New Phytol. (1999), **143**, 387–399

Mathematical modelling of morphogenesis in fungi: a key role for curvature compensation ('autotropism') in the local curvature distribution model

AUDRIUS MEŠKAUSKAS[1,2], LILYANN NOVAK FRAZER[1] AND DAVID MOORE[1]*

[1] *School of Biological Sciences, The University of Manchester, Manchester M13 9PT, UK*
[2] *Institute of Botany, Žaliųjų ežerų 49, 2021 Vilnius, Lithuania*

Received 11 November 1998; accepted 16 April 1999

SUMMARY

The assumption that the mushroom stem has the ability to undergo autonomic straightening enables a mathematical model to be written that accurately mimics the gravitropic reaction of the stems of *Coprinus cinereus*. The straightening mechanism is called curvature compensation here, but is equivalent to the 'autotropism' that often accompanies the gravitropic reactions of axial organs in plants. In the consequently revised local curvature distribution model, local bending rate is determined by the difference between the 'bending signal' (generated by gravitropic signal perception systems) and the 'straightening signal' (proportional to the local curvature at the given point). The model describes gravitropic stem bending in the standard assay with great accuracy but has the virtue of operating well outside the experimental data set used in its derivation. It is shown, for example, that the mathematical model can be fitted to the gravitropic reactions of stems treated with metabolic inhibitors by a change of parameters that parallel the independently derived physiological interpretation of inhibitor action. The revised local curvature distribution model promises to be a predictive tool in the further analysis of gravitropism in mushrooms.

Key words: mathematical models, computer simulation, signal transmission, *Coprinus cinereus*, gravitropism.

INTRODUCTION

The first attempt at deriving a mathematical model of the gravitropic reaction of mushroom stems (Stočkus & Moore, 1996) successfully simulated the gravitropic change in apex angle. The basic assumptions of this scheme, derived from Rawitscher (1932) and Merkys *et al.* (1972), were that changes in apex angle occurred as a result of four consecutive stages: (i) the physical change that occurs when the subject is disoriented, (ii) conversion of the physical change into a physiological change, (iii) transmission of the physiological signal to the competent tissue, and (iv) the growth response in which differential regulation of growth generates the change in apex angle. A combined equation was established that could generate simulated kinetics that imitated the reaction of mushroom stems quite well.

Although successful, the imitational models of Stočkus & Moore (1996) dealt with change in apex angle only. In these analyses the bending process forming the apex angle was decreased to a 'mathematical point', an abstraction equivalent to a dimensionless hinge. No attention was given to the shape of the stem as it reacted. It is evident from the observations that have been made, however, that there is a complex distribution of bending rates in mushroom stems during their tropic reactions (Kher *et al.*, 1992; Moore *et al.*, 1994). There are regions that, after reaching a particular angle, start to straighten. Almost 90% of the initial curvature is reversed by this process (called 'curvature compensation' by Kher *et al.* (1992)), so it cannot be ignored. Consequently, a more realistic model of the mushroom stem gravitropic reaction must describe the bending process in space as well as in time.

*Author for correspondence (tel +44 161 275 39093; fax +44 161 275 5656; e-mail david.moore@man.ac.uk).

In an earlier paper (Meškauskas *et al.*, 1998) we described the spatial organization of the gravitropic reaction in *Coprinus cinereus* as a descriptive mathematical model. This local curvature distribution model is able to simulate accurately the shapes assumed by real stems of *C. cinereus* fruit bodies that have been experimentally pinned to a horizontal support. By successfully simulating the morphology of experimentally manipulated tissue, the model approaches the desired aim of a mathematical description of fungal morphogenesis. This first model has difficulties, however, in accounting for the observation that during the gravitropic reaction the apical part of *C. cinereus* stems stops bending and begins to straighten well before reaching the vertical position, leaving the final realization of the gravitropic response to more basal subsections. In the first model this was accounted for by complexity in the signal perception mechanism. It was supposed that the bending stops and straightening begins after the subsection reaches some critical orientation in the gravity field because signal transmission through the subsection depends on the orientation of the subsection. A suitable mathematical expression was found empirically. Unfortunately, there are two problems with this. First, the expression applies specifically to stems displaced to the horizontal: to simulate gravitropic bending from different initial positions, a function in this equation must be fitted separately for each new initial angle. Second, the nature of the empirical solution (the ratio between the gradient of local bending rate along the stem and the local bending rate) is not readily understood in biological terms. Hence, this model is largely descriptive and cannot be used to predict the development of gravitropic reactions outside the parameter envelope from which it was derived, for example displacement angles other than the horizontal, or experimental manipulation of perception and/or signal transmission by the use of metabolic inhibitors. This deficiency implies that the first local curvature distribution model lacks some factor that is important for the spatial organization of the gravitropic reaction in real *C. cinereus* stems. Our further analysis leads us to conclude that curvature compensation is the relevant factor.

The key observation comes from experiments with clinostats, which equalize exposure to the otherwise unidirectional gravity vector (Hatton & Moore, 1992). These authors showed that *C. cinereus* stems that were gravitropically bent by initial exposure to the unilateral gravity vector were able to straighten when subjected to rotation on a clinostat. Hence, the stem seems to be able to sense its curvature and in the absence of the external tropic signal will restore the shape that existed before the tropic change. In plants, such a reaction is widely observed and has been called 'autotropism', although other terms have been used and their relative utilities and, indeed, strict accuracy are arguable (Stanković *et al.*, 1998). The term 'autotropism' is used widely in mycology to describe the tendency for hyphal tips to grow towards (positive autotropism) or away from (negative autotropism) other hyphae of the same mycelium, so to use the term here would be inappropriate. We shall continue to use the phrase 'curvature compensation' in the sense in which it was coined by Kher *et al.* (1992); that is, an autonomic straightening of a gravitropic bend.

Curvature compensation can be included in the local curvature distribution model by supposing that the actual bending rate is determined by the balance between the gravitropic and curvature compensation signals. The gravitropic signal is a function of the local *angle* in the signal perception site. The curvature compensation signal can be assumed to be a function of the local *curvature* in the signal perception site. Hence, the actual bending rate in any subsection can depend on the *difference* between the gravitropic and curvature compensation signals in this subsection. In the displaced stem, the gravitropic signal makes the subsection bend. In a straight stem displaced to the horizontal, the gravitropic signal is maximal and the curvature compensation signal is zero. As the stem bends, the gravitropic signal weakens (as the angle of displacement of the perception system lessens) but the bending enhances the curvature compensation signal. When the signals become equal the bending process stops. If the gravitropic signal continues to weaken (for example, by further approach to the vertical position because of the bending of more basal subsections), straightening begins. Thus, it is logical to expect the straightening of the apical part before the apex reaches the vertical position. On the clinostat, only the curvature compensation signal remains in a previously bent stem. If the gravitropic signal is perceived in the apex, it takes some time before the information 'no gravitropic signal' reaches any particular subsection. During this period, the subsection can still bend in the previous direction. Later, the bending direction is determined by the curvature compensation signal, which causes straightening. The experimental data of Hatton & Moore (1992) completely confirm this hypothesis: bending continues in the earlier direction for approx. 30 min after the stem is first placed on the clinostat. Later, gravitropic bending is replaced by straightening.

Here we describe a revised local curvature distribution model in which straightening is determined by local curvature, which is independent of the spatial orientation of the subsection. There is no need to assume any 'critical angle' below which the gravitropic signal changes markedly from positive (bending) to negative (straightening), so this model has a potential ability to predict the bending process from any initial position, from close to vertical to close to inverted vertical. None of the model parameters is specific to the initial orientation of the stem. This model is predictive, in that once fitted into the gravitropic reaction of a specimen for a certain angle it can simulate the bending process from any other angle. Furthermore, the simple wave equation is sufficient to describe the transmission of the hypothesized gravitropic signal so that the final form of this new model is simpler, making the biological interpretation of its parameters much easier. We show also that the new model successfully describes the gravitropic reaction of stems treated with metabolic inhibitors. This also confirms the credibility of the model, indicating possible links between the functions of the equations and actual physiological processes.

