



## MORPHOMETRIC ANALYSIS OF CELL SIZE PATTERNING INVOLVED IN GRAVITROPIC CURVATURE OF THE STIPE OF *COPRINUS CINEREUS*

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### ABSTRACT

During gravitropic bending of the stipe of *Coprinus cinereus* the majority of elongation occurred in the apical region of the lower surface of the stipe, although some elongation was seen throughout the stipe. The final rate of elongation was similar at both the upper and lower stipe surfaces but the lower surface achieved this rate first (close to the reaction time 25 min /2/), whilst the upper surface of the stipe only attained its final elongation rate after a period of acceleration of 150 min. Detailed morphometric analysis of cell size patterning in transverse sections revealed no significant differences in cross sectional area, spatial or proportional distribution of different cell types between the upper and lower regions of the gravitropic bend. Measurements of longitudinal cell size revealed significant differences in compartment size between the lower and upper region. Hyphal compartments of lower regions of the bend were on average four to five times longer than those of the upper region.

### INTRODUCTION

In the stipe (the stem of a basidiomycete mushroom fruit body) gravitropic bending is usually assumed to result from asymmetric distribution of growth, as represented by cell expansion. Using video and image analysis equipment we have investigated the kinetics and mechanics of gravitropic bending in the fruit body of *C. cinereus*. When pinned horizontally at its base, a stipe of the ink-cap fungus *Coprinus cinereus* will bend upwards, normally reaching the vertical within three hours. Stipe bending first occurs in the apical 15% of its length, then the position of the bend moves rapidly towards the base. Meanwhile, the stem elongates by 25%, mostly in its upper half. Bending raises the apex and as this approaches an angle of about 35° to the horizontal, curvature compensation (effectively reversing of the bend) adjusts the degree of bending so that the apex can be brought exactly vertical. Approximately 90% of bending is eventually compensated. This curvature compensation requires that the apical region is free to move. A stipe pinned at its apex reacted as usual (the stipe base being raised upwards) but this bending was not controlled and resulted in a stipe curled into an almost complete circle. Similar results have been observed in *Flamulina velutipes* /5/. This result suggests that curvature and curvature compensation are quite separate processes, and that compensation requires that the apex is free to move.

### METHODS

All experiments were performed on post-meiotic fruit bodies of *Coprinus cinereus* ranging from 50 to 60 mm in length, cultured as described previously /1/. Mushrooms were excised from the culture medium, decapitated and treated as appropriate to the experiment /2, 3/. After treatment, stipes were laid horizontally and recorded using a domestic quality video recorder for up to 6 hours. At the conclusion of experiments, stipes selected to undergo further microscopic analyses were fixed and embedded /4/. Transverse and radial longitudinal sections were cut 2 µm thick

and stained with toluidine blue. Microscopic analyses were carried out using an image analysis program (Skye Instruments).

## RESULTS AND DISCUSSION

### Morphometrics

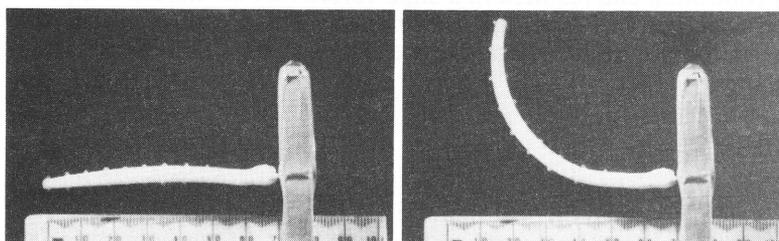
Using inert markers attached to the stipe, asymmetric elongation between the upper and lower surface of a gravitropically bent stipe was measured (Figs 1 and 2). The majority of elongation occurs in the apical region (although the extreme apex grows less). Elongation occurs throughout the stipe and is consistently greater on the lower surface than the upper in the ratio 3:2 (Fig. 2a). From Figure 2b, it can be seen that the lower surface elongates more, and reaches a maximum rate of elongation very close to the reaction time of 25 min /2/. The upper surface shows a period of acceleration before reaching an elongation rate similar to that of the lower surface after 150 min. This is quite different to *F. velutipes* where the upper side elongated very much less and at a much slower rate than the upper surface /5/. It is possible that this delay in elongation exhibited at the upper surface of the *Coprinus* stipe represents the mechanism of curvature compensation. This similar, but displaced, elongation rate is not observed in *Flammulina* /5/, and this may reflect the different lifestyles of the two organisms. Fruit body development and spore dispersal in *C. cinereus* can occur in as little as 24 h in contrast to *Flammulina* where fruit bodies may mature for several days before spore release.

