not bend to apex angle β.

Plant organs usually regain their straight appearance after showing gravitropic curvature. The change of the angular position was interpreted initially as the cause of straightening. However, some experimental results are not explainable simply by the change in angular position and the consequential response to gravity. Dolk (1936) showed that oat coleoptiles straighten even while being rotated on a horizontal clinostat. Firn & Digby (1979) showed that sunflower hypocotyls and maize coleoptiles began to straighten in the parts that had not reached the vertical position. The authors argued that this straightening is related to the curvature induced by gravitropic stimulus rather than to the stimulus itself; straightening was interpreted as a counter-reaction to the tropic curvature, opposite to the first curvature. Pickard (1973) also provided shadowgraphs of an oat coleoptile indicating that straightening progressed before any part of the coleoptile reached the vertical position. MacDonald *et al.* (1983) showed a similar straightening reaction

in cucumber hypocotyls and argued that the final set point angle achieved by an organ is a composite response of two tropisms, gravitropism and auto-tropism. In that paper, 'autotropism' was defined as an autonomic straightening response that was a consequence of the preceding gravitropic curvature. Hence, differently from other tropisms, autotropism is not a response to external stimuli: it is a response to internal stimuli arising from the curvature of the axial organ. Experiments with oat and wheat cole-optiles prompted Tarui & Iino (1997) to conclude that 'autotropic response actively counteracts the gravitropic response to straighten the coleoptile'.

In their recent review, Stanković *et al.* (1998) argue that this straightening is part of the gravitropic reaction itself rather than a separate process. They also argue strongly against the use of the word 'autotropism' to describe the phenomenon. It is not fitting for us to enter into either argument at the moment, but we would make two points. The first arises from our analyses of mushroom gravitropism (Meškauskas *et al.*, 1999). The word 'autotropism' is definitely not applicable to the straightening that occurs after gravitropism of mushroom stems be-cause it already has a long history of use in reference to interactions between hyphae in fungal colonies. We used instead the term 'curvature compensation' and it seems to us that this phrase is sufficiently free of implications as to mechanism to be equally valuable for application to the plant phenomenon.

In writing our mathematical model, the tropic bending process has been represented as being determined by the current spatial orientation of the stem and the stem curvature. Increase in curvature is viewed in the model as causing some 'straightening signal' (or factor). The current direction of bending is determined by the balance between the gravitropic signal (transmitted from the apex or locally per-ceived) and this straightening signal. Our second point, then, is that although we have no basis (in terms of available experimental data) to contribute to the argument on whether the straightening reaction should or should not be interpreted as a separate tropic response, our experience is that *sensing* the local curvature is a crucial component of a math-ematical model that gives very realistic simulations of plant gravitropism. We therefore suggest that the model could be a powerful tool for investigating the biological nature of 'autotropism'.

The revised local curvature distribution model is so effective in simulating the gravitropic behaviour of both fungal and plant axial organs that it might well prove to be a universal model. Future improve-ments might include:

● setting the perception function to assume a stationary angle other than the vertical to explain the gravitropic reaction of truly diagravitropic organs;

● incorporating a competence function to account for bending in organs in which the capacity for differential growth is known to be unequally distributed along the main organ axis, for example maize roots (Barlow & Rathfelder, 1985);

● finding a way to account for those rare cases in which plant organs growing plagiogravitropically seem to have naturally differentiated upper and lower flanks and behave differently depending on how those flanks are oriented when the organ is experimentally displaced as, for example, in *Ranunculus* stems (Zimmerman, 1924b).

Further, the model environment could be made more complex to simulate a wider set of manipu-lations, including reorientations during the bending process and behaviour on clinostats or in micro-gravity conditions. Obviously, all such improve-ments require adequate experimental data that are not yet available.

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