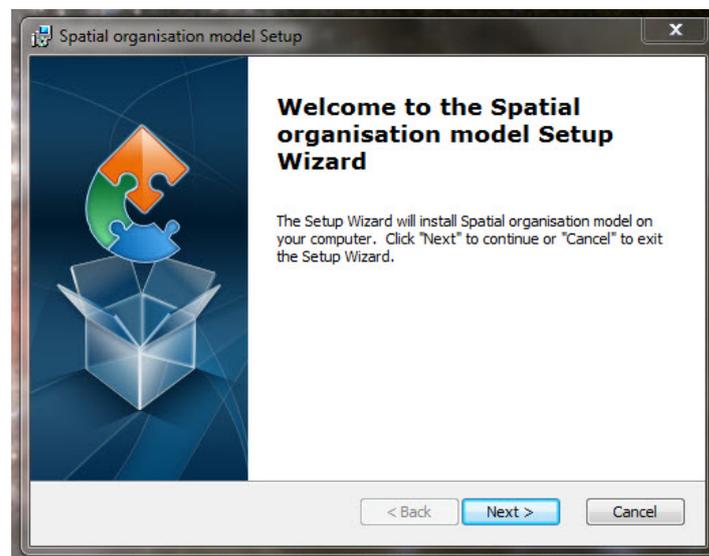


Spatial organisation model of the gravitropic reaction

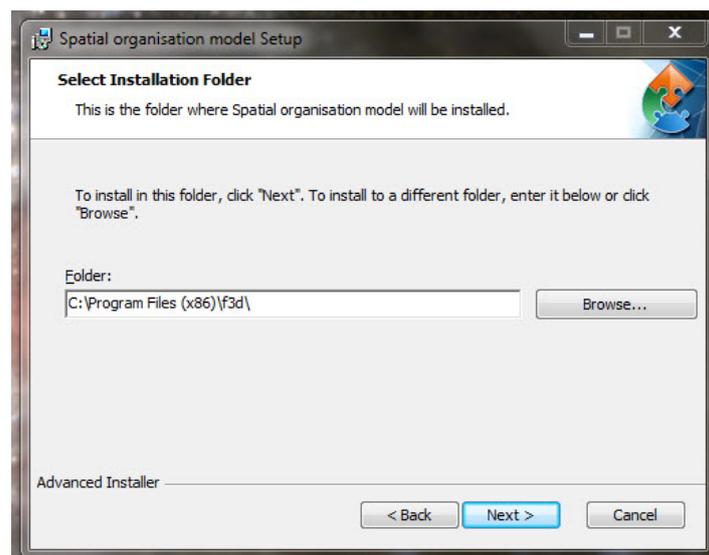
Installation

Download the **.msi** file named **spatial_only.msi**. Then navigate (in *File Explorer*) to the folder in which this file was saved and double-click on the file name to run the file [**OR** right-click on the **spatial_only.msi** file name and choose ‘**Install**’ from the menu that appears].

The installation file will display this announcement in the centre of your screen:



When you click ‘**Next**’, this screen appears:

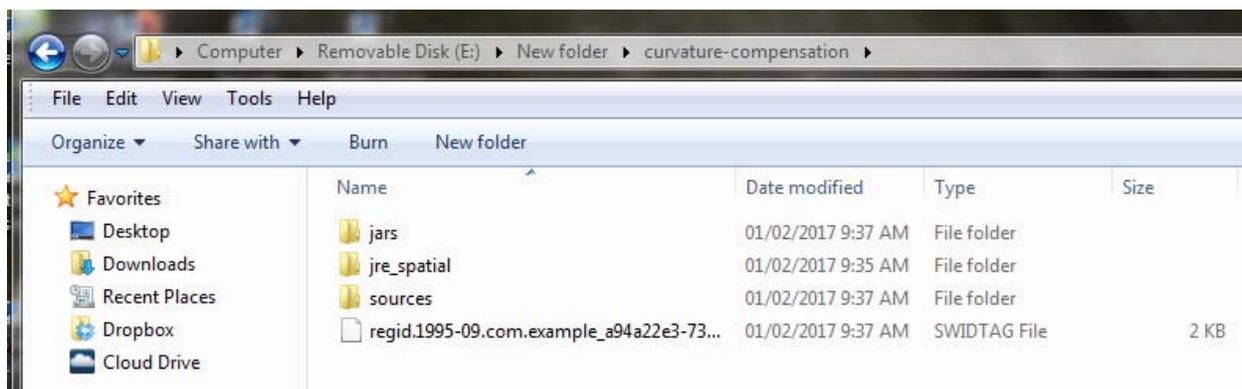


You can accept that offer to install the package into your existing ‘**C:\Program Files (x86)**’ folder, or click on the ‘**Browse...**’ button to find somewhere else. **BUT** as we’ve suggested already, we recommend downloading and installing on a USB flash drive, so we expect you

