

# Perception and response to gravity in higher fungi – a critical appraisal

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## CONTENTS

Summary	3	Gravimorphogenetic effects	9
General principles	4	Hypogravity-reduced gravitational fields	9
Graviperception in animals	5	Hypergravity-enhanced gravitational fields	14
Graviperception in plants	5	Perception of the gravitational stimulus	15
Graviperception and gravitropism in fungi	6	Communicating signals in mushroom fruit bodies	17
Gravitropism in hymenomycetes	7	Acknowledgements	21
		References	21

## SUMMARY

Considering that research on gravitropism in higher fungi has a history of over 100 years, the harvest of established fact is disappointingly meagre. We can be reasonably certain of the following.

Hymenomycete ‘mushroom’ fruit bodies (polypore and agaric) exhibit a number of tropisms of which anemotropism, gravitropism, phototropism and thigmotropism have been clearly demonstrated. At any one time, one tropism usually predominates but the inferior tropisms can be demonstrated if the predominating ones can be removed by manipulation of the growth conditions. In ascending order, the hierarchy appears to be: thigmotropism, gravitropism, anemotropism, phototropism. During the course of development of a fruit body different tropisms predominate at different times. The youngest fruit body initials grow perpendicularly away from their substratum. The nature of this tropism is completely unknown but perpendicular growth of fruit body initials has been remarked upon in experiments at a variety of light intensities and in gravitational fields from  $\pm 0$  to 4.5 g. The fruit-body primordium then becomes first positively phototropic but later negative gravitropism predominates. The switch between predominance of the two tropisms has been associated with the onset of sporulation in a number of different studies.

The major adjustment of the direction of growth in response to a tropic stimulus is made by the mushroom stem. It is the apex of the stem which makes the most immediate gravitropic response. Gravitropic growth curvatures are limited to the normal growth zones of the stem and seem to depend on re-allocation of available growth resources. If the fruit body is reoriented late in the growth of the stem, it may not be able to respond fully. In these cases gravitropic movements of the cap may still be able to bring the hymenophore back to the vertical. Mechanical forces may influence and contribute to the ‘gravitropic’ response but this has not been experimentally examined. The hymenophore (gill, tube or tooth) is positively gravitropic and responds independently of the stem. Bracket polypores do not show tropisms but exhibit gravimorphogenetic responses such that gross disturbance leads to renewal of growth to produce an entirely new fruiting structure suitably reoriented to the new spatial position.

One experiment performed on an orbiting space station suggests that, in the absence of a light stimulus, gravity may be required for initiation of fruiting in *Polyporus brumalis*. Otherwise, the indications from both clinostat and space-borne experiments are that the basic form of the mushroom (overall tissue arrangement of stem, cap, gills, hymenium, veil) in agaric and polypore alike is established independently of the gravity vector.

Abnormal stem growth has been observed in clinostat cultures of *Panus* (= *Lentinus*) *tigrinus* and *Polyporus brumalis*, but the morphogenetic event which seems most dependent on gravity is sporulation (in the broadest sense). Cultures of *P. brumalis* on orbiting space craft fail to produce the poroid hymenophore and in clinostat experiments on the ground even karyogamy was rare in similar cultures. *Coprinus cinereus* grown on the clinostat was able to produce apparently normal fruit body primordia which failed to produce spores and then aborted, forming a new flush of primordia on the old. Taken together with the clear association between observation of gravitropism and the onset of sporulation, the implication is that commitment to the meiosis–sporulation pathway both requires the gravity vector and couples it in some way to fruit-body growth.

There is no convincing evidence for a graviperception mechanism in fungi. There is no evidence for any

organised means of communicating the gravitropic stimulus once it has been perceived. Reports of three different experimental studies reveal the authors' conviction that the apparently coordinated expression of gravitropic response is in truth a common, but independent, response by the individual component hyphae of the structure concerned. There is some evidence that in the negatively gravitropic *Phycomyces* sporangiophore the vacuole floats in the protoplasm. If this is generally true it could affect protoplasmic volumes above and below the vacuole such that a greater proportion of the cell's potential for wall growth was adjacent to the lower wall. This is not only an attractive way of accounting for asymmetric wall growth, but since the relative density of the vacuole can presumably be controlled by regulation of water influx and efflux, it is also an attractive means of accounting for the control of gravitropic responses. *Phycomyces* also exhibits a response to the mechanical consequences of reorientation which is additional to (and different from) the longer term gravitropic response. If this also applies in higher fungi, it is possible that some of the apparent organization in the response of independent hyphae across a structure like a fruit-body stem of hymenophore could be a concerted response to altered distribution of mechanical loads rather than coordination of response by some morphogen or hormone chemical.

Key words: Gravi-perception, gravitropism, gravimorphogenesis, higher fungi.

#### GENERAL PRINCIPLES

'Which way is up?' is not an easy question to answer. To deal with the problem requires what Wilkins (1984) has called a gravity-sensing guidance system with three components: (1) a sensory mechanism to detect gravity; (2) a reaction mechanism which responds to signals from the sensory mechanism by initiating changes in growth pattern or muscle activity; (3) a communication mechanism to conduct the signals from the sensory mechanism to the reaction mechanism. The problems start with the very first of these steps, gravity sensing or, better, gravity *perception* (the term used by Larsen, 1962).