### Cellular analysis

The stipe of *Coprinus* is made up of two distinct populations of hyphae, narrow hyphae (cross-sectional area  $<20\mu\text{m}^2$ ) which make up between 23-54% of post meiotic stipes and inflated hyphae which constitute the remainder of the stipe /6/. An investigation into the nature of the two hyphal populations, their distribution and packing density in gravitropically bent stipes was carried out (Table 1).

	Lower region of the bend		Upper region of the bend	
	n	mean $\pm$ sem	n	mean $\pm$ sem
Mean cross sectional area of narrow hyphae ( $\mu\text{m}^2$ )	626	9.82 $\pm$ 0.6	881	9.43 $\pm$ 0.4
	664	9.01 $\pm$ 0.7	625	8.95 $\pm$ 0.4
	775	9.15 $\pm$ 0.8	474	9.67 $\pm$ 0.8
Mean cross sectional area of inflated hyphae ( $\mu\text{m}^2$ )	1442	176 $\pm$ 6.5	1442	183 $\pm$ 7.6
	1140	186 $\pm$ 9.0	1202	179 $\pm$ 9.4
	1195	184 $\pm$ 11.7	1194	190 $\pm$ 15.4
% narrow hyphae	3	30.5-39.1	3	28.8-41.5
Packing density	18	0.44 $\pm$ 0.02	18	0.47 $\pm$ 0.28

**TABLE 1.** Cell morphometric analysis of transverse stipe sections. Stipes were fixed 3 h after reorientation. Means represent values of 6 replicate sections, three stipes were measured, the values shown represent the upper and lower regions of corresponding stipes.