MATERIALS AND METHODS

Experimental material

The vegetative dikaryon of *Coprinus cinereus* (Fries) S. F. Gray was cultured on complete medium (Moore & Pukkila, 1985) in 9-cm Petri dishes in the dark at 37°C for 3–4 d. Fruiting bodies were obtained by inoculating the dikaryon on sterilized horse dung in crystallizing dishes, incubating at 37°C for 3–4 d in the dark and then transferring the dung cultures to a 26–28°C incubator with a 16 h light/8 h dark illumination cycle (white fluorescent lights, average illuminance 800 lx). The length of stem used at the start of observation was 40–48 mm. The standard assay of *C. cinereus* gravitropism involved removal of the cap followed by continuous video recording of the stem secured on a horizontal platform that was housed in a humidity chamber at room temperature. Video records give no evidence of rotation of the fruit body stem during either vertical growth or tropic bending. The numbers of stems involved in the experiments are given in the legends to the corresponding figures.

Image capture, analysis and mathematical model fitting

Images were captured from videotapes with Screen Machine Camera® version 1.1 peripherals and software at a resolution of 736 × 560 points per image (approx. 10 points mm^{-1}). Images were captured every 10 min up to 400 min, producing 41 images for each stem, except in experiments with the inhibitor A231817, for which images were captured up to 200 min, producing 21 images per stem.

Images were manipulated by 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. The distribution of local curvatures was calculated after polynomial regression by using Maple® V version 4.00b, as described in Meškauskas *et al.* (1998). The growth of the upper and lower sides of the *C. cinereus* stems was measured by using a custom program for image analysis, written in Borland Pascal version 7.0.

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 (that is, 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.

Inhibitor and water treatment

The effects of BAPTA (1,2-bis-(2-amino-5-nitrophenoxy)ethane-N,N,N',N'-tetraacetic acid), A231817 and cytochalasin D on the spatial organization of the gravitropic reaction of *C. cinereus* fruit body stems were tested. To perform the treatments, the stems were incubated vertically in

Table 1. *Tests for goodness of fit between observed stem shapes and stem shapes predicted by the revised local curvature distribution model in base-pinned* Coprinus cinereus *stems*

Parameter	Water	BAPTA 5 mM	Cytochalasin D 1 μM	Cytochalasin D 10 μM	A231817, 1 μM
Number of stems	9	7	9	9	16
Lack-of-fit mean square	0.00071	0.00057	0.00104	0.00339	0.00041
Pure error mean square	0.0059	0.0039	0.0081	0.0134	0.0009
df	1180, 10656	1244, 8736	1180, 10656	1180, 10656	604, 7296
Ratio	0.1199	0.1462	0.1279	0.2530	0.4566
Value z for which the distribution function $F(z) = 0.95$*	1.092	1.072	1.092	1.092	1.136

*All of these values indicate that differences between modelled shapes and observed shapes were not significant.

separate, end-sealed 1 ml pipette tips in the dark at room temperature (22 ± 1°C), totally immersed, for 2 h. After treatment, stems were rinsed briefly with distilled water (still in the vertical orientation); they were then turned to the horizontal position and video-recording was initiated. Controls were immersed in water. The exact numbers of stems involved in the experiments with each inhibitor are given in Table 1. Because BAPTA shifts pH significantly, the pH of the final BAPTA solution was adjusted to 6.2 ± 0.2 by titration. All chemicals were obtained from Sigma (Sigma-Aldrich Co. Ltd, Poole, Dorset, UK).

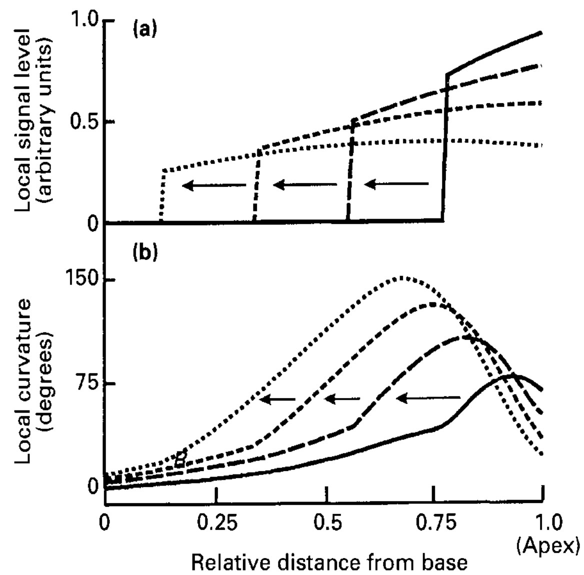

Fig. 2. Computer-generated distribution of the local level of the hypothetical gravitropic signal perceived in the apex (a) and the local curvature (b) during simulation of the gravitropic reaction of *Coprinus cinereus* stems. During the bending process the apex angle, and hence the signal level in the apex, decreases. The simulation was performed with parameter values from Table 2. The curvature distribution (b) can also be compared with directly measured data (Fig. 5b).

THE MODEL

Following Barlow *et al.* (1991) and Stočkus (1994a,b,c), we supposed that after reorientation the physiological signal arises in the apex, and that this signal at any time t is proportional to the cosine of the tip angle at that instant:

$$S_{\mathrm{Tip}}(t) = K_{\mathrm{S}} \cos[\alpha_{\mathrm{Tip}}(t)] \qquad \text{Eqn 1}$$

(the tip angle is measured as the angle from the horizontal (Fig. 1, curves labelled S)). After perception, the signal is transmitted towards the stem base. In our earlier model (Meškauskas *et al.*, 1998) the signal transmission function was unusual because it had to account for the fact that bend movement in real *C. cinereus* stems occurs with decreasing speed. However, when a curvature compensation signal is incorporated, this feature can be explained without further complication. The distribution of such a signal and the consequential distribution of local curvature is illustrated in Fig. 2. Accordingly, in this revised model the classic equation of signal transmission can be used, which assumes that the signal is transmitted as a wave whose magnitude decreases as it progresses. This is the most commonly used signal transmission function in gravitropic modelling (Johnson, 1971; Brown & Chapman, 1977; Stočkus 1994a):

$$S(\lambda,t) = S_{\mathrm{Tip}}[t-(1-\lambda)/\mathrm{v}]\, \mathrm{e}^{[-S_{\mathrm{DE}}(1-\lambda)]} \qquad \text{Eqn 2}$$

(λ is the relative distance from the base of the axial organ (0 = base, 1 = tip) and v is the signal

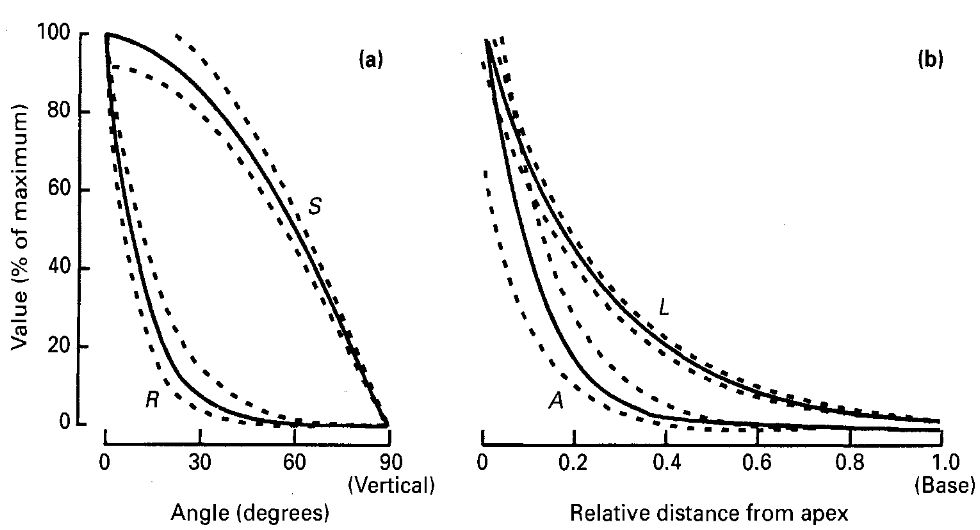

Fig. 1. Some dependences that can be calculated from the model parameters (Table 2) for *Coprinus cinereus* stems. (a) The gravitropic perception in the apex (S) and the local perception (R) as functions of the reorientation angle, which is the apex angle for S and the local angle for R. (b) The distribution of capacity for local perception of the gravitropic signal (L) and autotropic reaction (A) through the length of the stem. In both panels the vertical axis is given as a percentage of the maximum values that the two perception functions (S and R) acquire in the horizontal position (0°) and the two distribution functions (A and L) exhibit in the apex (position 0). The broken lines show 95% confidence intervals.

