Distribution of mechanical stress is not involved in regulating stipe gravitropism in *Coprinus cinereus*

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Removal of large segments of the apical part of the stipe of *Coprinus cinereus* (extending to about half its length) affected neither the ability of the stipe to show gravitropic bending nor its ability to compensate the curvature so induced and adjust to the vertical. However, gravitropic reaction time was directly proportional to the amount of stipe removed. Application of lateral loads of up to 20 g had no adverse effects on adjustment of the stipe to the vertical and continued vertical growth. It is concluded that sensing the distribution of extracellular mass and/or mechanical stress is unlikely to be a component of the control of gravitropic bending in *C. cinereus* stipes.

When placed horizontal, the basidiome stipe of *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray shows negative gravitropism by bending upwards. The gravitropic bend appears within 30 min of disorientation, initially occurring within the apical 15% of stipe length, then progressing basally to traverse 40% of the length of the stipe. Gravitropic bending raises the apex and, as this approaches an angle of about 45° to the horizontal, curvature compensation begins to adjust the degree of bending so that the apex can be brought exactly vertical (Kher *et al.*, 1992). The way in which curvature compensation is regulated is unknown but an attractive explanation is that the mass distribution of the raised portion of the stipe acts as a feedback device to minimize (or equalize) extracellular mechanical stresses; i.e. forces of compression and/or tension in the walls and/or plasma membranes of the stipe hyphae.

An extracellular mechanical component has been identified in the gravitropic reaction of sporangiophores of *Phycomyces* (Dennison, 1961) and this was subsequently shown to be a response to a stretch receptor (Dennison & Roth, 1967), presumed to sense mechanical stresses in the sporangiophore wall and/or membrane. Establishing if a similar mechanism exists in higher fungi is important, as it is possible that some of the apparent organization in the gravitropic responses of independent hyphae across multihyphal structures, such as basidiome stipes or hymenophores, could be a concerted response to altered distribution of mechanical loads across the tissues, rather than coordination by chemical morphogens or hormones (Moore, 1991). There is no point searching for morphogens if they are not likely to be there.

A relevant comparison is provided by experiments with peduncles of *Taraxacum officinale* (Clifford *et al.*, 1982), which showed that lateral loading for more than 5 min with a 2 g weight resulted in bending movements in the opposing direction to the stress vector. We have carried out similar experiments with stipes of *Coprinus cinereus* but find that normal gravitropic responses continue even under sustained lateral loads of up to 20 g.

MATERIALS AND METHODS

Culture conditions

All experiments were performed with the 'Meathop' dikaryon of *Coprinus cinereus*, originally isolated from a dung heap in Lower Meathop Hill in Cumbria. The vegetative dikaryon 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 sterilized horse dung with the dikaryon, incubating at 37° for 3-4 d in the dark and then transferring the dung cultures to a 26-28° incubator with an 8 h light/16 h dark illumination cycle (average illuminance 800 lx).

Videoanalysis of gravitropism

Mushrooms were removed from the cultures, the cap tissue discarded and the stipes measured and treated as appropriate to the experiments, being kept vertical throughout these procedures. Stipes were then fixed in a horizontal position to a platform in a humidity chamber, the standard gravitropism assay used by Kher *et al.* (1992). Stipe responses were videotaped with a domestic-quality VHS camera and VTR. Experiments were recorded continuously for 3 h; records were made for up to 8 h by changing tapes without disturbing the specimen. Video images were digitized and analyzed as described previously (Kher *et al.*, 1992).

Table 1. Effects of excising apical segments on the kinetics of gravitropic reactions of stipes of *Coprinus cinereus*

Amount excised (mm)	No. of stipes	Length at start (mm)	Reaction time (min)	Final tip angle	Final contact angle	Final base to bend (mm)
0	6	52·5±2·3	24·7±17·5	80·5±20·0	25·8±4·4	20·2±4·1
10	6	57·0±2·2	41·0±27·5	89·7±3·7	35·2±5·7	17·7±1·0
20	6	56·3±3·7	65·0±39·9	81·7±15·9	37·3±3·5	17·7±2·4
30	6*	58·7±1·9	112·0±28·7	61·7±16·1	32·0±5·7	19·5±1·1

Entries show mean±S.D. for the numbers of stipes indicated.

* Two of these stipes failed to show any gravitropic response.

All kinetic parameters derive from measurements made with an image analysis program of frames captured from video recordings, as described and illustrated by Kher *et al.* (1992), the 'final' measurements were made 8 h after the start of the experiment. The contact angle is the angle between the stipe bend and its horizontal support, the tip angle is the angle between the axis of the stipe tip and the horizontal. The reaction time is the time elapsed from the start of the experiment to the first evident gravitropic reaction and is obtained as the time axis intercept of plots of tip angle versus time for individual stipes.