**Fig 1.** Decapitated stipe with inert markers at 0 min (left) and 260 min (right) after horizontal placement.

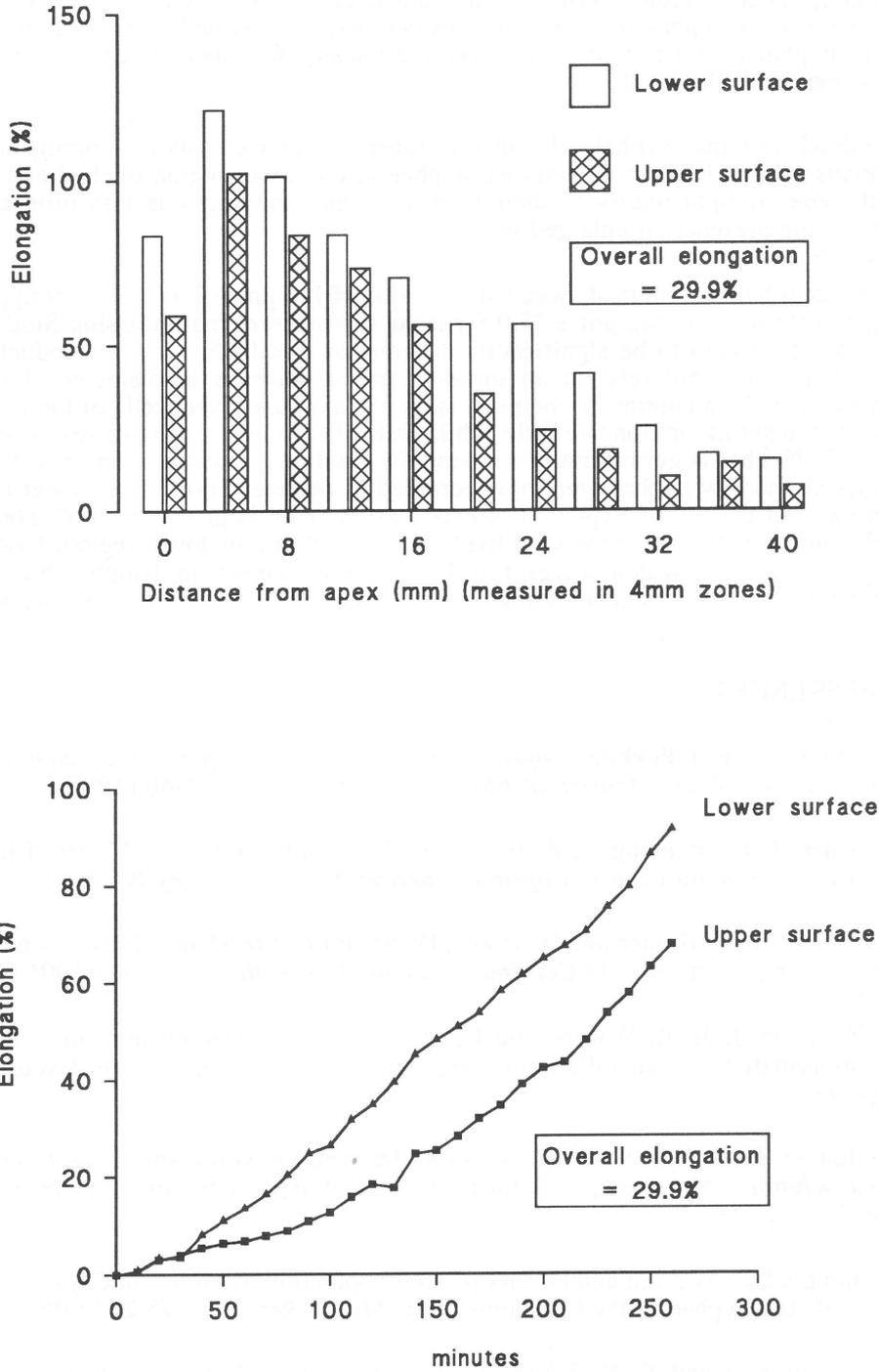


Fig 2. Elongation of *Coprinus* stipes during gravitropic bending. Top panel shows elongation attained at upper and lower surfaces of the 4 mm zones delimited by the markers after 260 min of gravistimulation. Lower panel shows the rates of elongation (measured at 10 min intervals) of the upper and lower surfaces of the stipes.

There were no significant differences seen between the upper and lower regions of the bend in cross sectional area of either narrow or inflated hyphae, nor was there a difference in the percentage of narrow hyphae. The packing density of hyphae (defined as the proportion of space taken up by hyphae in unit area of the section), was not significantly different between the upper and lower regions of the bend.

In longitudinal sections, hyphal cells in the upper region were short, tapering and hyphal compartments stained heavily. In contrast, hyphae in the lower region of the bend were very long, with septa at right angles to their long axes, and staining was less pronounced, due apparently to the presence of enlarged vacuoles.

Measurements of hyphal length showed a mean value of  $116 \mu\text{m} \pm 7.4$  ( $n=34$ ) for hyphae of the upper region compared to  $542 \mu\text{m} \pm 35.0$  for those of the lower region. Using Student's t-test, these values were found to be significantly different at  $P < 0.05$ . Clearly, production of the gravitropic bend does not rely on an increase and/or decrease in diameter of the hyphal compartment. This is in contrast to the plant stem of *Xanthium* where cells of the upper region were observed to shrink or elongate little, while cells of the lower region increased in diameter and length /7/. Neither is generation of the bend the result of expansion of narrow hyphae into inflated hyphae (narrow hyphae were not decreased in representation in the lower region, nor change in packing density of hyphae. It seems that the bend is generated in *C. cinereus* as a direct and unique result of an increase in hyphal length only in the lower region. Evidently, the intercalary wall growth which drives the 4 to 5-fold increase in length which is solely responsible for bending, is somehow regulated to lengthen the cells without increasing their girth.

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