transmission rate, which is constant for a wave equation). Hence the signal reaches any particular realization point delayed by time $(1-\lambda)/\nu$ and the signal level decreases exponentially during transmission (as determined by the constant S_{DE}).

If we were to suppose that the local bending velocity is simply proportional to this local signal level $S(\lambda,t)$, we would obtain an equation similar to model 4 in Stočkus & Moore (1996). However, for an exact simulation of the spatial development of gravitropic reaction in *C. cinereus* the description of the realization must be a function not only of $S(\lambda,t)$ but also of two additional signals.

The curvature compensation (that is, straightening) signal arises in the same point at which the bending process develops, and the level of this signal is assumed to be proportional to local curvature, C_L. The curvature compensation signal is not transmitted through the stem, but it is known that the compensation process is more highly expressed close to the apex than it is in more basal subsections (Meškauskas *et al.*, 1998). Compensation is polarized. In stems secured at the apex in the horizontal position, the base is raised by the gravitropic curvature but the basal part undergoes much less straightening than usually occurs at the apex. To produce the working model, this fact was accounted for by supposing that the distribution of the capacity for curvature compensation is not uniform and decreases exponentially in the basipetal direction. Then, the level of the straightening signal at position λ in a given period t is:

$$A(t,\lambda) = -A_U\, e^{[-A_D(1-\lambda)]}\, C_L(t,\lambda) \qquad \text{Eqn 3}$$

(parameters A_U and A_D determine the distribution of curvature compensation along the stem (Fig. 1, curves labelled A)).

Eqns 2 and 3 together give a mathematical model that can reproduce all *C. cinereus* stem shapes that occur during gravitropic reaction, but the lack-of-fit test indicates significant differences between simulations and reality in the time dimension. The bending process simulated by this simpler model is too slow in the beginning of the gravitropic reaction (over the first approx. 3 h) and too fast in the later stages. We therefore included the local perception of the tropic signal, which is confirmed both experimentally (Greening *et al.*, 1993) and by mathematical modelling (Meškauskas *et al.*, 1998). This means that the gravitropic irritation is perceived both at the stem apex and in the site of realization. The local perception function differs from the perception function in the apex and can be approximated as:

$$R_{Local}(\alpha_L) = e^{(-B_{DE}\alpha_L)} \qquad \text{Eqn 4}$$

(α_L is the local reorientation angle of the subsection). Similarly to $\cos(\alpha_L)$, this function is maximal when $\alpha_L = 0$ (horizontal position), but it can decrease much faster when α_L starts to increase (Fig. 1, curves labelled R). Hence it was supposed that the local perception has the most important role in the first stages of the bending process, when $\alpha_L < 45°$.

The distribution of capacity for local perception is not uniform and decreases exponentially from the apex in the basipetal direction. Hence the level of the perception at time t in position λ is:

$$R(t,\lambda) = B_S\, e^{[-B_D(1-\lambda)]}\, R_{Local}(\alpha_L) \qquad \text{Eqn 5}$$

(Fig. 1, curves labelled L). Hence, the realization of the gravitropic response, the local bending rate at point λ at time t, is:

$$dC_L(t,\lambda)/dt = K_W\,[S(t,\lambda)+A(t,\lambda)+R(t,\lambda)] \qquad \text{Eqn 6}$$

(K_W is the realization constant).

By summarizing the equations shown above and expressing local angle through local curvature, we obtain the final equation below ($t \geqslant 0$; $0 \geqslant \lambda \geqslant 1$, initial condition $C_L(\lambda,0) = 0$ (straight stem), boundary conditions $C_L(t,0) = C_L(t,1) = 0$):

$$\frac{\partial}{\partial t}C_L(\lambda,t) = \Big\{K_S \cos\Big[\int_0^1 C_L\Big(t-\frac{1-\lambda}{\nu},\, x\Big)dx\Big]\, e^{-S_{DE}(1-\lambda)} - A_U\, e^{-A_D(1-\lambda)} C_L(t,\lambda) + B_S\, e^{-B_D(1-\lambda)}\, e^{-B_{DE}\int_0^\lambda C_L(t,x)dx}\Big\} K_W \qquad \text{Eqn 7}$$

By using this equation, the program in C++ was written to obtain numeric solutions.

RESULTS

Fitting the model to experimental data

The fit of the model to the gravitropic reactions of untreated *C. cinereus* stems is shown in Fig. 3. The values of the parameters, established by using the method of dichotomy (see the Materials and Methods section), are given in Table 2. A 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 1 shows that the model fits the experimental data; deviations were not statistically significant.

One of the interesting results seen in Table 2 is the quite large value (8.65) for the constant A_D that determines how fast the capacity for curvature compensation decreases in the basipetal direction. Such a value means that curvature compensation is mainly a characteristic of the apical 20–30% of the stem (Fig. 1, curves labelled A). For example, the capacity for curvature compensation in the middle of the stem is approx. 1.3% of the value at the apex. For more basal subsections it is even lower. Similarly, the ability to perceive the gravitropic signal

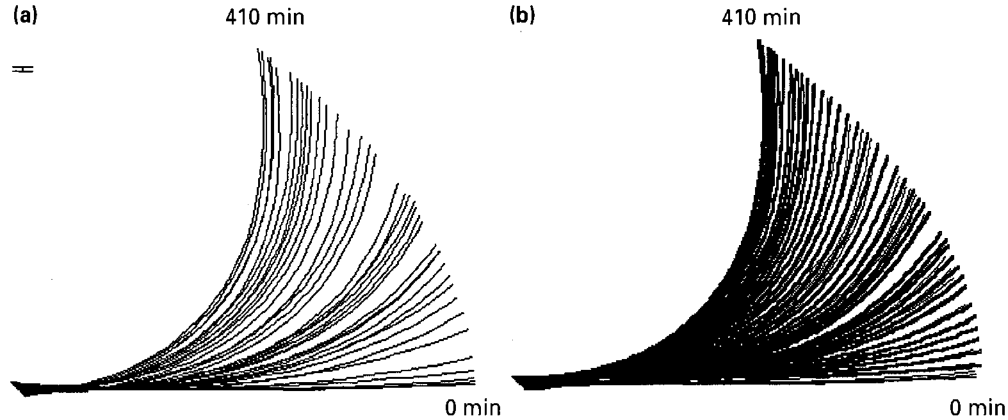

Fig. 3. Comparison of (a) averaged bending of base-pinned *Coprinus cinereus* stems with (b) the images generated by the model shown as Eqn 7. Calculated values of the parameters are given in Table 2. In (b), computer-generated images (thick lines) are plotted over the experimentally observed images (regular lines). Times of subsequent images differ by 10 min. The error bar at the top left of (a) defines the maximal SD of the mean for experimentally observed shapes. The goodness of fit was tested by the lack-of-fit test (see Table 1).