Removal of apical segments prior to reorientation

Whilst still vertical, up to 30 mm of the apical portion of the stipe was completely removed before the stipe was pinned through the basal bulb to a balsa wood platform. The platform was reoriented to the horizontal and placed in the humidity chamber. Stipes used were between 50 and 60 mm in length prior to excision of the apex (Table 1).

Application of lateral mechanical stress to vertical stipes

Fine fishing line was tied to the apex of stipes which were held vertical in Gilson pipette tips inserted in blocks of plant oasis (polyurethane foam used for floral displays) (see Fig. 3). The line was passed over a horizontal support, this being adjusted to be level with the stipe apex, and attached to a known weight. All experiments were carried out in a covered humidity tank, the oasis being soaked and standing in a water layer. Observations were made by video recording and by conventional photography.

RESULTS AND DISCUSSION

Kher *et al.* (1992) showed that when the stipe was pinned through its apex to a horizontal support, gravitropic bending occurred as usual (the stipe base being raised upwards) but it was uncontrolled and resulted in a stipe curled into an almost complete circle. This implies that the apex is the seat of the control process which adjusts the position of the stipe in relation to the gravitational vector and must be free to move for the negatively gravitropic bending response to be constrained to bring the apex of a disoriented stipe back to the vertical.

This distinction between gravitropic bending and curvature compensation is an important one which has not been made previously, but the notion that the stipe apex is involved in regulation of gravitropism at all conflicts with some statements in the literature. Cox & Niederpruem (1975) claim that the stipe apex does not control gravitropism on the basis of experiments which showed that segments of stipe incubated upside down showed negative gravitropic curvature. Jeffreys & Greulach (1956), working with *Coprinus sterquilinus*, showed that the portion of stipe remaining after removal of the upper 25 or 50% responded gravitropically, and claimed that this 'indicates that growth in the lower part of the stipe is not governed by a hormone transported from the upper portion'. The overall conclusion reached by Jeffreys & Greulach (1956) was that 'each hyphal strand responds individually... Because the strands are aggregated, this results in a unit action by the stipe.' This latter comment grossly underestimates the degree of sophistication of stipe structure, recently described (Hammad, Watling & Moore, 1993*a*), and the co-ordination of tissue growth throughout the basidiome which has been detected (Hammad *et al.*, 1993*b*). None of this published work on gravitropism in *Coprinus* was either sufficiently systematic or quantitative, and it would not have predicted the results of experiments in which the stipe apex was pinned to the horizontal (Kher *et al.*, 1992).

Gooday (1974) showed that removal of the apical 3 mm of the stipe of *Coprinus cinereus* had no effect on elongation, so if freedom of apical movement is necessary to regulate gravitropic bending, perhaps the immediate question is: what constitutes 'the apex'?

To address this question apical segments 10, 20 and 30 mm long were removed from 50-60 mm stipes prior to their being laid horizontal in the standard gravitropism assay. Results are recorded in Table 1 and one such experimental series is illustrated in Fig. 1. As usual (Kher *et al.*, 1992), the data are very disperse, but it is quite clear that even when more than half of the stipe was removed the remaining segment usually responded gravitropically, though stipes from which a 30 mm long section had been removed failed to reach the vertical within the 8 h observation period. Indeed, two of them failed to respond, so perhaps the whole of the gravitropically responsive region was excised in these two cases. The only consistent influence of apex removal was on the timing of the first visible gravitropic response (= reaction time). Clearly, the structural stipe apex is not necessary for the gravitropic response to occur in the majority of stipes, but the amount of stipe excised correlated directly to the reaction time of the stipe: the greater the portion of stipe removed, the longer it took for a response to gravity to be observed (Fig. 2).

Thus, although it would appear that most of the apical region (extending to more than half the total length of the stipe) is capable of producing a gravitropic response, the apex is, nevertheless, topographically important in regulation of the