are running the **spatial_only.msi** file from a flash drive in a USB port on your machine. The little window that currently shows **C:\Program Files (x86)\f3d** is actually a text edit window, so you can position your cursor in it and start typing the address of the place you want to install the *Spatial Organisation modelling program*. I'll assume your USB drive will be drive E: (you may have to **Browse** to use File Explorer to confirm that) and suggest you type **E:\curvature-compensation** into that edit window. With that done, click on the 'Next >' button and the Installer will do everything for you. It will keep you informed of progress and announce when it's finished.

- You'll note that I've not accepted the invitation to install into a folder called **\f3d**, preferring my own name **\curvature-compensation**. I've done that for two reasons: one is to show you that the program doesn't mind which folder it's installed into (and will eventually run from), **but** don't mess with the names of the other folders the **.msi** file installs! The second reason is that I like to know explicitly what my folders contain.

When the installation is complete, use File Explorer to *Browse* to, and look inside, the folder you've just created (**\curvature-compensation**) and you'll find it contains:



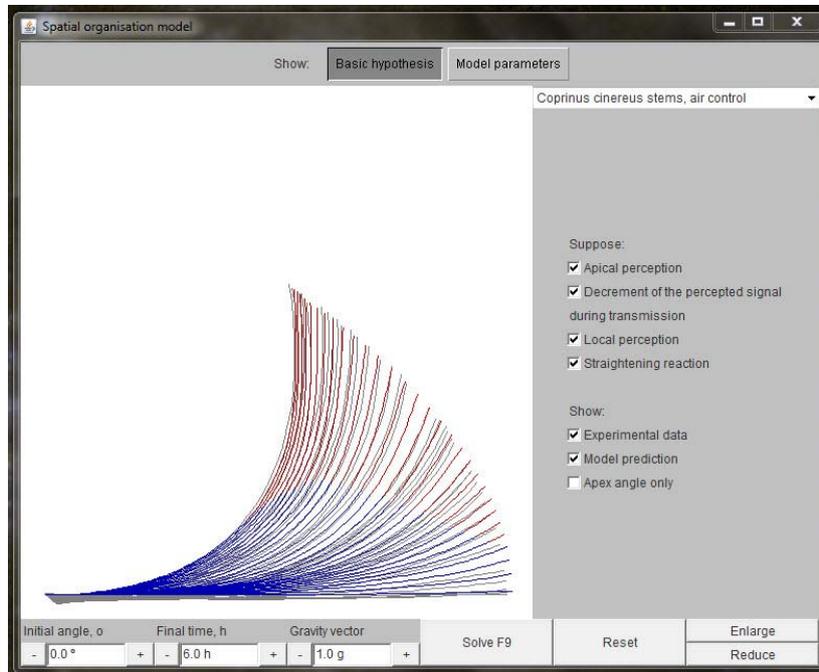
The SWIDTAG (software identification tag) is a management file that need not concern you. It is an XML-format file that identifies the software to Windows. The file folder called **sources** is an archive containing a ZIP file (**compensation.zip**), which holds all the .java files for the program, and a **.txt** copy of the **GNU General Public License** [<https://www.gnu.org/>] under which this software is distributed. The file folder called **jre_spatial** contains all the working components of the program.

The folder you need to look at is the one called **jars**; have a look in that and you'll see that it contains a single file called **compensation.jar**, which is an 'executable jar file' that will launch the *Spatial Organisation modelling program*.

- Before you do launch the program here's another recommendation: create a **shortcut to compensation.jar** to save yourself the hassle of navigating through all these folders to launch it. Like this: settle your cursor on **compensation.jar** to highlight it in the folder window, then **right-click** and choose 'Create shortcut' from the menu that appears. The shortcut will appear in the same window beneath **compensation.jar**, and you can then drag-and-drop the newly created shortcut from that folder window to your **Desktop**, where it will acquire a snazzy 'cup of steaming java' icon.