Environmental stimuli programme and control the initiation and development, growth and behaviour of many organisms – plants and animals as well as mushrooms and structurally simpler fungi. Mobile organisms respond to such impulses by tactic movements, but in sessile organisms, especially plants and fungi, their effects are often evident in tropisms – directional growth movements in response to directional stimuli. Usually a growth curvature results from differential growth on the two sides of the organism or structure. Generally speaking, growth towards the stimulus (positive tropism) results when the side closest to the stimulus grows least; in negative tropisms the region closest to the stimulus grows most and causes the structure to bend away from the stimulus. The stimuli include touch or contact with solid objects (hapto- or thigmotropism), chemicals (chemotropism), airflow (anemotropism), light (phototropism) and gravity (gravitropism).

Perception of gravity is potentially quite different from perception of other stimuli. Asymmetric responses to light, wind, temperature or chemicals can all be ascribed to the effects of differential exposure to a unilateral impulse, e.g. one side of the plant is relatively shaded from the incident light, wind or heat, so grows more or less than the other side. But gravity cannot be 'shaded' and over the scale of

living organisms there is no gravitational gradient. If the stimulating field is uniform then the response must be non-uniform, and since the force of gravity acts only on mass the perception system must depend on gravity establishing an asymmetric distribution of matter within the organism.

Gravity-induced asymmetries have been suggested to include (a) differences in hydrostatic pressure between 'top' and 'bottom'; (b) differences in forces of compression or extension between the 'top' and 'bottom' of rigid structures; (c) changes caused by movement of extracellular or intracellular structures relative to immovable parts of the cell or tissues. Most of the classic and best known responses to gravity depend on some variation of category (c); (a) and (b) are usually discounted for a number of reasons. Response to differences in hydrostatic pressure in plants is usually discarded on the basis that it is likely to be extremely small in relation to hydrostatic pressures due to turgor. Audus (1962) calculates the hydrostatic pressure differential across a 20  $\mu\text{m}$  plant cell to have a value about two millionths of the turgor pressure. Such minute perturbations are unlikely to provide the basis for an effective perception system. Differences in compression or tension stresses as a cause of gravitropisms are usually discarded on the grounds that the response to gravity occurs even when the organ is supported along the whole of its length. This fact has not always been demonstrated and, in particular, much of the published work with mushroom fungi is open to interpretation along these lines and will be discussed below. For the moment let us consider more carefully the most general case, that gravity perception depends on movement of extracellular or intracellular structures relative to immovable parts of the cell or tissue.

Theoretically, either the pressure of a weight or the shear force generated by the movement of a weight could be used to produce gravi-perceptive signals. Considering a fixed weight, pressure is at a maximum when the supporting surface (which is presumed to be detecting the pressure) is horizontal.

As the detector is tilted, the magnitude of the pressure decreases, reaching zero when the detector is vertical. If tilted past the vertical, the force experienced by the detector becomes a 'negative pressure' (tension) as the weight hangs from the detector surface, reaching a maximum when the detector is upside-down. In contrast, consider a mobile weight on a surface able to detect the shear force generated if the weight moves across the surface. The shear is zero when the detector surface is horizontal (and the weight stationary), but increases as the tilt increases and gravity causes the weight to move across the surface. Further, the *direction* of the shear force across the surface depends on the direction of tilt. As the vertical position is reached shear reaches a maximum, then declines in magnitude, but without changing its direction, approaching zero as the detector surface reaches the horizontal. Consequently, pressure causes effects in the detector which decline as the tilt increases. This is independent of tilt, and pressure changes sign when the detector surface tilts to the other side of the vertical. On the other hand, shear force increases with tilting angle, is directional, and changes direction when tilting to either side of the horizontal. Contemplating the function of a gravity perception system in the normal state (noting that experiments tend to greatly exaggerate phenomena under study), if an organ is normally held vertically then presumably the perception system will be adapted to recognise and correct for fairly small deviations from the vertical. In other words amplification of small deviations is likely to be an advantage. This leads to the conclusion that a shear detector that is horizontal when the organ is in its normal orientation would provide the most effective gravity perception. Such a mechanism would produce signals that increase in magnitude on increased tilt away from normal, and would offer directional sensing that could be incorporated into a negative feedback system.

#### GRAVIPERCEPTION IN ANIMALS

The majority of animals have just such a mechanism, for their graviperceptive organs detect changes in orientation relative to the vertical, producing a (neural) signal which is used to control posture and movement. Most metazoans have such organs though they are lacking (i.e. they have not so far been detected) in sponges, many phyla of worms and many insects. Statocysts are the gravity perceptive organs of invertebrates. They are fluid-filled sacs lined with hairs, some of which support an aggregate of crystalline grains (the statolith). Displacement of the hairs by the statolith stimulates sensory neurones. Thus these organs serve as shear detectors and so provide a gravitational reference for detecting the position or movement of the body or parts of the body. This is similar to the otolith organs

which are paired in the inner ears of vertebrates. In these the movement of hairs embedded in an otolith (composed of calcified crystals) provides information on the orientation and movement of the head. It has been suggested that the statocyst of coelenterates may have been the first true sensory organ in animals (Hyman, 1940; Bullock & Horridge, 1965). Perception of gravity is clearly a very ancient sensation.