Table 2. *Model parameters obtained by optimizing the fit of the revised local curvature distribution model to the gravitropic reaction of* Coprinus cinereus *stems*

Parameter	Model feature, defined by this parameter	Units	Calculated value
K_S	Perception in the apex	Relative	1.00 ± 0.08
B_{DE}	Local perception	degree^{-1}	0.085 ± 0.02
B_S	Distribution of ability to perceive the gravitropic irritation along the stem	Relative	2.4 ± 0.15
B_D (exponent of B_S)	Distribution of ability to perceive the gravitropic irritation along the stem	Relative	4.00 ± 0.1
A_U	Distribution of ability for autotropism	Relative	0.0225 ± 0.0075
A_D (exponent of A_U)	Distribution of ability for autotropism	Relative	8.65 ± 0.85
S_{DE}	Signal decrement during transmission	Relative	1.575 ± 0.075
V_T	Signal transmission speed	h^{-1}	0.259 ± 0.0017
K_W	Local bending rate dependence on local signal level	degree h^{-1}	85.0 ± 3.0

locally is also mainly a feature of the apical two-thirds of the stem (Fig. 1, curves labelled L).

The signal transmission rate that emerges from this analysis is 0.259 h^{-1} (stem length is set to unit length) or approx. 12 mm h^{-1}. This is much faster than passive diffusion and could indicate the existence of some active system for signal transmission. It takes approx. 3 h for the signal to reach the base of the stem, where it maintains approx. 20% of its initial value. Signal decrement is quite significant.

Comparison of the growth in the upper and lower sides during the gravitropic bending of C. cinereus *stems*

The growth of lower and upper sides during the gravitropic reaction of *C. cinereus* stems is shown in Fig. 4. Both sides of vertical stems grew continuously at a rate of approx. 0.085 mm min^{-1}. In stems reoriented to the horizontal, the growth rate was about half this during the first 170 min, after which growth rate increased on both sides. The final length achieved at 400 min after reorientation was close to the length of vertical controls. Uniform decrease in growth rate supports the view that the gravitropic signal can be perceived throughout the stem. Greening *et al.* (1997) analysed incremental growth rates

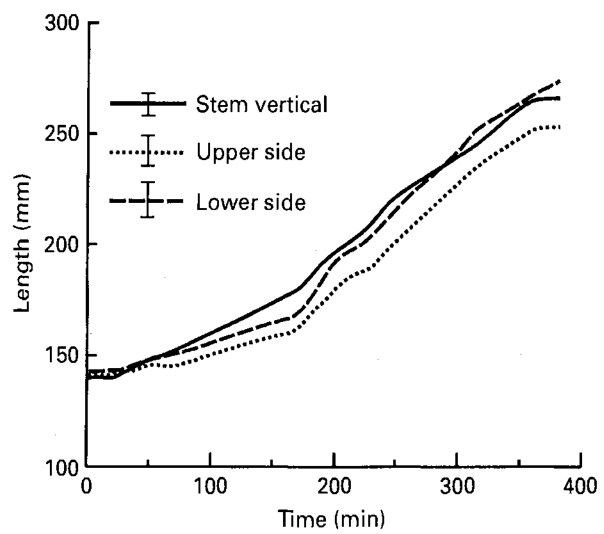

Fig. 4. The growth of the upper and lower sides of *Coprinus cinereus* stems during the gravitropic reaction. Growth was measured for the whole length of each side. The error bars in the upper part of the figure represent the maximum SD of the mean for each curve.

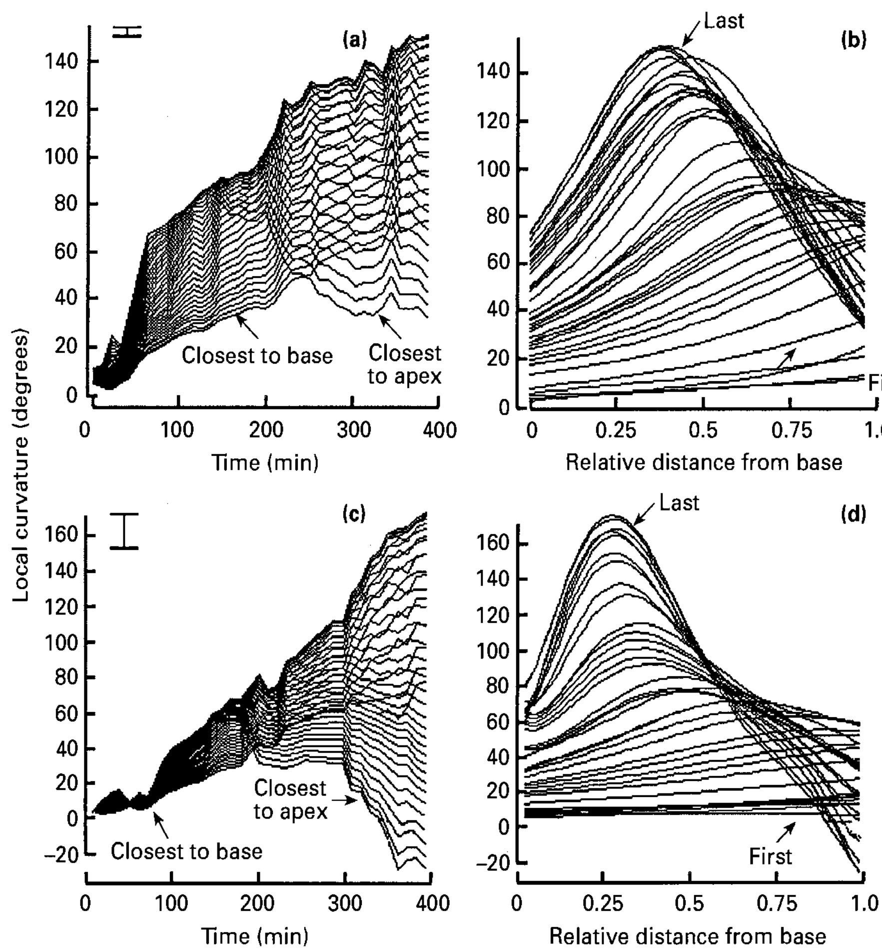

Fig. 5. The effect of incubation in water (2 h in vertical position before turning to the horizontal) on the distribution of local curvature over the length of the stem for base-pinned *Coprinus cinereus* stems. (a,b) Untreated control; (c,d) water treatment. (a,c) Time-telescoped projections (each curve represents the changing curvature of a different subsection of the stem); (b,d) space-telescoped projections (curves represent distribution of curvature over the length of the stem at 10 min intervals). The distance from the base is given in arbitrary units of stem length. The error bar at the top left of (a) and (c) represents the maximum SD for both projections in each case. Note that as the apical regions of the stem start to straighten (shown as smaller local curvature in the last and later lines of (b) and (d)), the corresponding lines in (a) and (c) curve downwards (from approx. 130 min).

and showed that the gravitropic bend is caused by the lower side accelerating its growth rate more rapidly than the that of the upper side.

Fitting the model to the gravitropic reactions of inhibitor-treated C. cinereus *stems*

Inhibitors of specific cellular processes can be used to establish links between components of the mathematical model and actual physiological processes.

To perform the inhibitor treatments described here, the stems were incubated for 2 h in the vertical position in inhibitor solution. A water treatment was used as the control and the effect of this on the development of local curvature is shown in Fig. 5. The data are shown in two ways (and the same sort of display is used in Figs 6, 9, 11 and 13). Fig. 5a,c are time-telescoped projections in which each curve represents the changing curvature of a different subsection of the stem during the time course of the experiment. Fig. 5b,d are space-telescoped projections in which the curves show the distribution of curvature over the whole length of the stem at 10 min intervals. Fig. 5 shows that the water treatment slowed the beginning of the bending process in comparison with untreated stems and that the subsequent straightening of the water-treated stems was more vigorous.