Launch the Spatial Organisation modelling program

Launch the program with a double-click either on your new shortcut to apex.jar or directly on apex.jar in its own folder. The following view will appear in a new window (which you may want to resize to suit your preferences):

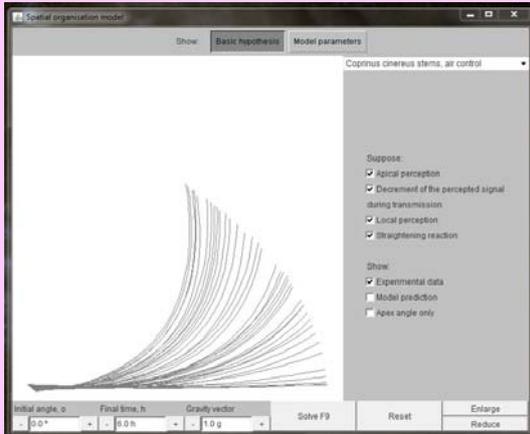


IF THIS WINDOW DOESN'T APPEAR when you double-click compensation.jar, it's because you don't have Java™ installed. This is quick and easy (and free) to do. Go to the download section of the **Java.com** website [at <https://java.com/en/download/manual.jsp>] and choose the flavour of **Java** appropriate to your browser and operating system; download and install. [The only limitation you might meet is that the Windows 10 'Edge' browser does not support plug-ins and will, therefore, not find and install **Java**. **Internet Explorer 11** and **Firefox** will run **Java** on **Windows 10**, and will therefore, select and install the most appropriate version of Java for Windows 10. Our programs do not require a browser to run, so **once Java is installed** these browser preferences are not relevant to us.]

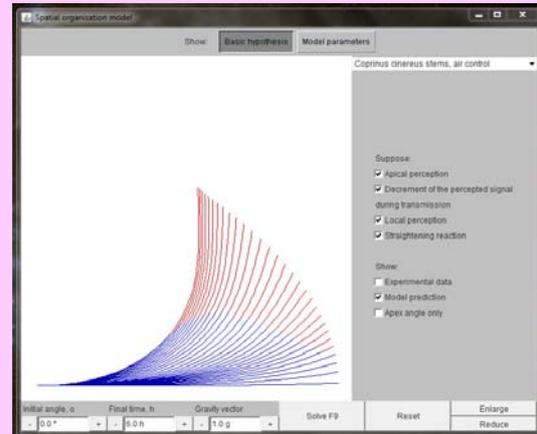
Now, let's get back to that **start-up screen**. It's showing you experimental data, and the results of modelling gravitropism, of the subject shown across the top of the panel on the right-hand side of the window ('*Coprinus cinereus*, air control').

In the main window, the experimental data and model predictions are shown as the **profiles of the stems** (= mushroom stipes) of the small ink cap mushroom *Coprinopsis cinerea*, though when the program was written this organism was called *Coprinus cinereus* and this is the name that appears here. You're seeing the profiles reduced to a depiction as a single line (representing the centre-line of the stems) viewed from the side, with the base of the stem on the left, and its apex on the right. The real experimental observations are drawn as a series of **GREY** lines, and the Java simulation **predictions** are drawn as lines made up of sections coloured **BLUE** where gravitropic curvature predominates, and coloured **RED** in the apical

regions where curvature compensation becomes effective and returns the section(s) to being geometrically straight.



Displaying experimental data only



Displaying model predictions only

You will notice a set of three check-boxes towards the bottom of the right-hand panel; these allow you to control what is displayed in the main window. By default, the start-up screen shows *stem profiles* of both *model predictions* and *experimental observations*, and accordingly, both ‘*Experimental data*’ and ‘*Model prediction*’ check-boxes are checked at start-up. Try clicking on one of them to remove the check-mark **and** remove that set of data. Do that alternately to inspect each case independently.

If you click (to activate) the ‘*Apex angle only*’ check-box, the main display changes to a graph with its vertical axis graduated in angular degrees (note that 0° = horizontal, 90° = vertical), and horizontal axis showing time in hours. If both ‘*Experimental data*’ and ‘*Model prediction*’ check-boxes are checked then this plot shows experimental observations as discrete square symbols (**black**) **and** the model prediction as a **red** line. Remove either (or both) of these by ‘unchecking’ the appropriate check-box. Before we go further into the modelling controls, you will understand this better if we show you how the experimental data were obtained.

In our standardised experiment, fruit body stems (stipes) of the mushroom *Coprinopsis cinerea* were placed horizontally (fixed with a mapping pin through their basal bulb or with a piece of adhesive tape wrapped around the basal bulb) on a balsa wood support in a moist chamber and observed continuously using video recordings, followed by computer analysis of the videotaped images to analyse the kinetics of the gravitropic response.

