

## KINETICS OF STIPE GRAVITROPISM IN THE MUSHROOM FUNGUS *COPRINUS CINEREUS* UNDER THE CONDITIONS OF MICROGRAVITY SIMULATION PROVIDED BY CLINOSTAT TREATMENT

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**Abstract**—The extent of the gravitropic response, measured as the angle of the stipe apex at maximum curvature, was dependent upon the gravitational exposure time. The reaction time did not depend on exposure time, and interruption of gravitational exposure by a period of clinostatting allowed the gravitational stimulus to decay. It is concluded that the gravitropic impulse is an 'all-or-nothing' signal in *Coprinus cinereus*, that perception and response probably occur in the same tissue regions, and that sustained exposure to the unidirectional gravity vector is necessary for the normal gravitropic response. The presentation time was found to be 7 min. Immediately after reaching maximum curvature, stipes placed on the clinostat after various gravity exposure times 'relaxed' by 5°. This relaxation suggests that gravitropic bending is a two-stage process with an initial, reversible, phase of plastic bending being followed by a 'fixation' phase providing the gravitropic stimulus has been maintained.

The kinetics of mushroom stipe gravitropism in *Coprinus cinereus* have been characterised recently [1-4]. Although research on fungal gravitropism has been in progress for more than a century [1], the first report of the use of a clinostat to estimate the presentation time (the minimum gravistimulation time needed to evoke a response) for any fungal gravitropism was published in 1992 by Hatton & Moore [5]. This was very much a preliminary report giving constructional details of the clinostats used and estimating the presentation time of the stipe to be 9.6 min. In the research reported here we have carried out a more detailed analysis of the response of *C. cinereus* stipes on clinostat treatment, including an assessment of the response to different periods of gravitational exposure.

### 2. EXPERIMENTAL

The 'Meathop' dikaryotic strain of the basidiomycete fungus *Coprinus cinereus* (Schaeff.: Fr) S. F. Gray *sensu* Konr. was used for all experimental work. Culture conditions and procedures used were described in Refs. 2 & 5.

Most use was made of the Type II clinostat [5] fitted with a container in which a number of stipes could be mounted. The standard procedure used fruiting bodies approximately 50-70 mm in height. These were gently removed from the mycelium in a photographic dark-room illuminated with red safety light. The mushroom caps were removed and the initial length of the stipe measured. The stipe was pinned through its basal bulb to a balsa-wood support, which was then placed in the clinostat chamber. Two to five stipes could be mounted in each chamber, depending on size and experimental requirements. During all these preparative operations the stipe was kept vertical.

Gravistimulation was started by orienting the chamber to the horizontal. This orientation was maintained for varying lengths of time before clinostat rotation (at 2 rpm) was started. In the analyses

that follow, *gravistimulation time* is the time from initial reorientation to the horizontal to the start of rotation. Clinostat rotation was continued for up to 6 h.

A number of control experiments were performed under identical conditions except that clinostat rotation was not started so that the stipe was subject to constant gravistimulation. Apart from being mounted on a clinostat, these control experiments are directly comparable with the experiments reported by Kher *et al.* [2]. In another set of control experiments, clinostat rotation was started immediately on orientation to the horizontal and the angle of the stipe tips was measured to see if rotation alone imparted directional information to the stipe.

The whole operation from initial orientation was video-taped with a domestic-quality VHS video recorder and camera illuminated with a red safety light (this avoided phototropic affects as fungal phototropisms are sensitive to blue light). Experiments were performed at the ambient temperature of the dark room which varied between 21 and 25°C.

Stipe morphology throughout the experiment was determined by pausing the video in 'freeze frame' and tracing the images from the screen onto overhead projection acetate. Correct alignment of the rotating clinostat image in frames used for measurement was assured by reference symbols marked on the clinostat chamber. Measurements were made at initial orientation (= zero time then at five min intervals from 15 min to 60 min after initial orientation (coinciding with the period of maximum response [2]) and at 15 min intervals thereafter.

Length and angle measurements were obtained from the tracings using a Summagraphics MM1201 graphics tablet interfaced to a PC running 'AutoCAD'. The raw data from this analysis were processed through Microsoft 'Excel 4' spreadsheet on a PC. True lengths were derived by comparing the tracing lengths with the initial image, since the initial length was recorded at the start of the experiments. Angular measurements were referenced against the stipe support which was taken as the horizontal. For each

stipe the following measurements were made:

(i) angle of stipe apex to the horizontal. Since restoration of the stipe apex to the vertical (i.e.  $90^\circ$  with respect to the horizontal) is the function of the gravitropic process, this measurement indicates the extent of completion of the true biological response. However, it should be noted that in the normal situation the apex angle is a product of both gravitropic bending and curvature compensation [2] and apex angle may consequently reflect interplay between a number of processes.