#### GRAVIPERCEPTION IN PLANTS

In a great many plants gravity controls the orientation of roots, stems and leaves in relation to the vertical and also the pattern of the organism as a whole in the sense that the correct orientations must be achieved immediately the seed germinates. Noll (1892) was the first to suggest that plants might sense gravity by means of a statocyst-like mechanism, similar to that of animals, which depends on sedimentation of microscopic particles. It is now generally assumed, but by no means fully proved, that the mechanism of graviperception in plants is the sedimentation of starch-containing amyloplasts (statoliths) in specific cells (statocytes) in the columella (central core) of the root cap and in various locations in shoots [e.g. near vascular bundles or the inner cortical or endodermis layers of the stem (Wilkins, 1984; Poff & Martin, 1989)].

Although many orders of magnitude slower than the neural responses in animals, plant organs can detect changes in orientation very quickly in comparison with their normal growth rates. This is expressed in the *presentation time* which is the minimum time of stimulation required to provoke a gravitropic response. The presentation time can be as low as 10–15 s, but an average value is 4 min. Centrifugation experiments show that the presentation time is inversely related to the gravitational field strength, and it is also significant that successive sub-threshold stimuli can be summed, response being evoked when total exposure is equal to the presentation time (Audus, 1962). The importance of the presentation time is simply that it is within this time period that the graviperception occurs. Response to graviperception (issue of signals, patterning of growth, etc.) is characterized in the *reaction time*, which is the time from first reorientation of the organ to the appearance of the tropic growth curvature; this can vary between about 10 min and many hours. The presentation time can be used as the basis of calculations aimed at identifying which cell organelles might be the statolith particles and this is one line of evidence which indicates that amyloplasts are the effective particles (Audus, 1962, 1969, 1979). Morphometric analysis of organelle redistribution after roots have been placed horizontally supports the crucial role of amyloplasts in graviperception, but also indicates that movement of the nucleus towards the proximal end of the cell (that

is, the end of the cell furthest from the root tip) is also correlated with the gravitropic response (P. Moore, 1986).

Sedimenting amyloplasts showed no consistent contact with any other cellular structure (P. Moore, 1986), so it is not at all clear how graviperception occurs. Sievers and his co-workers (Sievers & Volkmann, 1972; Volkmann & Sievers, 1979; Sievers & Heyder-Caspers, 1983) maintain that amyloplasts are effective in *Lepidium* roots because they exert pressure on membranes of the endoplasmic reticulum. A major reason for this suggestion is the short presentation time (only 12 s) in this material, which must rule out sedimentation over large distances. However, Audus (1962) had already calculated that the pressure exerted by amyloplasts is extremely small and pressure sensing is, theoretically, a poor basis for a graviperceptive guidance mechanism (see above). Nevertheless, the distribution of amyloplasts along the lower wall(s) of the statocytes is the feature most frequently remarked upon in discussions of gravitropism in plants, implying that their pressure or adjacency to those walls (or membranes internal to them) is an essential part of graviperception.

In lentil (*Lens culinaris*), roots grown in microgravity in orbit during a Spacelab mission, amyloplasts were more uniformly distributed through the cell than in 1 g controls (Perbal & Driss-Ecole, 1987), but these authors suggest that a network of microfibrils, which seems to bind the nucleus to the cell periphery, may be able to respond to acceleration. An observation which appears to have been given very little attention is that sedimenting amyloplasts in maize roots are associated with a surrounding region of cytoplasm which stains intensely with toluidine blue in sections of resin-embedded material (P. Moore, 1986); the author stresses that this differential staining was unique to the early stages of amyloplast sedimentation, being observable only in material fixed immediately after reorientation. One wonders if the effect may be an expression of the activity of shear detectors. The colour of the staining is not described, but in any case toluidine blue staining reactions vary with preparation methods (Bergeron & Singer, 1958; Feder & Wolf, 1965; O'Brien, Feder & McCully, 1965). However, it is a basic dye which reliably stains anions in the presence of resin embedding media. This observation indicates a transient increase in polyanions in the vicinity of the amyloplast as sedimentation commences. Perhaps some conformational change in microtubules or microfilaments around the amyloplasts is generated as they begin to sediment.

Although there is still doubt about what is causal and what is consequential metabolic changes in response to reorientation of plant organs in the gravitational field include asymmetric auxin dis-

tribution; redistribution of auxin-regulated RNA and movement of calcium towards the lower side of the organ (Lee & Evans, 1985; P. Moore, 1985; Harrison & Pickard, 1989; McClure & Guilfoyle, 1989).