These experiments were done in the early 1990s; in those almost prehistoric days before digital cameras, digital TV and digital computer monitors. Our video images were analogue-TV VHS recordings, and were digitised with a PC computer using the Skye S1733 video digitising card and Skye S1730 image analysis software [Skye Instruments Ltd still prospers (<http://www.skyeinstruments.com/>) although ‘video digitiser’ no longer features on the website]; the (analogue) video images being sampled at 10 min intervals. For photographic illustrations, analogue video frames were digitised with a ‘*Screen Machine*’ real-time video digitiser card and software, and transferred to film (Ilford 100 ASA black & white) with a Polaroid Image Recorder (for traditional wet development!).

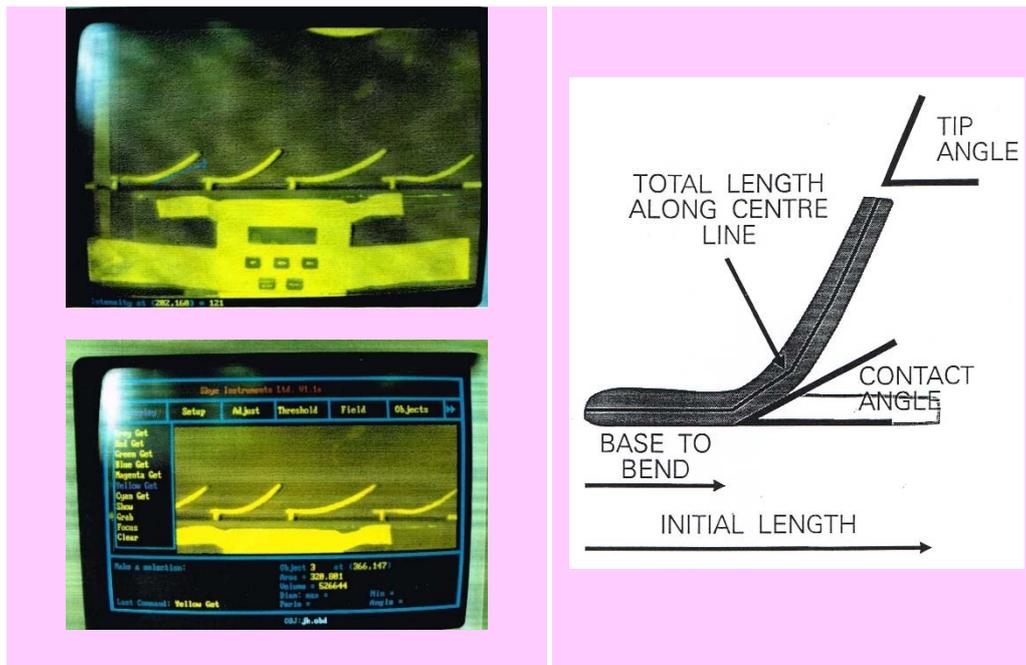


Figure 1 (Left). Display of the (analogue) video image as seen on the TV monitor (**top**), and the corresponding frame digitised using image analysis software (**bottom**). The observation method uses red + infrared illumination, because we wanted to avoid the mushroom's biological sensitivity to blue light, and it also made good use of the infrared-sensitivity of the analogue video cameras available at the time.

Figure 2 (Right). Growth and gravitropic parameters measured (at ten minute intervals) by image analysis. The total length was measured along an imaginary line which bisected the stem; bend movement was measured as the distance from the base to the bend; the tip angle was the angle the apex made with the horizontal and represented the sum of the upwards bend impulse and bend compensation; and the contact angle, measured at the junction of the lower edge of the stem with the horizontal support.

We found that the stem became gravireceptive after the cap had completed meiosis, beginning to bend within 30 minutes of being placed horizontal. Stem bending first occurred in the apical 15% of its length, and then the position of the bend moved rapidly towards the base, traversing 40% of stem length in 2.5 h. Meanwhile, the stem elongated by 25%, mostly in its upper half but also in basal regions. If the **apex** was pinned in the horizontal position the stem base was elevated but overshot the vertical, often curling through more than 300°. When the **base** was pinned to the horizontal (considered analogous to the normal situation of a fruit body disturbed by the action of animals in the environment), 90% of the initial bend was compensated as the stem brought its apex accurately upright, rarely overshooting the vertical. The **apex** had to be free to move for this *curvature compensation* to occur.

Experimental observations on the spatial organisation of the gravitropic curvature and model development

To study the exact shapes assumed by the gravitropically bending *Coprinopsis cinerea* stems, the video images captured from the tapes at 10 minute intervals were digitised and transformed into tables of co-ordinates of points for **50 subsections of each stem during 400 min of gravitropic reaction**. Data for 14 independent replicate stems were combined statistically to obtain an understanding of their average behaviour. **Figures 3 & 4** show a sample of the **experimental observations**.