(ii) Distance from the base of stipe to the gravitropic bend. In constantly gravistimulated stipes the position of the gravitropic bend moves towards the stipe base. This is a *base to bend* measurement, i.e. the movement of the bend is *not* related to the apical growth of the stipe.

(iii) Overall length of stipe.

Statistical tests were applied to the data as appropriate. Mean values were compared by a Student's *t*-test (two samples assuming equal means). In addition, a modification of the F-test was used to compare regression slopes.

### 3. RESULTS AND DISCUSSION

#### 3.1 Legth comparison

The lengths of all stipes were recorded at the beginning of each experiment, with growth rate being determined from the image tracings. The mean initial length of stipes used in this study was  $57.8 \pm 1.4$  mm ( $n = 72$ ), with a mean growth rate of  $3.98 \pm 0.52$  mm  $h^{-1}$  ( $n = 71$ ) during the first hour of the experiment. There was no correlation between growth rate and initial stipe length (correlation coefficient,  $r = 0.026$ ) and there was no significant difference between any of the growth rate measurements.

#### 3.2 Zero gravistimulation controls

Stipes which were rotated immediately after being mounted on the clinostat acted as zero gravistimulation controls. Apex angles of seven stipes treated in this way varied between  $-12^\circ$  and  $+9^\circ$  to the horizontal with no significant bias in direction of orientation (Fig. 1). This indicates that clinostat rotation alone does not provide any significant directional stimulus to stipes placed in the experiment chamber and that the treatment can be regarded as being effective in avoiding the effects of the unilateral gravity vector, i.e. at this rotation rate the clinostats used in this study provided an adequate simulation of microgravity.

#### 3.3 Clinostat experiments

##### 3.3.1 Effect of varying gravistimulation time

In the work reported here, the response kinetics of 78 stipes were examined on the clinostat with gravistimulation periods varying from 0 to 50 min. A number of experiments were performed with the clinostat stationary (= constant gravistimulation). The time course of the typical response to constant gravistimulation [2], illustrated in Fig. 2, consists of a lag phase (the reaction time, during which re-orientation

is perceived and a response implemented), after which the tip angle increases at a rapid and uniform rate which eventually decelerates as the tip angle approaches  $90^\circ$  to the horizontal. After this angle is attained, small adjustments may be seen to keep the tip vertical. To get an estimate of reaction time for each stipe tested, a linear response rate phase was identified by eye for regression analysis to calculate the reaction time by extrapolation (Fig. 2). This

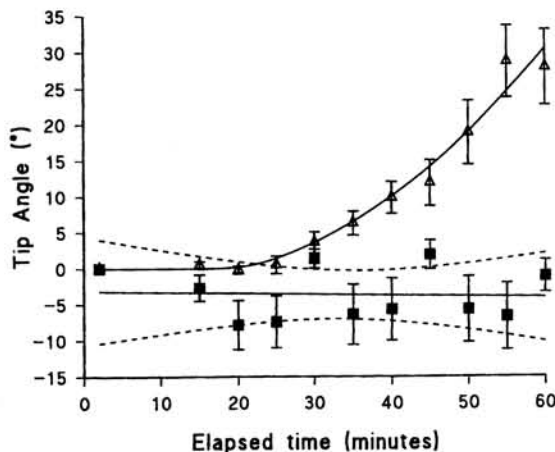


Fig. 1. Comparison of the initial response of a population of constantly gravistimulated stipes (open triangles,  $n = 12$ ) with a population subject to constant clinostat rotation immediately following reorientation of the horizontal ( $n = 7$ ). Tip angle was used as a measure of response. Whilst the stationary (constantly stimulated) stipes make a rapid negative gravitropic response, the rotated stipes show no obvious directional orientation. The slope of a regression line ( $-0.015$ ) with 95% confidence limits (dotted lines) fitted through the points demonstrates that clinostat rotation does not impart any directional bias to the stipe apex angle.