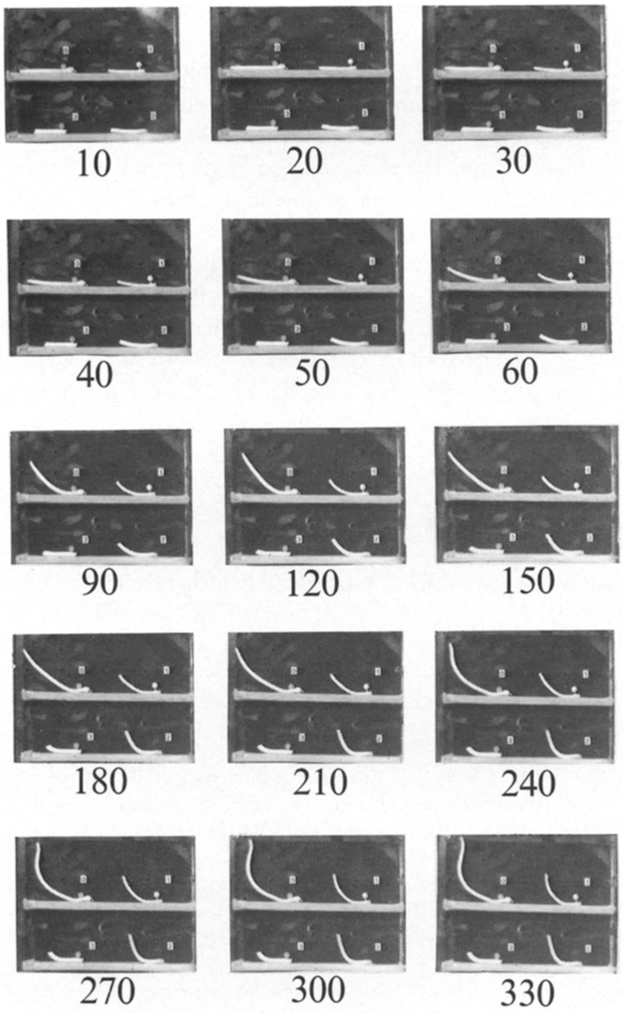

Fig. 1. Gravitropic responses of *Coprinus cinereus* stipes from which apical segments had been removed prior to reorientation. The panel shows a series of stills from a video record (numerals indicate elapsed time in min) of a chamber containing four stipes; the intact control stipe is at top left and is compared with (reading clockwise) stipes from which 10, 20 and 30 mm apical segments were removed. The control stipe was 50 mm long at the start of the experiment.

normal reaction; the stipe is polarized. Importantly, removal of the apical segments did not impair the ability of the stipe to adjust to the vertical; i.e. curvature compensation, as well as gravitropic bending, seemed to occur normally in these specimens.

One way of accounting for this might be to suppose that although the whole of the upper half (or even the entire stipe) may contain hyphal cells able to produce the gravitropic 'reaction', the proportion of such gravitropically competent cells is successively reduced in zones further from the true apex, or the presentation time of those cells is lengthened the further from the apex they are located. This could be understood in terms of diffusion of a chemical signal which might be produced autocatalytically or might be cumulative in its effect, but much the same result could be obtained if the competent cells reacted to some purely physical factor like change in the distribution of mass. However, if curvature compensation depends on sensing some consequence of the mass distribution as the apical portion of stipe is raised, the mechanism must be extremely flexible to be able to adjust to an experimental situation in which most of that part of the organ has been surgically removed.

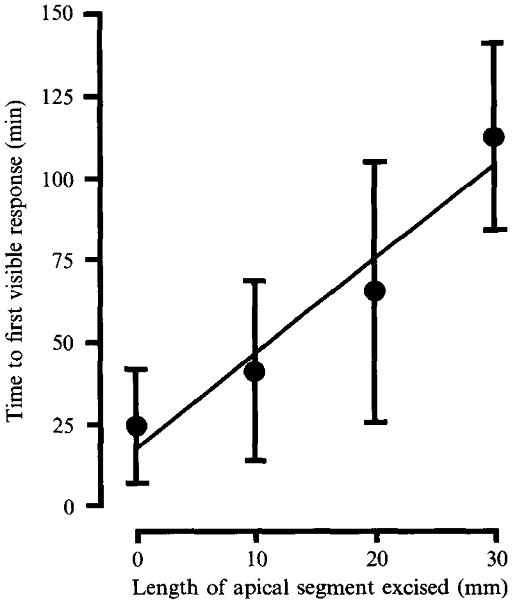

Fig. 2. Relation between reaction time of stipes and amount of the apical part which had been removed. Linear regression, $y = 2{\cdot}86(x) + 17{\cdot}79$; $r = 0{\cdot}97$.