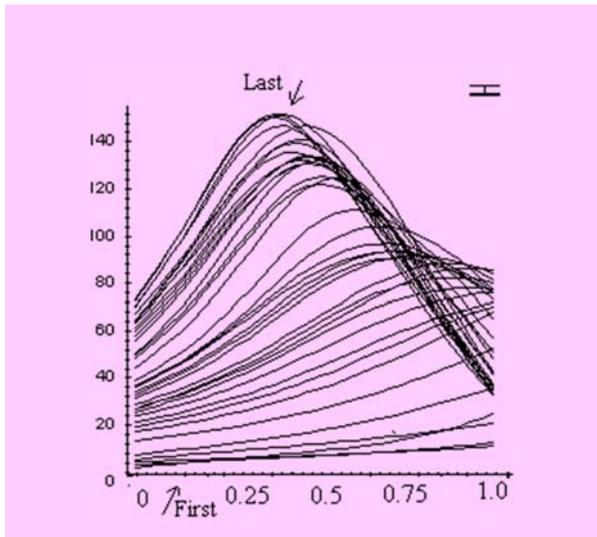


Figure 3. The distribution of local curvature over the length of the stems of *Coprinopsis cinerea* pinned to a horizontal platform through their base. Curves represent sets of observations made at 10-minute time intervals. Vertical axis shows local curvature in angular degrees. Replicate stems varied slightly in length and were normalised to the same overall length (= 1.0) so the horizontal axis shows the relative distance from the base of each of the 50 subsections calculated for each stem. Distance from the base (position zero) is given in arbitrary units of the normalised stem length. The error bar at top right represents the maximal standard deviation of the set of (14) replicates used for the figure.

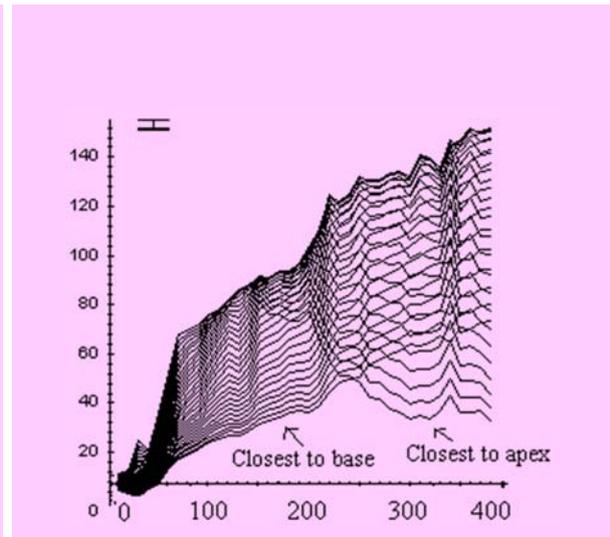


Figure 4. The distribution of local curvature over the length of the stem for base-pinned *C. cinerea* stems. Each curve represents the changing curvature of a different stem subsection. Error bar at top left represents maximal standard deviation. Vertical axis shows local curvature in angular degrees; the horizontal axis shows the elapsed time in minutes (time zero = time when the stem was reoriented from vertical to horizontal).

It was observed that **base-pinned stems first bent from the apex**, but later the curvature of this part decreased, and **in the late stages the apex became nearly completely straight again**. Subsections **located about one third of stem length from the base** determined the **main part of the final upward curvature**.

This straightening of tissue which was the first to curve was called **curvature compensation**; and a schematic illustration is shown in **Figure 5**. In summary: after being turned to the horizontal position, the gravitropic bending process begins quite close to the apex; but well before the apex reaches the vertical position, the apical part begins to straighten.

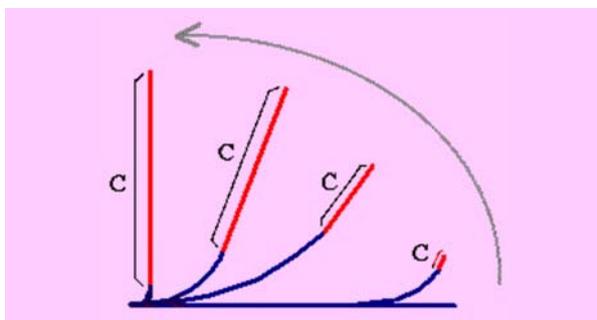


Figure 5. Diagrammatic representation. During the bending process the straightening progresses in the basipetal direction. When the stem is restored to the vertical in the final stages of the gravitropic reaction, most of the curvature is concentrated near the base (and the part which bent first is now straight).