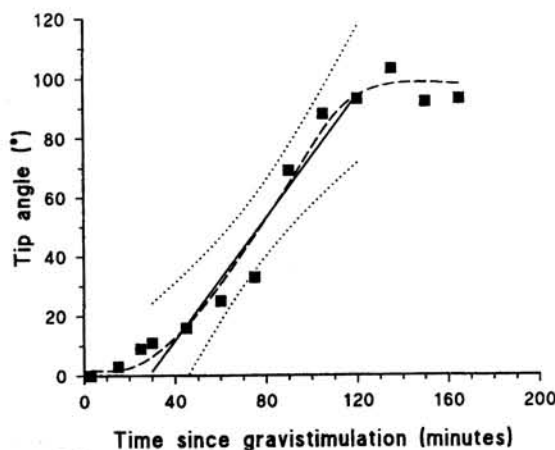


Fig. 2. Response of *Coprinus cinereus* stipe to constant gravistimulation, expressed as the angle between the tip and the horizontal. At time zero, gravistimulation was started by placing the stipe on a horizontal platform. The mean response curve is shown as a dashed line and comprises an initial lag period (the reaction time) which is followed by a rapid response of near constant rate until the apex approaches the vertical. Reaction time was assessed by extrapolation of a regression of the linear portion of the response curve (shown as a solid line with 95% confidence limits indicated as dotted curves).

method was used routinely after trial calculations had indicated that alternative methods (e.g. [6]) did not provide any reduction in variability.

A typical response to a defined period of gravistimulation followed by clinostat rotation is shown in Fig. 3. The early part of the response is similar to that of a constantly gravistimulated stipe, with a lag phase and a linear response region. However at the end of the linear response region the tip angle reaches a maximum value, then decreases slightly. This slight decrease in tip angle occurred consistently, and we call it 'relaxation'. After relaxation the tip angle varied more widely the longer clinostat treatment was continued. For example, for the set of 12 stipes which were gravistimulated for 35 minutes the standard error range was 13% of the mean at the time maximum tip angle was attained, but was 74% of the mean after a further 90 minutes on the clinostat. However, stipes remained gravisensitive throughout, as could be demonstrated by stopping the clinostat (Figs 3 & 4) when a normal gravitropic response ensued. Elongation continued unaffected by clinostat rotation.

Tip angle measurements for each stipe were plotted against time from the start of the experiment. From these plots the following parameters were

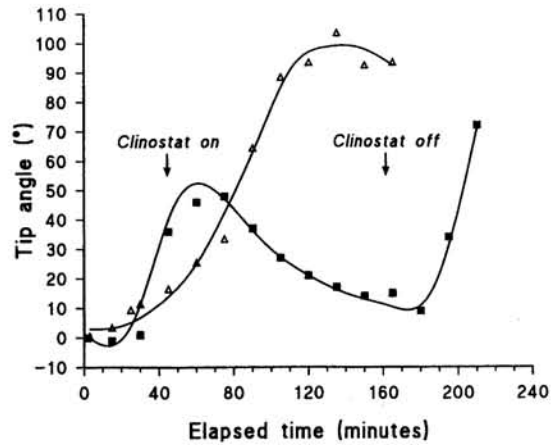


Fig. 3. Comparison of the response of a constantly gravistimulated stipe (triangles) and one subjected to clinostat rotation after 45 minutes gravistimulation. The latter shows a similar initial response to the constantly gravistimulated stipe, but differs in reaching a maximum tip angle before 'relaxing' back towards the horizontal. The clinostat was stopped at 165 minutes and the stipe shows a gravitropic response. This experiment demonstrates that clinostat rotation is sufficient to negate gravistimulation and that sensitivity to gravistimulation is not lost during clinostat rotation.