#### GRAVIPERCEPTION AND GRAVITROPISM IN FUNGI

##### *General background*

Gravitropism is widespread in fungi (Banbury, 1962) and has been the subject of investigation for a century or more, yet unlike the other two major kingdoms of eukaryotes, only *observations* of the phenomenon can be described – very little of any substance can be said about the *mechanisms* involved. There are a number of reasons for this. Among these is that, although research on gravitropism in mushrooms was in progress at the turn of the century, the most acute analytical work has been done on tropisms in structurally simpler fungi, particularly on the sporangiophore of *Phycomyces*, and no serious attempt has been made to unite the two bodies of observations. Another reason for the lack of evidence of progress in the fungi has been their confusion with plants for most of this century (and before) which led both to diminished effort on fungal research and to the expectation that similar effects should be revealed and comparable data obtained. The inevitable result is that the problem of understanding the whole process of graviperception and gravitropism is far more acute in fungi than in either animals or plants because in fungi (*a*) there is no obvious candidate for a gravity sensor in the cell and (*b*) there is no obvious structural, mechanical, electrophysiological or chemical means for communicating the response of such a sensor through the structure. Yet there is ample evidence for gravitropic response.

Mushrooms and toadstools are the most characteristic spore distribution structures of the basidiomycete fungi. The mechanism of spore release in these organisms is intolerant of water so the spore producing tissue (the hymenophore) must be protected from the rain. The mushroom is an umbrella and the spore-bearing surface must be on the lower side of the cap. Evolutionary strategies adopted to increase the surface area of the hymenophore have been to fold it to produce plates (called 'gills' and characteristic of agarics) or extend it into tubes (in the polypores) or spines or teeth (hydroid forms) lined with hymenium (the layer of spore-producing cells). A consequence of such strategies is that spores must fall vertically between those spines and gills or through the tubular pores to escape from the protecting cap to be dispersed on air currents beneath the fruit body. It follows from this that the surface of the hymenophore must be positioned absolutely vertically if spore fall is to occur suc-

cessfully and with the minimum of spore entrapment within the cap.

Numerical data on this point seem to come entirely from A. H. R. Buller. He states that a tilt on the gills of just 5° causes a loss, through entrapment, of half the spores of the Mushroom (*Psalliota campestris* = *Agaricus campestris*), that when *Polystictus* (= *Trametes*) *hirsutus* was tilted to an angle of 30° spore liberation '...almost entirely ceased' (Buller, 1909, pp. 38, 40), and that a tilt from the vertical of only 1° leads to the loss of spores from 80% of the length of the tubes of '*Fomes fomentarius*' (Buller, 1922, p. 132). Statements like these emphasize the importance of the vertical orientation to effective spore release and clearly show the enormous selective advantage which a graviperceptive mechanism would have as a factor coordinating morphogenesis of these organisms.

It is no surprise, then, that the structure of the mushroom fruit body has long been known to be extremely dependent on gravity. The most striking reactions were reported by Schmitz (1842, 1843) and Sachs (1865, 1877) established the basic principles that agaric stipes are negatively gravitropic and the gills positively gravitropic. More detailed experimental work on the topic was done in the early part of this century (Buller, 1905, 1909, 1922, 1924; Hasselbring, 1907; Streeter, 1909) and there have been a number of studies at regular intervals since then. Most of the research bears literally on tropic responses to gravity, rather than on the potentially more subtle morphogenetic responses.

#### *Gravitropism in hymenomycetes*

Gravitropism is the most obvious and readily observed manifestation of the effect of gravity on hymenomycetes. The exact nature of the gravitropic responses seems to differ between the different groups. Gorovoj, Kasatkina & Klyushkina (1987) identify three types of such mechanisms.

1. In most of the gill and polypore fungi with soft quick-growing fruit bodies the gills or the walls of the tubular hymenophores have a longitudinal section in the form of a wedge-shape narrowing downward. Apart from a few exceptions like *Panaeolus*, spore formation and release in such fungi occur simultaneously over the whole surface of the hymenophore for a relatively long period. The wedge-shaped section and strictly vertical orientation of gills and tubes permit the spores to fall out freely. In these fungi the hymenophore may be the main centre regulating gravitropic growth, as on the one hand the hymenophore has its own orientation mechanism assuring positively gravitropic growth, while on the other hand there is evidence that the hymenophore may influence growth of the stem.

2. The second pattern is exhibited by species of *Coprinus* (and, presumably, their relatives although

these are untested). Here, the gills are parallel-sided and spore formation and release are synchronised over a relatively short period of time and in zones at the exposed edges of the gills. Sporogenesis is accompanied by autolysis of those parts of the gill which have released spores, and by the advancement of the spore maturation zone from top to bottom (i.e. margin to apex) of the cap (Rosin, Horner & Moore, 1985). Buller (1922, p. 240) says that the gills of Coprini are not gravitropic and if this is truly the case then it is the stem alone which is responsible for spatial orientation of these fungi.

3. Finally, bracket fungi which have very tough, solid consistency are probably best considered to exhibit gravimorphogenetic rather than gravitropic reactions. After their position has been changed, for example by a fall of the tree on which they grow, the direction of growth changes in the meristemoid zones and only the newly formed parts of the fruit body assume a vertical position. The fruit body as a whole, though, is not gravitropic. Rather, in this instance gravity exerts a morphogenetic effect, determining the overall shape and form of the fruit body as it develops (Ingold, 1953). The fruit-body primordium is an undifferentiated spherical mass. If this emerges on a vertical tree trunk it grows out horizontally to form the 'bracket'. If it forms on the lower side of a branch the fruit body develops a circular form with a central attachment to the tree; rather like an inverted mushroom. This gravimorphogenetic effect is evident when the branch is disturbed sufficiently to reorient the fruit body drastically. When this happens the existing fruit-body tissues cannot adjust; instead, new tissues are formed so that a fresh crop of hymenial tubes can be directed downwards (illustrated in Buller, 1922, p. 110).