To determine the order of magnitude of the mass distribution which might be involved, stipes were pinned in their centre of the balsa wood platform before reorientation to the horizontal; after they had completed their gravitropic response they were bisected transversely and the fresh weight of each half determined. Centre-pinned stipes usually bent into a U-shape. Interestingly, the mean reaction time of the apical half was over 17 min faster than that of the basal, indicating, again, that the stipe is polarised. Over a sample of 18 stipes, the tip of the apical half made an angle of 60 ± 20° (means ± S.D.) with the horizontal within 3 h, and the end of the basal half reached an angle of 66 ± 17·5° in that time. The fresh weights of these two halves were 151 ± 53 mg for the apical segment and 118 ± 30 mg for the basal. Clearly, if the mass of the raised portion of stipe is involved in regulating the degree of stipe bending then the system which senses mass distribution must be responsive normally to overall masses in the range 100–200 mg and to less than 10% of these values in the experiments in which large apical segments were removed.

Following these experiments, lateral loads were applied to vertical stipes which were secured at their base, to determine whether mechanical stress applied across the diameter would affect the symmetry of stipe growth. Initial experiments involved transient applications of weights of up to a few hundred mg, but no response ensued (i.e. the stipes continued their vertical growth without deviation). Nor was any reaction seen when the weights were increased to 1–2 g, which is the range covering the fresh weights of the basidiome caps removed from the experimental stipes. A response was only found when the stipe was loaded with enough weight to bend

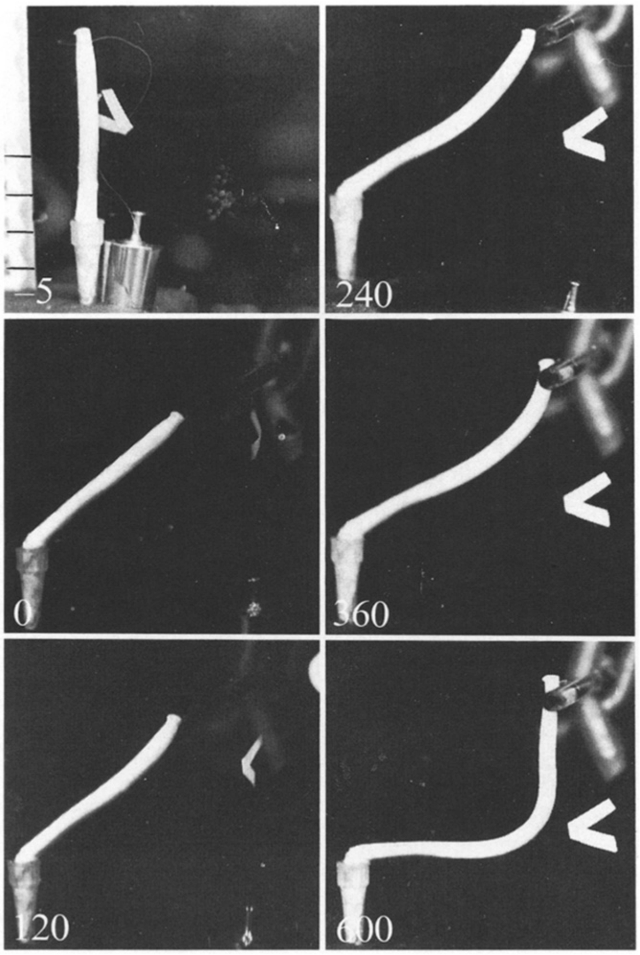

Fig. 3. A series of photographs recording the response of a stipe to a lateral load of 20 g; numerals indicate elapsed time (min). The image at top left shows the experimental assembly 5 min before application of the load. The stipe is firmly wedged into a micropipette tip and tied at its apex to the weight with monofilament fishing line which has a piece of tape attached to it to improve visibility. At time 0 the weight was suspended over a glass rod clamped beside the stipe tip to apply a horizontal force to the stipe (which consequently bent at an angle of 45°). Subsequently, the stipe underwent a normal gravitropic response, returning to the vertical and growing vertically despite the sustained 20 g lateral load.

it sufficiently from the vertical to trigger the gravitropic response. This required application of weights between 10 and 20 g, depending upon the stature of the specimen. Such loading was followed by an apparently normal gravitropic reaction. The stipe apex was returned to the vertical and vertical growth was sustained, despite the continued 10-20 g lateral load (Fig. 3).

Taken together, these experiments imply very conflicting requirements for any mechanism which might be postulated to detect disorientation and adjust back to the vertical by reacting to asymmetrical distribution of mechanical stresses in the extracellular structure of the stipe. Adjustment to the vertical despite excision of large apical sections of the stipe implies sensitivity to very small mass differences, certainly within the µg to mg range. On the other hand, application of at least a 10000-fold greater lateral load had no effect, though it would be expected to add so much noise to so sensitive a system as to swamp any supposed mass distribution detection mechanism. Therefore, we believe that these experiments show that gravitropic detection and response in *Coprinus cinereus* do not involve detection of externally altered mass or stress distributions.

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