However, fitting the model to the gravitropic reaction of water-treated stems shows that only two parameters were changed (Table 3). The apical region, where the curvature compensation is most significant, was longer (A_D smaller) and the realization of the gravitropic response in the bending site was slower (K_W smaller). The gravitropic reactions of inhibitor-treated stems were compared with the water-treated controls rather than untreated stems (Table 3).

The effect of BAPTA. BAPTA is specific chelator for calcium ions. Calcium chelators suppress the gravitropic reaction in plant roots (Lee *et al.*, 1983) and coleoptiles (Medvedev *et al.*, 1991). In all such cases the effect of calcium chelators can be reversed by subsequent treatment with calcium ions. In our experiments also it was shown that 1 h of treatment

Table 3. *Changes required in parameters of the local curvature distribution model to account for stem gravitropic behaviour of* Coprinus cinereus *in response to different experimental treatments*

Parameter	Change required	Percentage of control	Maximum deviation for the new optimal value
Effect of water treatment in comparison with untreated control			
K_W	85.0 → 61.5	72.3	±23.5
A_D	8.65 → 4.65	53.7	−2.24, +∞*
Effect of other treatments in comparison with water-treated control			
BAPTA 5 mM			
V_T	0.259 → 0.125	48.3	±0.04
S_{DE}	1.575 → 1.2	76.1	−1.2, +1.8†
B_S	2.4 → 0.45	18.7	±0.6
A_D	4.65 → 8.65	185.8	−5.00, +∞*
Cytochalasin 1 μM			
K_W	61.5 → 47.5	77.2	±12.5
Cytochalasin-D 10 μM			
V_T	0.259 → 0.003	1.1	−0.003, +0.09†
K_W	61.5 → 47.6	77.3	±12.4
A231817, 1 μM			
K_W	61.5 → 20.0	32.5	±6.0

Changes were taken into consideration only if the new optimal value differed by more than 10% from the control.
*Infinite value means the absence of autotropism.
†The optimal value is different from the average of minimal and maximal values. The minimal and maximal values are the boundary values between which the model still satisfies the lack-of-fit test.

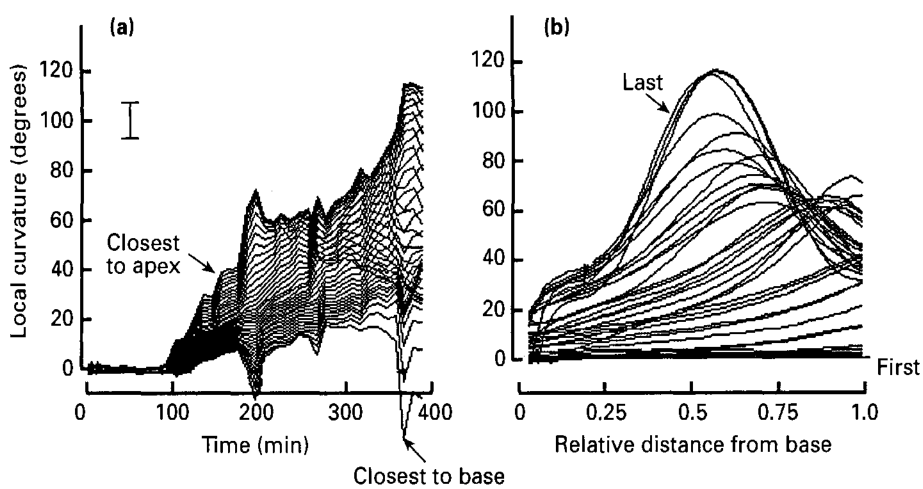

Fig. 6. Distribution of local curvature over the length of the stem for BAPTA-treated (5 mM, 2 h) *Coprinus cinereus* stems. (a) Time-telescoped projection (each curve represents the changing curvature of a different stem subsection); (b) space-telescoped projection (curves represent 10 min time intervals). The distance from the base is given in arbitrary units of stem length. The error bar represents the maximum SD for both projections.

with BAPTA followed by 1 h of treatment with 10 mM $CaCl_2$ resulted in no effect on the spatial organization of the *C. cinereus* gravitropic reaction (results not shown). BAPTA delays the bending process and changes the curvature development pattern in *C. cinereus* stems (Novak Frazer & Moore, 1993). The BAPTA-treated stems also curl beyond the vertical in very late stages of gravitropic reaction when control stems have returned and remain at the vertical (Novak Frazer & Moore, 1993).

One of the most obvious effects of 2 h of treatment with 5 mM BAPTA, seen in Fig. 6 (in both projections) was a significant initial delay of gravitropic bending. In the first 90 min of the gravitropic reaction the stem remained almost completely straight, and only after that time did the bending process begin. In some models of gravitropic reaction (Stočkus, 1994a) the initial bending delay is closely related to signal transmission rate. It might be that the treatment decreases this parameter. Although the compensation process is clearly seen, it is smaller than in the water-treated control stems (Fig. 5). This causes the region of maximum curvature to remain closer to the apex.

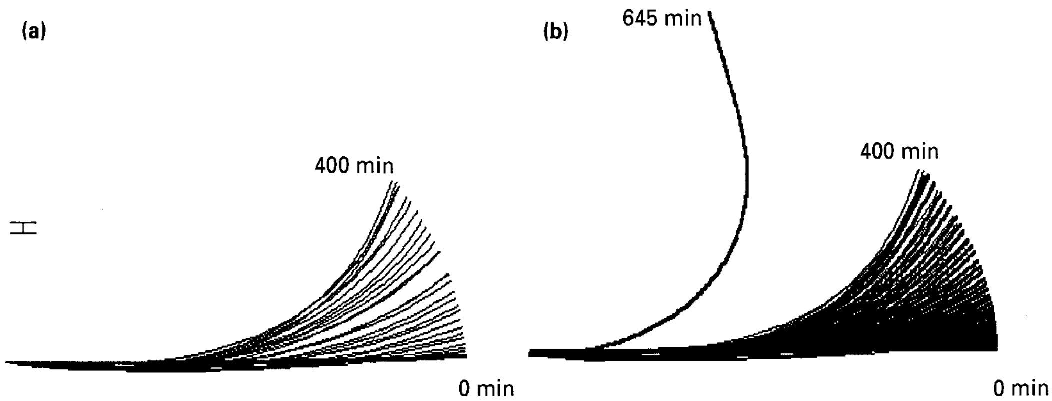

Fig. 7. Comparison of (a) averaged bending of BAPTA-treated (5 mM, 2 h) *Coprinus cinereus* stems with (b) the images generated by the model shown as Eqn 7 with parameters given in Table 3. In (b), computer-generated images (thick lines) are plotted over the experimentally observed images (regular lines). Times of subsequent images differ by 10 min. The error bar in (a) defines the maximum SD of the mean for experimentally observed shapes. The goodness of fit was tested by the lack-of-fit test (see Table 1). The computer-generated images also include a shape for a very late stage of the gravitropic reaction (10 h from reorientation).