This variety of ways in which the hymenomycetes adapt to gravity is paralleled by the variety of tropisms exhibited during morphogenesis of these fungi. The very smallest fruit body initials grow perpendicularly away from the surface on which they arise independently of the direction of light or gravitational signals (Buller, 1905, 1909; Plunkett, 1961; Schwantes & Barsuhn, 1971). Similar behaviour has been claimed for the gills of *Lentinus lepideus* (Buller, 1905) and pores of *Polyporus squamosus* (Buller, 1909) which first grow perpendicularly to the hymenophore surface but in the course of further development show a pronounced gravitropism. These reactions are analogous to the avoidance reactions of *Phycomyces* sporangiophores (Johnson & Gamow, 1971; Lafay, Matricon & Bodere, 1975) and may be similarly based on negative chemotropisms to products of mycelial metabolism or to water activity (Gamow & Böttger, 1982).

As they develop further, the stems of ground agarics (gilled mushrooms) seem generally to be non-phototropic but show a marked negative gravi-

tropism whereas lignicolous and coprophilous hymenomycetes are often both phototropic and gravitropic (Plunkett, 1961). Anemotropism can also be demonstrated (Badham, 1982). Usually, an initial period of light seeking growth in the earliest stages of development is followed by negative gravitropism. Buller (1909, p. 48) ascribed this to 'a remarkable change... in the physiological properties of the stem', i.e. from being primarily positively phototropic the growth control apparatus is rendered almost insensitive to light but negatively gravitropic. The combined effect of gravi- and phototropisms in fruit bodies of *Polyporus brumalis* (where the young stem does not have a cap) was studied by Plunkett (1961) who showed that though several tropisms may be expressed during fruit body development, one of them usually predominates at any given stage but if the effect of the predominant tropism is experimentally diminished the weaker reactions begin to show. The stems of *P. brumalis* fruit bodies whose caps had not developed clearly showed negative gravitropism, but when exposed to unilateral illumination they turned towards the light source. Plunkett screened the stems with black paper disks and found that in the shadow of the disk gravitropism predominated, but when they grew out of the shadow phototropism became the dominant reaction again.

Plunkett (1961) concluded that 'geotropism is only dominant under conditions of low light intensity when it may be presumed that the phototropic mechanism is understimulated' and that the cap acts as a light-screen, simply shading the upper, photoreceptive, part of the stem. Whether this can apply generally to agarics is questionable. Plunkett (1961) does seek to extend the interpretation to *Coprinus* species maintaining that a considerable part of the growing region of the stem is not covered by the rudimentary cap. Yet, most of the true stem is enclosed within the undeveloped cap, only the bulbous base being exposed (Moore, Elhiti & Butler, 1979). Further, it is clear that in sporangiophores of *Phycomyces* phototropic and gravitropic responses interact (Varjú, Edgar & Delbrück, 1961) so that the direction of growth at equilibrium is at '... a compromise angle between gravi- and phototropic tendencies'. These authors specifically excluded shading as a modifier of the phototropic effect, ascribing this rather to a changed distribution of light on the photoreceptive surface due to the lens effect of the cell. Interplay between gravi- and phototropisms is no great surprise, therefore, but the problem of comparing observations from the fungal literature is that hymenomycete fruit bodies exhibit many distinct modes of development which differ in the sequence and timing in which tissues appear as well as in the extent and structural contribution made by the component tissues (Reijnders, 1963; Watling, 1985). So few studies have been made that close comparisons are probably as mis-

guided as would be an attempt to judge the aeronautical ability of pigs from the behaviour of dolphins.

Gravitropism of the stem provides the 'coarse' adjustment, the 'fine' adjustment resides in the spore-bearing tissues (the hymenophore) which are positively gravitropic. Buller (1922, p. 151) states that spines (= teeth) of *Hydnum* are positively gravitropic, but detailed attention has been restricted to the gravitropism of gills (Buller, 1909) and pores (Plunkett, 1961).

Buller (1909, pp. 51–52) showed that all the gills of the Mushroom, '*Psalliota campestris*', could readjust to the vertical providing the cap was not tilted beyond 30°. Adjustment of the gills was detectable with a microscope within 1 h, and by the naked eye within 2 h. Buller's description of his interpretation of the process: 'Each gill grows faster on its upper side than on its lower side and thus gradually curves downwards, so that a large part of it comes to look once more directly toward the earth' (Buller, 1909, p. 50) accords with Banbury's report, in an appendix to his review paper (Banbury, 1962), of otherwise unpublished observations that the gill response involves a gradual curvature extending over the whole width of the gill. Banbury stated that in many specimens only the lower third of the gill reached the vertical and 'the appearance argues against any idea of a restricted "motor" region, and suggests a gravitational response by the individual component hyphae throughout the gill'.