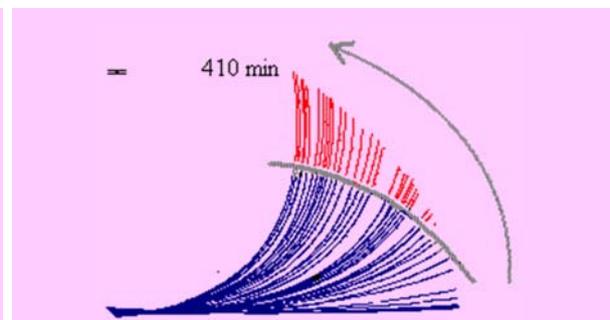


Figure 6. Experimental observations. Lengths of several replicate stems were normalised to unity to generate mean values; mean error bar displayed at top left. Statistical analysis indicates that the straightening process also occurs in more basal sections, but is less pronounced.

Figure 6 shows actual experimental data for *Coprinopsis cinerea* stems. The straight part is marked in red and separated from the rest by a grey line.

Figure 7 indicates how the curvature of the stem can be defined mathematically through the **local angle** (α_L), curvature (C_L), distance from the stem base (λ) and **x** and **y** co-ordinates of the point of interest.

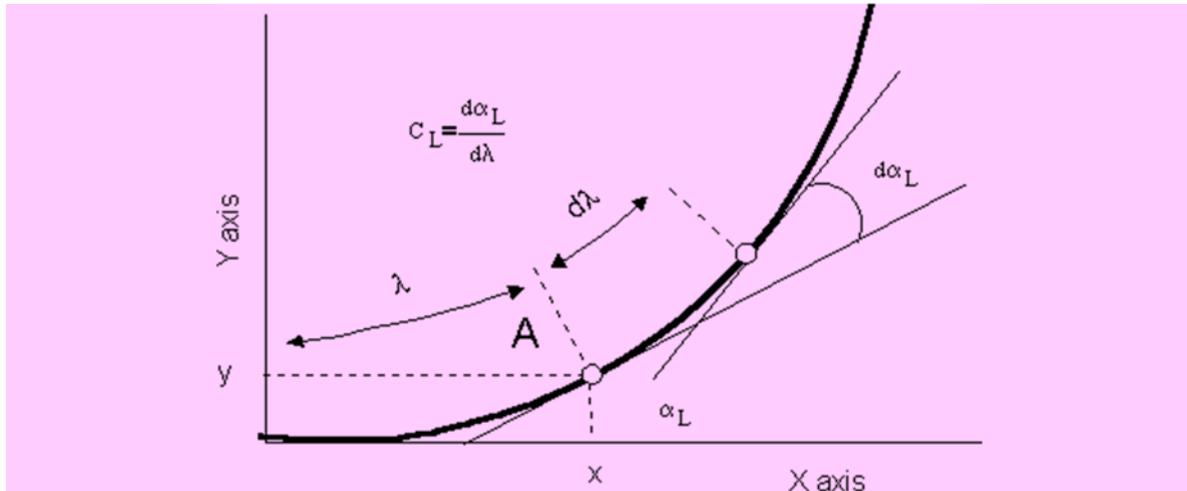


Figure 7. Curvature is a ratio, calculated using the formula shown in the top left quarter of the figure. It is true as $d\lambda$ tends to zero. The parameter $d\alpha_L$ is mathematically defined as the **angle of contingence**. All parameters illustrated are for the point A.

The **compensation signal** arises at the same point where the bending process develops; that means it is not transmitted through the stem. This signal was introduced to explain the straightening of the apical part of the living stem before it is restored to the vertical. The level of this signal was assumed to be proportional to **local curvature**, C_L (defined in **Figure 7**).

Observation shows that compensation is much more strongly expressed close to the apex than in lower (but still apical) subsections. The model takes this fact into account by assuming that the distribution of ability to straighten (= the compensation reaction) is not uniform and exponentially decreases towards the stem base.

The level of the compensation (straightening) signal at position λ at any given time t is:

$$A(t, \lambda) = A_U \cdot e^{-A_D(1-\lambda)} \cdot C_L(t, \lambda) \quad (1)$$

here parameters A_U and A_D determine the distribution of autotropism along the stem.