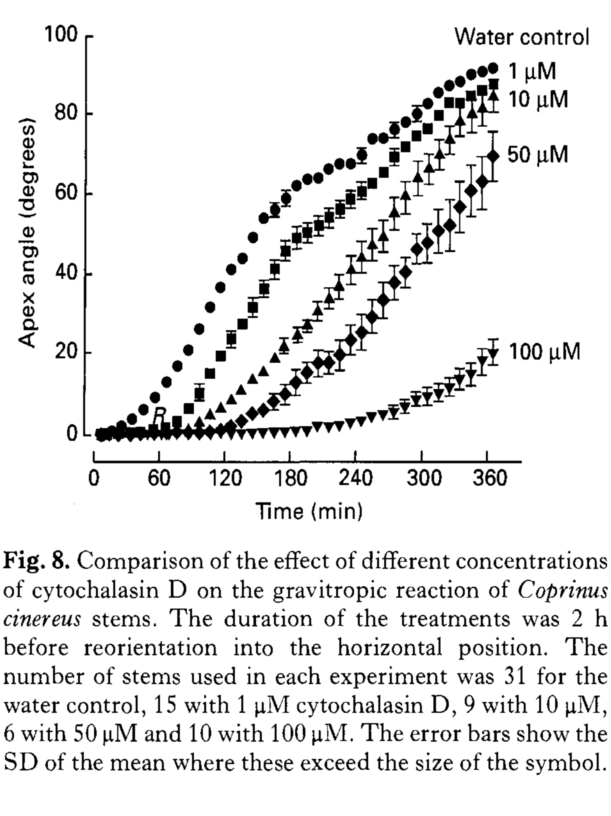

Fig. 8. Comparison of the effect of different concentrations of cytochalasin D on the gravitropic reaction of *Coprinus cinereus* stems. The duration of the treatments was 2 h before reorientation into the horizontal position. The number of stems used in each experiment was 31 for the water control, 15 with 1 μM cytochalasin D, 9 with 10 μM, 6 with 50 μM and 10 with 100 μM. The error bars show the SD of the mean where these exceed the size of the symbol.

The revised model is able to simulate the gravitropic reaction of BAPTA-treated stems, including the curling beyond the vertical in late stages (Fig. 7). In comparison with the water-treated control, the model indicates a lower signal transmission rate (V_T), significant inhibition of local signal perception B_S (to a level only 18.7 % of the control), enhancement of curvature compensation and a slight decrease in the signal decrement during transmission that can be linked with the decreased transmission rate (Table 3). The bending past the vertical in the model is caused by slow signal transmission rate: the bending part is late to respond to the fact that the vertical position has already been reached. The model confirms the interpretations of Novak Frazer & Moore (1996) that calcium chelators affect signal transmission during the gravitropic reaction in *C. cinereus* but not its perception at the apex.

The effect of cytochalasin D. Cytochalasin D disrupts the actin microfilament networks in fungal hyphae (Kanbe *et al.*, 1993), including those in the fruiting bodies of *Flammulina velutipes*, in which it causes a loss of 80 % of stem gravitropic bending capability (Monzer, 1995). The lowest concentration of cytochalasin D that caused noticeable changes in the gravitropic reaction of *C. cinereus* stems was 1 μM (Fig. 8); 10 μM caused further qualitative changes, and 50 and 100 μM caused further quantitative changes. The effects of both 1 μM and 10 μM cytochalasin D in a 2 h treatment regime were analysed (Fig. 9). Exposure to 1 μM cytochalasin D for 2 h did not change the spatial organization of the gravitropic reaction significantly. As is seen in Fig. 10 and Table 1, the revised model fits the gravitropic reaction of such stems extremely well. The only parameter needing modification was the realization constant K_W (Table 3).

Low concentrations of cytochalasin D might interfere with the final steps of the realization of the bending process without significant effect on signal perception and transmission. However, the realization constant K_W is not completely independent of the constants of local perception (B_S), local curvature compensation (A_U) and signal perception in the tip (K_S). An alternative hypothesis could therefore be that K_W remained unchanged but K_S, B_S and A_U decreased to the same extent (22.8 %). Eqn 7 would return the same values in these two circumstances. Therefore it is also possible to suggest the hypothesis of Monzer (1995), that cytochalasin D at low concentration inhibits the perception of signals involved in the gravitropic reaction (see Eqn 6).

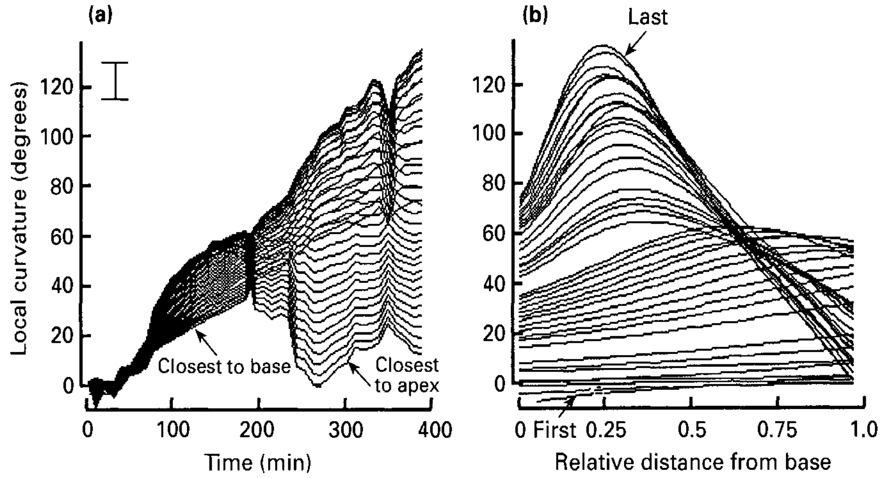

Fig. 9. The distribution of local curvature over the length of the stem for *Coprinus cinereus* stems treated with cytochalasin D (1 μM, 2 h). (a) Time-telescoped projection (each curve represents the changing curvature of a different stem subsection); (b) space-telescoped projection (curves represent 10 min time intervals). The distance from the base is given in arbitrary units of stem length. The error bar at the top left of (a) represents the maximum SD for both projections.

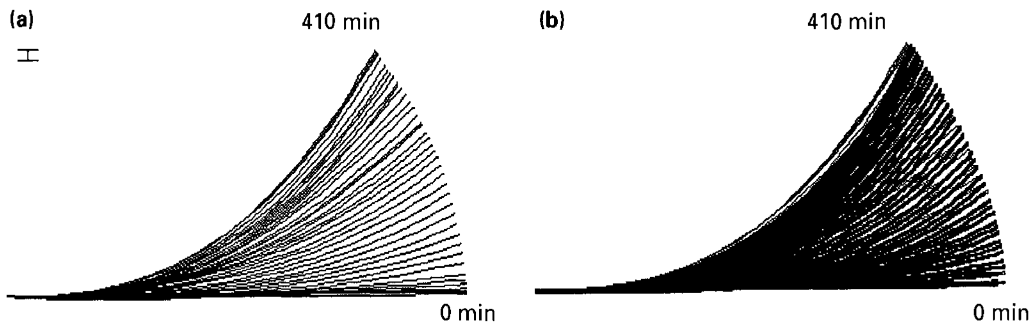

Fig. 10. Comparison of (a) averaged bending of *Coprinus cinereus* stems treated with cytochalasin D (1 μM, 2 h) with (b) the images generated by the model shown as Eqn 7 with parameters given in Table 3. In (b), computer-generated images (thick lines) are plotted over the experimentally observed images (regular lines). Times of subsequent images differ by 10 min. The error bar at the top left of (a) defines the maximum SD of the mean for experimentally observed shapes. The goodness of fit was tested by the lack-of-fit test (see Table 1).