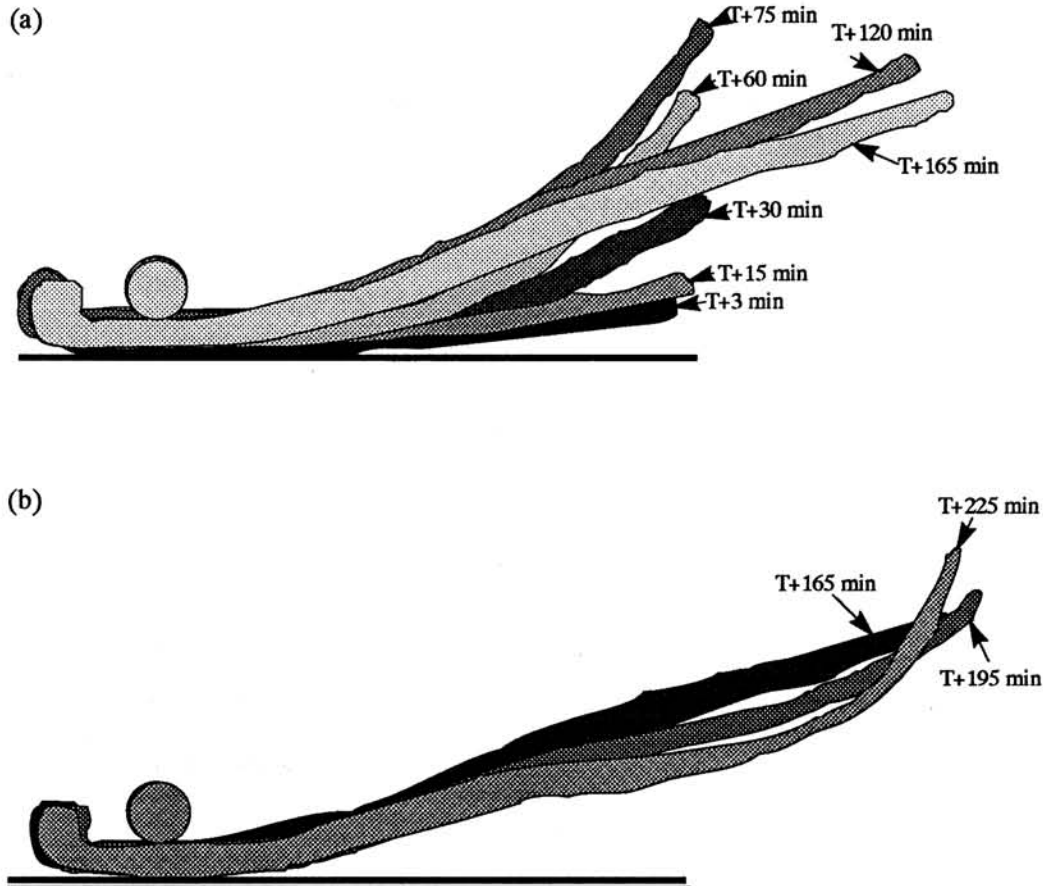


Fig. 4. Morphometric tracings from video images of the stipe for which numerical data is plotted in Fig. 3. In (a) the response up to 165 minutes after initial gravistimulation is shown. Note that the bend starts near the tip and moves towards the base. Maximum tip angle is reached at approximately 75 minutes after which the tip angle starts to decrease. In (b) the response to gravistimulation after the clinostat was stopped is shown. As in the initial response, bending occurs first near the tip and moves backwards. Elongation of the stipe continued throughout the period of the experiment.

obtained. A graphical representation of all the data is given in Fig. 5.

(a) A linear rate of response generally occurred during the 25-60 min period after initial reorientation although for some stipes it could start as early as 15 min. There was no correlation between rate of response and gravistimulation time. The mean response rate was  $0.69^\circ \text{ min}^{-1}$ .

(b) The mean reaction time was  $21.06 \pm 5.24$  minutes, comparable to the value (25.4 min) calculated by Kher et al. [2] for stationary stipes. There was no significant correlation between reaction time and gravistimulation time.

(c) The tip angle 15 min after the maximum was reached averaged  $4.9 \pm 0.53^\circ$  less than the maximum, irrespective of the maximum value and irrespective of gravistimulation time. After this, time, however, tip angle randomised.

(d) Perception time can be calculated by plotting a measurement of response, such as tip angle against  $\log_{10}$  stimulation time; the intercept of the regression line with the time axis indicating the perception time. A response proportional to log stimulus has been demonstrated in other sensory systems, such as photoreception in the eye and sound reception in the ear [7, 8]. This relationship is known as