The proposition that **perception of the gravitropic signal** takes place in the stem apex is enough to create the mathematical model that can reproduce all stem shapes observed to occur in *Coprinopsis cinerea* during the gravitropic reaction of live stems. However, it can't simulate the process as it develops in time (goodness of fit tests indicated significant differences between model-predicted and observed shapes). The bending process, simulated by this simple model, is too slow in the beginning of the gravitropic reaction (in approximately the first three hours) and too fast in the later stages.

To account for this, we included the *local perception* of the signal as a parameter. After adding this parameter, we achieved **exact simulations**. The **local perception function** differs significantly from the perception function in the apex and can be approximated as

$$R_{Local}(\alpha_L) = e^{-B_{DE} \cdot \alpha_L} \quad (2)$$

Similarly to $\sin(\alpha_L)$, this function is maximal when $\alpha_L = 0$ (horizontal position), but it can reduce much faster when the parameter α_L starts to increase. Hence it was supposed that local perception plays the most important role in the initial stages of the bending process, when the α_L is less than 45 degrees.

The essential condition to satisfy goodness of fit tests was also to assume that the distribution of ability for local perception is not uniform, but *exponentially decreases* from the apex and towards the base. Hence the level of the local perception at the time t in the position λ is:

$$R(t, \lambda) = B_S \cdot e^{-B_D(1-\lambda)} \cdot R_{Local}(\alpha_L) \quad (3)$$

The *realisation of the gravitropic response*, the local bending speed in point λ at time t is:

$$\frac{dC_L(t, \lambda)}{dt} = K_W \cdot (S(t, \lambda) + A(t, \lambda) + R(t, \lambda)) \quad (4)$$

where K_W is the realisation constant. The final equation describing the spatial organisation of curvature during the gravitropic reaction is shown in **equation 5** below:

$$\frac{\partial}{\partial t} C_L(\lambda, t) = \left[K_S \cos \left(\int_0^1 C_L \left(t - \frac{1-\lambda}{v}, x \right) dx \right) e^{(-S_{DE}(1-\lambda))} + A_U e^{(-A_D(1-\lambda))} C_L(t, \lambda) + B_S e^{(-B_D(1-\lambda))} \left(e^{-B_{DE} \int_0^\lambda C_L(t, x) dx} \right) \right] K_W$$

($t > 0$; $0 \leq \lambda \leq 1$, initial condition $C_L(\lambda, 0) = 0$ (straight stem);
boundary conditions $C_L(t, 0) = C_L(t, \lambda) = 0$)

Equation 5

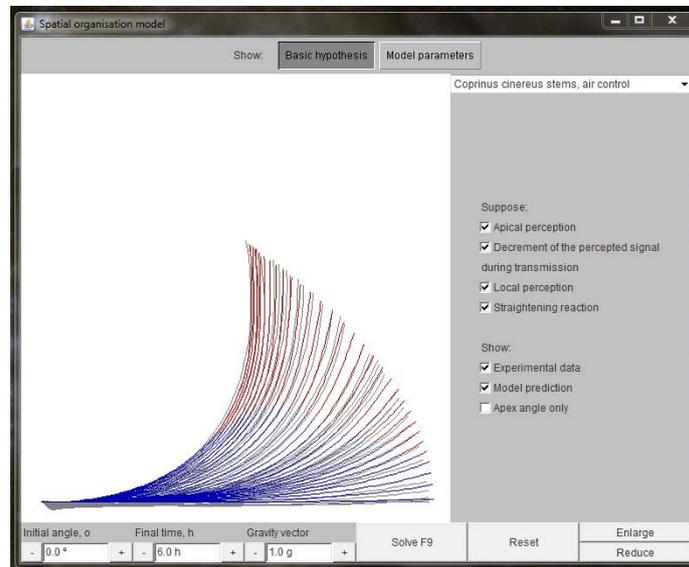
In subsequent tests the *simulated shapes of the bending stem coincided almost exactly with experimentally observed images*.

The point of giving all this detail here is that the parameters of **apical perception**, the **decrease in the perceived signal during transmission**, **local perception** and the **straightening reaction** are all immediately available on the *modelling screen*. This allows

the user to manipulate any or all of these parameters to investigate their influence on the gravitropism reaction.