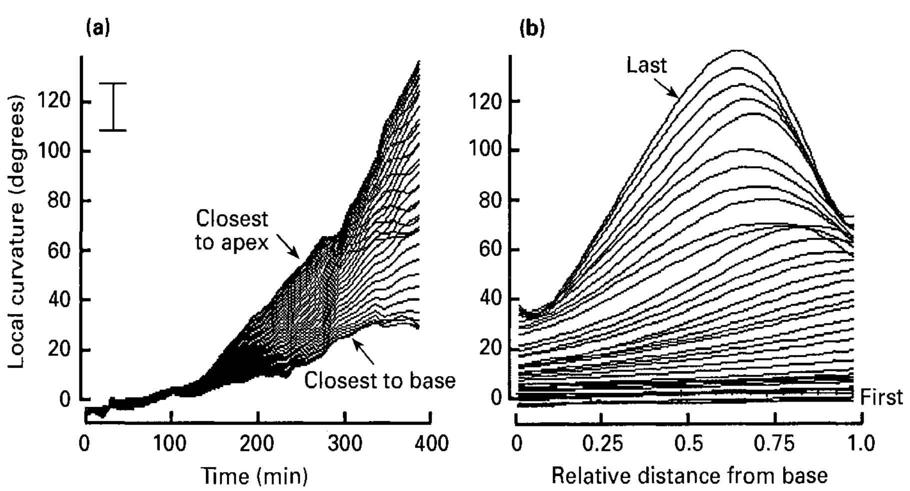

Fig. 11. The distribution of local curvature over the length of the stem for *Coprinus cinereus* stems treated with cytochalasin D (10 μM, 2 h). (a) Time-telescoped projection (each curve represents the changing curvature of a different stem subsection); (b) space-telescoped projection (curves represent 10 min time intervals). The distance from the base is given in arbitrary units of stem length. The error bar at the top left of (a) represents the maximum SD for both projections.

Cytochalasin D at 10 μM significantly changed the spatial development of the gravitropic response (Fig. 11). As shown in the time-telescoped projection (Fig. 11a), the gravitropic reaction was significantly slower, especially during the first 200 min. The space-telescoped projection (Fig. 11b) shows that

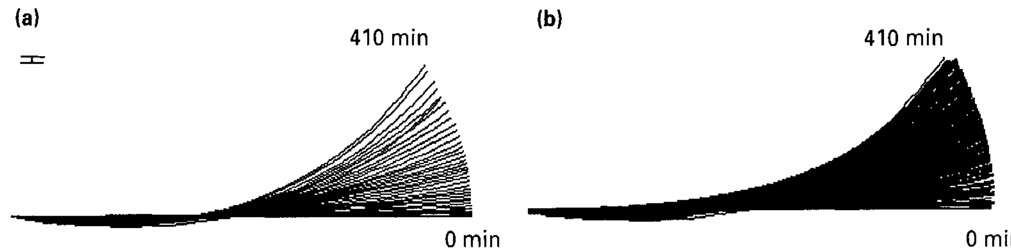

Fig. 12. Comparison of (a) averaged bending of *Coprinus cinereus* stems treated with cytochalasin D (10 µM, 2 h) with (b) the images generated by the model shown as Eqn 7 with parameters given in Table 3. In (b), computer-generated images (thick lines) are plotted over, and because of the accuracy of the model largely obscure, the experimentally observed images (regular lines). Times of subsequent images differ by 10 min. The error bar at the top left of (a) defines the maximum SD of the mean for experimentally observed shapes. The goodness of fit was tested by the lack-of-fit test (see Table 1).

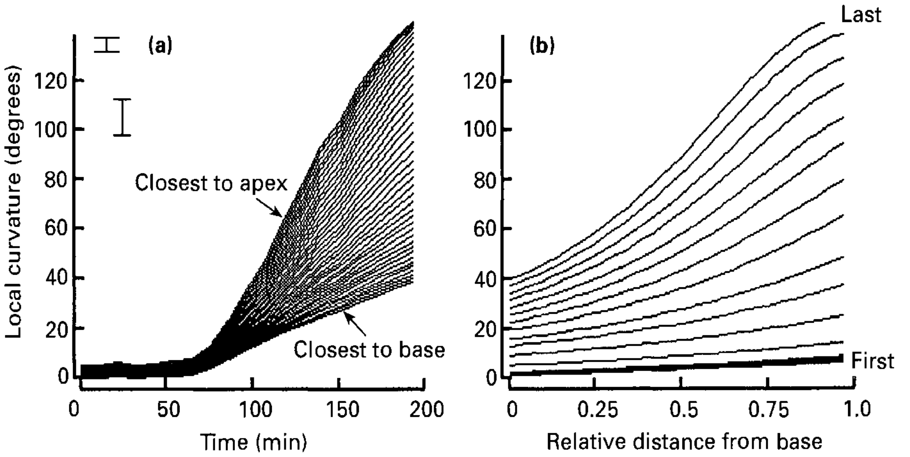

Fig. 13. The distribution of local curvature over the length of the stem for *Coprinus cinereus* stems treated with A231817 (1 µM, 2 h). (a) Time-telescoped projection (each curve represents the changing curvature of a different stem subsection); (b) space-telescoped projection (curves represent 10 min time intervals). The distance from the base is given in arbitrary units of stem length. The error bar at the top left of (a) represents maximum SD for both projections. Note that stem straightening was minimal.

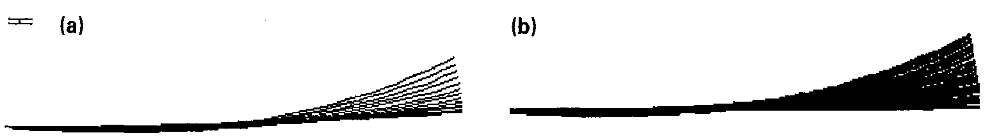

Fig. 14. Comparison of (a) averaged bending of *Coprinus cinereus* stem treated with A231817 (1 µM, 2 h) with (b) the images generated by the model shown as Eqn 7 with parameters given in Table 3. In (b), computer-generated images (thick lines) are plotted over, and because of the accuracy of the model largely obscure, the experimentally observed images (regular lines). Times of subsequent images differ by 10 min. The error bar at the top left of (a) defines the maximum SD of the mean for experimentally observed shapes. The goodness of fit was tested by the lack-of-fit test (see Table 1).

the region of maximum curvature remained much closer to the apex throughout than it did in the control. This effect was even more significant than in BAPTA-treated stems (Fig. 7).

Fitting the revised model to these gravitropic reactions requires a decrease in the signal transmission rate to 0.2 mm h^{-1}, approx. 1.1% of the initial value (Fig. 12, Table 3). Consequently, signal transmission is largely blocked, and the role of the local signal perception is enhanced in such treated stems, especially in the earlier stages. Cytochalasin D did not suppress local perception. The realization constant (K_W) was decreased to approximately the same value as seen with 1 µM cytochalasin D, so the saturation point might have been reached at this lower concentration.

In summary, low concentrations of cytochalasin D inhibit the bending process or perhaps the perception of all three signals involved in the gravitropic reaction (apex and local perception of reorientation, and local curvature perception) to the same extent. Higher concentrations of cytochalasin D additionally suppress signal transmission.

The effect of A231817. A231817 forms specific Ca^{2+} channels in the membranes, so it can decrease the calcium activity gradient on the outside of plasma membranes and also abolish the cytoplasmic sequestration of calcium ions. The revised model could be fitted to the gravitropic reaction under the influence of A231817 after decreasing the realization constant K_W (Table 3, Figs 13, 14). This implies that the calcium ionophore A231817 decreases the bending rate (it also suppresses the random oscillations of the bending rate and decreases the variance between the bending of different stems). Stem straightening was minimal (Fig. 13), but it is not possible to say whether this was an independent effect of the ionophore (perhaps inhibiting curvature compensation) or was a consequence of the decreased tropic bending rate.

DISCUSSION

The revised local curvature distribution model described here successfully accounts for curvature compensation (straightening before the apex reaches the vertical position) with the following assumptions:

- the bending process is influenced by the curvature compensation signal, which causes straightening if the gravitropic signal is decreased 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 curvature compensation signal at any point in the stem is proportional to the local curvature in that point, and the ability of the stem to generate this signal decreases from the apex toward the base (Fig. 1);
- the gravitropic signal perception system in the stem apex generates a signal proportional to the cosine of the apex angle;
- this signal is transmitted towards the stem base, weakening as it progresses, at a constant rate of approx. 12 mm h^{-1}. This simple assumption is sufficient to explain the gravitropic curvature of stems over the first 6 h. In very late stages this model predicts an overshoot of approx. 5° beyond the vertical and several decreasing oscillations about the vertical position. If signal transmission through subsections is assumed to be blocked after the subsection reaches orientations close to the vertical (85–95°), no overshoot is predicted);
- together with the perception of the gravitropic signal in the apex, a system of local gravity vector perception exists, and the ability to detect the gravity vector locally decreases from the apex toward the base (Fig. 1).