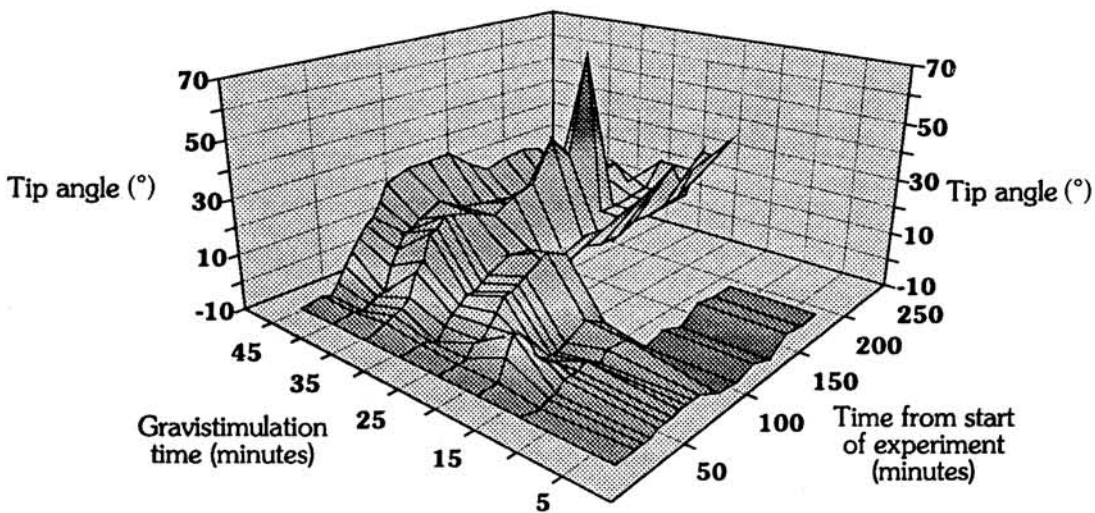
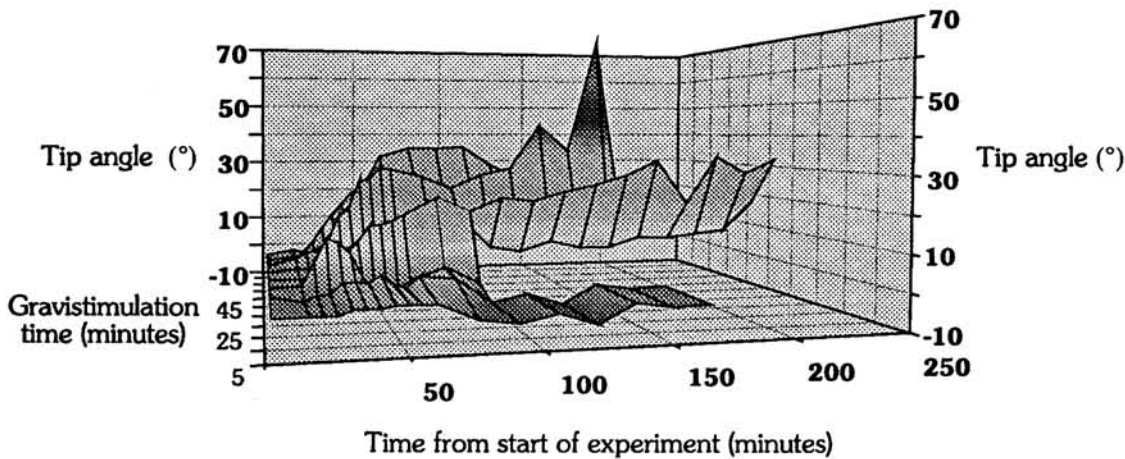


Fig. 5. A compilation of all of the data obtained from 78 stipes gravistimulated for various periods prior to clinostat treatment. The data are shown as a three dimensional surface (gravistimulation time x elapsed time x tip angle) and two views of the data space are illustrated. The top view emphasises that the same reaction time (evident as a lag phase) and the same rate of response follow whatever the gravistimulation time. The lower view illustrates that maximum tip angle was proportional to gravistimulation time, that tip angles decreased slightly ('relaxed') in all cases after the maximum, and that subsequent angles varied progressively more widely as clinostating was continued to 150 minutes and beyond.

the 'Weber-Fechner' law [9]. Such a system allows a wider dynamic range over which a sensor can encode a response to a stimulus. Experiments on root graviperception [10, 11] show that the rate of response conforms with this relationship in plants. In *C. cinereus*, however, both the rate of response and the reaction time were the same for all gravistimulation times, but the maximum tip angle reached was proportional to  $(\log_{10})$  gravistimulation time ( $r=0.56$ ,  $n=67$ ). The perception time calculated from maximum tip angle measurements was 7.04 min (Fig. 6).

### 3.3.2 Effect of interrupting gravistimulation

With the perception time established as about 7 min, this length of exposure was used to assess additivity and the effect of interrupting gravistimulation. For this test the maximum tip angle attained was determined for three sets of stipes exposed to three different gravistimulation regimes:

(i) 7 min gravistimulation, followed by continuous clinostat rotation;

(ii) 7 min gravistimulation, 7 min clinostat rotation, 7 min gravistimulation, followed by continuous clinostat rotation;

(iii) 14 min (uninterrupted) gravistimulation followed by continuous clinostat rotation.