Operating the simulation model

Let's remind ourselves about the **Spatial Organisation modelling program** start-up view:



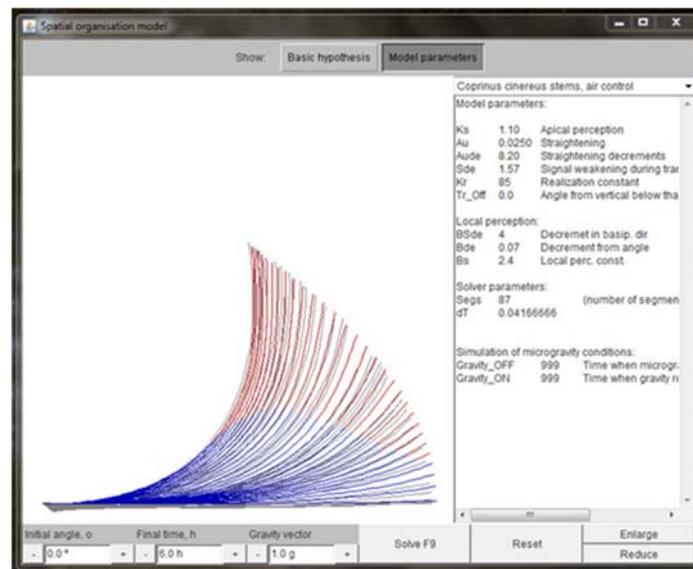
Notice the following points about this screen:

- The parameters we have just been discussing, **apical perception**, **decrease in the perceived signal during transmission**, **local perception** and **straightening reaction** are all accessible through check-boxes in the right-hand panel. They can be switched **on** and **off** immediately by clicking on the check-box (when the box shows the check mark , the parameter is **on**). Remember: change it, then 'press' **Solve** to make the simulation react.
- The standard experiment (see **Figure 1** above) starts with re-orienting the stems to the horizontal; so, the initial angle (see the box in the bottom left corner?) is **zero**. You can change that. Try changing the initial angle, then 'press' (click) the button **Solve** (bottom middle) [or press function key F9] and the program will plot its prediction.
- You can also change the time span (remember, change it then press **Solve**).
- More challenging for the experimental scientist is a change of the gravity vector (the third little window). You might like to find out, for example, how a fruit body would react to being placed horizontal on the surface of **Mars** ($g = 0.38$), **Saturn** ($g = 0.92$) or **Jupiter** ($g = 2.36$). **The model will predict** how the *Coprinopsis* stem will respond in each of these circumstances. To test those predictions, you might like to ask if Richard Branson has ever thought of taking mushrooms into space. Best of luck!

Let's now examine the top of the start-up screen:

- At far right is a drop-down menu that allows you to choose between a (small) number of built-in experimental models. Click on the triangle and the menu will show you what is available. There are six experiments dealing with gravitropic responses of 'Coprinus cinereus' (= *Coprinopsis cinerea*) mushroom fruit body stems, and one featuring wheat coleoptiles. The 'C. cinereus' experiments were done to investigate the effects of metabolic inhibitors and are detailed in these publications:

- 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. DOI: [http://dx.doi.org/10.1016/S0953-7562\(09\)80512-1](http://dx.doi.org/10.1016/S0953-7562(09)80512-1). [CLICK HERE to download the full text.](#)
- 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** (6/7): 87-90. DOI: [http://dx.doi.org/10.1016/0273-1177\(95\)00616-M](http://dx.doi.org/10.1016/0273-1177(95)00616-M). [CLICK HERE to download the full text.](#)
- At top, centre of the window are two buttons, one labelled **Basic hypothesis** and the other, **Model parameters**. These are toggle-buttons; when one is on, the other is off.
- The program starts up with **Basic hypothesis** in the **on** position (appearing to be in the ‘down’ position) and the right-hand side panel displays the set of check-boxes we have already described.
- When you click (‘press’) **Model parameters** this changes the contents of the right-hand data window to show numerical values of all the parameters used in the simulation modelling.



- This **Model parameters** window is a **text editing window**; so, you can change the numerical values just by typing in new ones. Remember, make the change then press (click) the button **Solve**. The program will then make a new prediction for the revised parameter set and plot it (with the **same** set of experimental data, unless you switch that display off).
- You can make changes to each (and all) of the parameters on this panel to investigate their influence on gravitropism systematically. And when you want to go back to the default values, just click (‘press’) the **Reset** button (bottom, right). There’s a **lot** of experimentation you can do here.

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. DOI: <http://dx.doi.org/10.1046/j.1469-8137.1998.00252.x>. [CLICK HERE to download the full text.](#)

Meškauskas, A., Novak Frazer, L. & Moore, D. (1999). Mathematical modelling of morphogenesis in fungi: a key role for curvature compensation (‘autotropism’) in the local curvature distribution model. *New Phytologist*, **143**: 387-399. DOI: <http://dx.doi.org/10.1046/j.1469-8137.1999.00458.x>. [CLICK HERE to download the full text.](#)

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