The overwhelming advantage of this revised local curvature distribution model is that it can be used predictively to fit situations that were not part of the context used to design the original model. This includes angles of disorientation other than the horizontal displacement used in our standard experiments. More importantly, we show here, by examining how the model fits data from experiments with metabolic inhibitors, that the model is capable of revealing effects that exactly match conclusions reached from purely physiological considerations. Significantly, by its very nature as a mathematical description, the model returns quantitative values for the physiological effects that it detects.

It is the inclusion of a function describing curvature compensation that enabled this revised local curvature distribution model to be produced, yet the nature of curvature compensation is far from clear. Possibly, the 'rest' state of the system (in our case, a straight stem) is determined by internal controls in a 'default' state to which they tend to return after the environmental change (in our case, disorientation from the vertical) that caused the initial disturbance decreases below a particular level. The implication is that the disturbed state is unstable and needs continued reinforcement to be maintained. The situation is different from the alternative 'trigger' hypothesis that environmental change switches the system into an alternative stable state.

A phenomenon similar to curvature compensation has often been observed in plant gravitropic reactions. Plant organs usually regain their straight appearance after showing gravitropic curvature. 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 was related to the curvature induced by the gravitropic stimulus rather than to the stimulus itself. The straightening was conceived of as a counter-reaction to the tropic curvature. Pickard (1973) showed that straightening progressed before any part of the oat coleoptile reached the vertical position, and MacDonald *et al.* (1983) found a similar straightening reaction in cucumber hypocotyls. These authors argued that the final set point angle achieved by an organ is a composite response of two tropisms, gravitropism and autotropism. In that paper, 'autotropism' was defined as an autonomic straightening response that resulted as a consequence of a preceding gravitropic curvature. Hence, differently from other tropisms, autotropism is not a response to external stimulus but to the internal stimulus arising from the curvature of the organ. In their review, Stanković *et al.* (1998) argue that this straightening is part of the gravitropic reaction itself (a conclusion with which we cannot agree). However, Stanković *et al.* (1998) state that '... Autotropic straightening partially returns the organ to the orientation that existed before it was exposed to a new signal gradient ... The organ seems to have a spatial 'memory' of the previous growth vector established by an earlier environmental, regulatory stimulus ... The identity of this spatial memory is both intriguing and completely unknown.' With those sentiments we do agree.

ACKNOWLEDGEMENTS

A. M. thanks the British Mycological Society and the Federation of European Microbiological Societies for the award of a FEMS Fellowship, and the Royal Society of London for the award of a Fellowship under the NATO Postdoctoral Fellowship Programme.

REFERENCES

Barlow P, Brain P, Butler R, Parker J. 1991. Modelling of the growth response of gravireacting roots. *Aspects of Applied Biology* **26**: 221–225.

Brown A, Chapman D. 1977. Effects of increased gravity force on nutations of sunflower hypocotyls. *Plant Physiology* **59**: 636–640.

Firn RD, Digby J. 1979. A study of the autotropic straightening reaction of a shoot previously curved during geotropism. *Plant, Cell and Environment* **2**: 149–154.

Greening JP, Holden J, Moore D. 1993. Distribution of mechanical stress is not involved in regulating stipe gravitropism in *Coprinus cinereus*. *Mycological Research* **97**: 1001–1004.

Greening JP, Sánchez C, Moore D. 1997. Coordinated cell elongation alone drives tropic bending in stems of the mushroom fruit body of *Coprinus cinereus*. *Canadian Journal of Botany* **75**: 1174–1181.

Hatton JP, Moore D. 1992. Kinetics of stem gravitropism in *Coprinus cinereus*: determination of presentation time and 'dosage–response' relationships using clinostats. *FEMS Microbiology Letters* **100**: 81–86.

Johnson A. 1971. Geotropic responses in *Helianthus* and their dependence on the auxin ratio – with a refined mathematical description of the course of geotropic movements. *Physiologia Plantarum* **24**: 419–425.

Kanbe T, Akashi T, Tanaka K. 1993. Effect of cytochalasin A on actin distribution in the fission yeast *Schizosaccaromyces pombe* studied by fluorescent and electron microscopy. *Protoplasma* **176**: 24–32.

Kher K, Greening JP, Hatton JP, Novak Frazer L, Moore D. 1992. Kinetics and mechanics of stem gravitropism in *Coprinus cinereus*. *Mycological Research* **96**: 817–824.

Lee J, Mulkey T, Evans M. 1983. Reversible loss of gravitropic sensitivity in maize roots after tip application of calcium chelators. *Science* **220**: 1375–1376.

MacDonald IR, Hart JW, Gordon DC. 1983. Analysis of growth during geotropic curvature in seedling hypocotyls. *Plant, Cell and Environment* **6**: 401–406.

Medvedev S, Maksimov G, Markova I. 1991. Function of calcium ions in the regulation of gravitropism. *Eksperimentinė Biologija* **4**: 92–101.

Merkys A, Laurinavičius R, Jarošius A. 1972. The Cholodny–Went theory and the development of physiology of tropic plant movements. In: *Regulation of Plant Growth and Nutrition. Proceedings of the symposium 'Results of investigations of plant physiology and biochemistry during 1966–1970'*. Minsk, USSR: Science Press (in Russian), 84–95.

Meškauskas A, Moore D, Novak Frazer L. 1998. Mathematical modelling of morphogenesis in fungi: spatial organization of the gravitropic response in the mushroom stem of *Coprinus cinereus*. *New Phytologist* **140**: 111–123.

Monzer J. 1995. Actin microfilaments are involved in cellular graviperception of the basidiomycete *Flammulina velutipes*. *European Journal of Cell Biology* **66**: 151–156.

Moore D, Greening JP, Hatton JP, Novak Frazer L. 1994. Gravitational biology of mushrooms: a flow-chart approach to characterising processes and mechanisms. *Microgravity Quarterly* **4**: 21–24.

Moore D, Pukkila P. 1985. *Coprinus cinereus*: an ideal organism for study of developmental biology. *Journal of Biological Education* **19**: 31–40.

Novak Frazer L, Moore D. 1993. Antagonists and inhibitors of calcium accumulation do not impair gravity perception though they adversely affect the gravitropic responses of *Coprinus cinereus* stipes. *Mycological Research* **97**: 1113–1118.

Novak Frazer L, Moore D. 1996. The role of calcium accumulation and the cytoskeleton in the perception and response of *Coprinus cinereus* to gravity. *Advances in Space Research* **17**: 87–90.

Pickard BG. 1973. Geotropic response patterns of the *Avena* coleoptile. I. Dependence on angle and duration of stimulation. *Canadian Journal of Botany* **51**: 1003–1021.

Rawitscher F. 1932. *Der Geotropismus der Pflanzen*. Jena, Germany: Gustav Fisher.

Stanković B, Volkman D, Sack F. 1998. Autotropism, morphogenesis and gravity. *Physiologia Plantarum* **102**: 328–335.

Stočkus A. 1994a. Basic assumptions and comparison of three gravitropic response models. *Advances in Space Research* **14**: 145–148.

Stočkus A. 1994b. Diagravitropic response in primary plagiogravitropic corn roots. *Environmental and Experimental Botany* **34**: 87–94.

Stočkus A. 1994c. Modelling of gravitropic response. 2. Competence function. *Biologija* (Vilnius) **1**: 73–77.

Stočkus A, Moore D. 1996. Comparison of plant and fungal gravitropic responses using imitational modelling. *Plant, Cell and Environment* 19: 787–800.