In poroid forms, the growing 'rims' of the pores are positively gravitropic, able to reorient the pore if the fruiting structure is disturbed, though this may be submerged in a phototropic response (Plunkett, 1961). By arranging illumination from below, Plunkett (1961) was able to induce the formation of inverted caps – i.e. the pores were formed on the upper side and a normal hymenium developed. Evidently, basal illumination changes the sign of the gravitropic response. However, it is clear that this switch to positive gravitropism on inversion is not general. Inverted cultures of the resupinate polypore *Phellinus contiguus* produce disorganized masses of tissue rather than inverted hymenophores (Butler & Wood, 1988). However, Plunkett (1961) does stress that his cultures needed to be a few degrees away from the vertical in order to respond gravitropically and where such features as this are not monitored comparisons are difficult.

Interactions between tropisms in the agaric *Psilocybe cubensis*, especially response to wind velocity, have been studied by Badham (1982). At initial stages of development the stems bent so that the fruit bodies grew into the air flow (positive anemotropism) but as spores began to be formed negative gravitropic curvature of the stem occurred. Badham (1982) suggests that in the first phase the stem is elongating towards the direction of greatest 'evaporative de-

mand'. The anemotropism being interpreted as being due to distribution of water vapour around the stem in the airflow: the windward side being exposed to greater 'evaporative demand' than the leeward. Although Badham's experiments were performed at low light intensity he suggests that this growth response to water activity could be a component of phototropic responses as incident light might heat the surface of the tissue and increase evaporation of water (or other gases) from the exposed surfaces. Light is required for the initiation of *P. cubensis* fruit bodies (Badham, 1980) and for spore formation. As the latter phase coincides with the onset of negative gravitropism in the stem, Badham (1982) suggests that a substance produced in the gills modifies the predominating anemotropism of the stem so that the response to gravity dominates.

The natural and experimental switches between dominating tropisms show that they are different and interactive. In some instances (as in Plunkett, 1961) they can be shown to interact in ways dependent on simple physical factors. In others (as in Badham, 1982) a morphogenetic change in one tissue seems to be at least coordinated with a change of tropism in another. The vegetative hyphae of cultures which give rise to gravitropic, phototropic and anemotropic fruit bodies do not themselves show such tropisms though their genetic descendants within those structures do. Thus, the tropic response is an attribute which can be regulated.

In summary, the stems of both agaric and aphyllorphorean fruit bodies are negatively gravitropic and their hymenophores are positively gravitropic. These responses can be quite rapid. Buller (1909, 1922) detected the gravitropic adjustment of the gills of *Agaricus bisporus* within an hour and states: 'If one turns a [*Coprinus*] fruit-body from a vertical to a horizontal position, the stipe begins to turn up the pileus within about three minutes after first receiving the geotropic stimulus' (Buller, 1909, pp. 69-70). At the other end of the spectrum, the responses can be maintained for very long periods. In bracket polypores the tubes which bear the hymenial surfaces are positively gravitropic. The tubes often extend vertically downwards for considerable distances; spores of these fungi may have to fall many centimetres before emerging into the air beneath the bracket. Since many of these fruit bodies are perennial, this gravitropic growth may continue for up to 10 yr (Buller, 1922, p. 112).

#### *Gravimorphogenetic effects*

*Introduction.* There are many aspects of early mushroom morphogenesis which *might* be influenced by gravity, including the polarity of the primordium, the relative positions of stipe, hymenophore and cap tissues and the initial distribution of agaric gills, but these could all equally well be controlled by non-

gravitational cues. The key experimental approach is to remove suspected cues and study the progress of events in their absence. The major stumbling block to decisive experimentation on this problem is that the influence of gravity can only be escaped in orbital laboratories. A number of earthbound devices can help. Centrifuges offer a means of increasing the apparent gravitational field, and the clinostat equalises the effect of gravity around its horizontal axis of rotation, to some extent (discussed below) imitating the zero-gravity state.

*Hypogravity-reduced gravitational fields.* Hasselbring (1907) investigated the effect of growth on a clinostat on development of *Polystictus* (= *Pycnoporus*) *cinnabarinus*, *Schizophyllum commune* and *Coprinus* spp. In each case the experiments involved rotating 'cultures' of the organisms for some months. For *P. cinnabarinus* and *S. commune*, sticks and branches bearing fruit bodies were collected from the field and brought into the laboratory, placed into a moist chamber and 'rotated on the horizontal axis of the klinostat'. *P. cinnabarinus* failed to produce normal bracket-like fruit bodies (with a dorsi-ventral polarity) in these conditions, but formed cushions with the tubular hymenophore developed over the whole surface of the cushion. The fruit body of *S. commune* was also affected by clinostat-growth: gills developed on what would be the dorsal (upper surface) of a normal fruit body. Dung cultures of *Coprinus*, which were grown on the clinostat from inoculation with spores, produced several crops of fruit bodies which were '... normal in every way. Their direction of growth was perpendicular to the substratum, the actual direction being horizontal, since the pots were rotated on the horizontal axis of the klinostat.' This is the only clear statement about the orientation of fruit bodies on the clinostat. Interpretation is made more difficult because its speed of rotation is not stated and, more importantly, it is stated that of the sticks and branches brought into the laboratory 'only a few gave results', so this is far from being an objective study. Reijnders (1963, p. 316) confirmed Hasselbring's results for *Coprinus*, reporting that in cultures of *C. narcoticus* which he grew on a clinostat from spore germination through to fruit body maturity it was not possible to discover the least effect on development.