All other experimental and analytical procedures were identical to those described above. Maximum angles attained by stipes from sets (i) and (ii) were not significantly different from one another and the overall mean was  $7.2^\circ \pm 10.2^\circ$  ( $n=15$ ). The maximum angles attained by stipes in sets (i) and (ii) were both significantly different from that attained in set (iii) which was  $19.3^\circ \pm 7.0^\circ$  ( $n=8$ ). From this we conclude that interruption of the gravistimulation period by 7 min clinostatting is sufficient for the initial stimulus to decay.

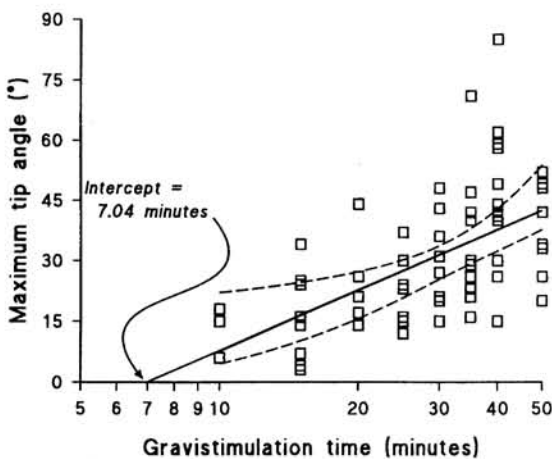


Fig. 6. Estimation of perception time in *Coprinus cinereus* from the 'dose-response' relationship between gravistimulation time and maximum tip angle. Perception time is given by the intercept of the regression line with the time axis (95% confidence limits for the regression line are plotted as dashed lines).

## 4. CONCLUSIONS

Gravity perception in *C. cinereus* occurs at an intracellular site (there being good evidence that the stipe is insensitive to externally-applied mechanical loads [3]), and is completed within approximately 7 min from the start of gravistimulation. Under normal circumstances perception probably occurs near the apex, which exerts apical dominance over all other gravireceptive cells in the stipe although the extent of the gravireceptive region is considerable [3]. The physical susception of the gravitational stimulus results in the production of a physiological impulse. The lack of correlation between bending rate and gravistimulation time indicates that a uniform impulse is sent from the perception site in an 'all or nothing' manner. This is a major contrast with the situation in plant root gravitropism, where the rate of response does depend on gravistimulation time [10, 11], and suggests that totally different mechanisms operate in the two systems. Since, in *C. cinereus*, the response rate is independent of gravistimulation time, it would appear that the duration of the bending impulse, rather than its strength, determines the magnitude of the response which can be attained. Certainly, the interruption experiments indicate that the gravitropic impulse decays rapidly.

These data suggest that sustained exposure to the gravity vector is necessary for sustained gravitropic bending of the stipe. The reaction time was the same irrespective of the extent of gravistimulation, suggesting that the post-perception events are the same whether the gravitropic impulse is strong or weak. Mechanistically, this could result either from the need for the impulse to traverse a specific length of stipe before eliciting a response, or from a requirement for a specific length of time (reaction time-presentation time = about 14 min) for the gravitropic response to be organised. Excision of parts of the stipe apex has been shown to increase reaction time [3], so we discard the first possible mechanism (which would predict a decreased reaction time in such experiments).

Consequently, the most likely mechanism is that perception and response occur in the same region of the stipe, the former requiring 7 min and the latter 14 min to become established.

An unexpected observation was that shortly after the maximum tip angle had been attained, the angle decreased by a uniform amount. This may be similar to the autotropisms seen in various plant organs [12, 13], although oat coleoptile autotropisms were observed only in space-flown material, not in clinostat-treated seedlings [13]. We favour a more mechanistic interpretation of relaxation in *Coprinus* stipes, believing it to be a result of initial plasticity of the bend. This interpretation implies that gravitropic bending of the stipe is a two-step process: the first step setting up tissue tensions (this could be a polarisation of ions, or a real physical stress) which establish anelastic, reversible, bend which, if maintained for a sufficient length of time becomes fixed in the second step as the cell walls are cross linked and/or supplemented by fresh wall synthesis. This also has parallels in plants, specifically in the gravitropic response of *Zea mays*, where short periods of gravistimulation lead to transitory responses in coleoptiles, gravitropic curvature becoming fixed by longer exposures [14];

and where there is a loss of gravitropic curvature in roots a few minutes after removal of the gravistimulus (the 'springback' phenomenon) [15].

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