Plunkett's clinostat experiments with *Polyporus brumalis* (Plunkett, 1961) were limited and mainly show the dominance of the phototropic response in this organism. He obtained fruit bodies with a clear differentiation between upper and lower surfaces of the cap on cultures which were incubated on the clinostat from inoculation. He states: 'In the two fruit-bodies studied extremely shallow hymenial tubes, 1 mm deep, were produced but developed no further'. Earlier in this section of his paper is the statement 'The results given relate to cases where no

obvious arrest to development occurred' – objectivity is again in doubt. Badham (1982) put cultures of *Psilocybe cubensis* bearing young primordia on a clinostat and rotated them until they matured (for about 72 h). No morphogenetic changes were reported but results are sparsely described and the experiments were done in a wind tunnel with an air flow parallel to the axis of rotation of the clinostat so another tropism was being investigated at the time.

The most recent clinostat experiments have been described by Gorovoj, Kasatkina & Laurinavichius (1989) who experimented with *Polyporus brumalis*, *Panus* (= *Lentinus*) *tigrinus* and *Coprinus cinereus*. These are undoubtedly the most carefully executed clinostat experiments with fungi which have been published. The clinostat turntable was rotated at 2 rpm at a temperature of  $22 \pm 1$  °C; illuminated cultures were exposed to about 1500 lx and dark-grown cultures were wrapped in black paper immediately after inoculation. Experiments were repeated at least three times, vibrational controls were included, as were controls with a vertical clinostat rotating at the same speed to exclude centrifugal effects. These tests showed that mechanical loads caused by rotation do not cause any noticeable changes in the morphology of fruit bodies of *Polyporus brumalis*. Cultures were placed on the clinostat at the initial 'rudiment' stage of fruit-body formation so that the whole process of morphogenesis occurred under the conditions of altered gravity. Immediately after removal from the clinostat the material was chemically fixed.

The report includes detailed description of the morphologies and morphogenesis of the various polymorphisms observable in normal development of fruit bodies in control cultures. Appreciation of the spectrum which constituted the 'normal' phenotype is essential if the consistent effects of an experimental treatment are to be recognized, but it is particularly important in the case of *Polyporus brumalis* which seems to be prone to producing abnormal fruit bodies even under normal growth conditions. Gorovoj *et al.* (1989) record adhesion and blending between fruit bodies where they touched, saying that this seems typical for the aphyllorphorean fungi. The most drastic deviations from normal morphogenesis led to complete abortion in the formation of fruit bodies; rudiments may fail to develop further or may develop into short finger-shaped protrusions with widened tops, and even a rudimentary cap, which then abort. Combined adhesion and abortion led to massive conglomerates of the stems of fruit bodies without caps growing upwards in a dense bunch, and to 'clavarioid' branched stems which could reach considerable dimensions – up to 4–5 cm with a branch width of about 5 mm, but no signs of formation of the cap. The upper part of every branch showed negative geotropism from the very beginning of its de-

velopment. Clavarioid fruit bodies are frequently formed by *Lentinus*, *Lentinellus* and *Polyporus* (R. Watling, personal communication). Deviations in morphogenesis of fruit body caps of *P. brumalis* were observed fairly often by Gorovoj *et al.* (1989); seemingly, the development of a cap may stop at any stage. These deformities are most probably caused by internal factors and not by the conditions of cultivation because aberrant forms can be found in the same culture with normal fruit bodies.

On the clinostat, fruit-body initials of *Polyporus brumalis* were first oriented perpendicularly to the surface of the mycelium; in later growth they bent in arbitrary directions. Most of the rudiments aborted at the stage of round globes or finger-shaped outgrowths (these sometimes reaching up to 2 cm in length). Other rudiments continued morphogenesis, many producing caps. The size of the stem increased considerably, in most cases stems bent in random directions and as this brought different fruit bodies into contact, adhesions and accretions between fruit bodies were more frequently observed. Some cases of fruit bodies with a wide stem were observed; no such distortions were observed in the control. On the clinostat, as in the control, deformed fruit bodies were found in the same test-tube with relatively normal ones. The most characteristic and significant effect of clinostat growth on morphogenesis of *P. brumalis* was that the differentiation of the tubular hymenophore was completely retarded.

In a series of five experiments, each with four replicates, no sign of the typical tubular hymenophore, or even of the network of dissepiments which signals its first appearance, were observed on the smooth lower surface of the cap. This result parallels those obtained by earlier workers. It implies that the unilateral gravity vector is required for formation of the tubular hymenophore and directs attention to the dissepiments as the most likely sites of location of the cellular mechanisms of gravi-perception. Gorovoj *et al.* (1989) note that in normal development it is only in dissepiments where hyphal bundles are orientated in the direction of the gravity vector.

Gorovoj *et al.* (1989) examined the microscopic anatomy of rudiments and fruit bodies at all stages of development during growth on the clinostat. No major differences were seen in the structure of the subhymenium of fruit bodies grown on the clinostat compared with control and, in general, the structure of the stem of experimental fruit bodies did not differ from that in the control. The major difference was in the hymenium, the cells of which were stopped in their development at the dikaryon stage. Karyogamy was rarely observed and basidia with spores were infrequently seen. Gorovoj *et al.* (1989) also report a cell type not observed in the control, described as being bottle-shaped and with a thin elongated 'nose'. Nuclear divisions were occasionally seen in these



cells and after division, the noses elongated and came to protrude up to 10–15  $\mu\text{m}$  above the level of the hymenium surface. Then a nucleus moved along the elongated nose; when it reached the tip this expanded into a round head of 2–3  $\mu\text{m}$ . The authors suggest that clinostating leads to a distortion of morphogenesis of the basidia which form only one sterigma with an underdeveloped basidiospore, or that morphogenesis of cystidia is induced.

In experiments to determine how the degree of differentiation of the hymenophore depended on the duration of clinostating, Gorovoj *et al.* (1989) placed samples on the clinostat with fruit bodies in different stages of development. The first samples consisted of mycelium lacking rudiments; the second had rudiments up to 5 mm in size already formed; and in the third series cultures with fruit bodies whose caps had begun to differentiate were clinostated. Clinostating continued until mature fruit bodies were obtained. The outcome was that slightly different levels of differentiation of the hymenophore were obtained. In the first series the mature fruit bodies had a smooth hymenophore surface; in the second series of cultures most of the fruit bodies had the lower surface of the caps covered with blebs (the very earliest stage in formation of dissepiments) and in some places the cap even had a small network of dissepiments; and in the third series the fruit bodies had a network of dissepiments on the lower surface of the cap. However, formation of the normal tubular hymenophore did not occur in any of the samples.

Normal morphogenesis of *Lentinus tigrinus* was more uniform than *Polyporus brumalis* although the proportions between the cap and the stem were quite variable. In length a stem may exceed the diameter of the cap by more than a factor of two; or a cap may be twice as large in diameter as the length of its stem. As in *P. brumalis*, at early stages of development fruit-body rudiments were oriented perpendicularly to the surface of the mycelium from which they originated. As soon as cap differentiation became evident, however, this strict orientation was no longer observed and young fruit bodies could tilt aside in random directions. The lower surface of the cap was at first absolutely smooth. Then ribs appeared on this surface and developed into radial folds interconnected by numerous bridges. Thus, in these early stages, the *Lentinus tigrinus* hymenophore resembles the convoluted 'embryonic' hymenophore described in *Copinus cinereus* and *Volvariella volvacea* (Chiu & Moore, 1990a & b). As the hymenophore begins to differentiate, *Lentinus tigrinus* begins to show gravitropic responses. Stems of fruit bodies begin to bend so as to bring their caps to a horizontal position. In mature fruit bodies the hymenophore is oriented horizontally and the gills strictly vertically irrespective of the position of the stem.

In clinostat experiments with *Lentinus tigrinus* the

greatest influence was on morphogenesis of the stem. The initial stages were affected in the sense that numerous branches were formed (the number of such outgrowths could exceed ten), as though the single growth zone expected at the tip of the stem was proliferating and new growth zones were being organised quite abnormally in the lower regions of the stem. On many primary branches, secondary branches formed. When in contact these also adhered. In addition, there were many cases where the stem, having reached the length at which a cap would normally form, produced branches rather than a cap. Thus, it seems that in this organism the 'absence' of a gravity stimulus on the clinostat results in a loss of polarity and apical dominance over short distance – the growth zone at the tip proliferating into branches, and basal regions of the stem are no longer inhibited from branching. There also appears to be some dependence on gravity for differentiation of cap tissues. When fruit bodies with caps were formed they mostly had a thick unbranched stem which sometimes had localized swellings or was bent; in one case the stem was bent through 180 degrees. In all these cases, caps had a gilled hymenophore on their ventral surface but all were irregular in form in some way.

Little morphological variability was observed by Gorovoj *et al.* (1989) in *C. cinereus* grown under normal, stationary, conditions. On the clinostat, no normal, spore-yielding, fruit bodies were obtained. Otherwise, the morphological effects of clinostat exposure in *C. cinereus* were variable. In some cases outward morphology was close to normal; some grew perpendicularly to the surface of the substratum, some bent aside. Some primordia on the clinostat had an elongated base; some stems were greatly elongated and bent, and clusters of fruit body initials were often formed on the lower half of the stem. A consistent anatomical distinction between control and clinostat-grown fruit bodies was the unusual narrowness of the gills in the latter. Generally speaking, therefore, in *C. cinereus* the 'absence' of gravity on the clinostat did not impair progress through the initial stages of development; all the main parts of the fruit body – cap, stem, hymenophore, veil – were formed, but all of them remained underdeveloped. It seems that the unilateral gravity vector is required for the culminating stages of sporulation and maturation and in its absence fruit-body development stops prior to spore formation. It is important to emphasise that the failure of maturation of these primordia is the most significant result; their lack of spores and formation of clustered initials are probably consequential symptoms. A large number of sporeless mutants of *C. cinereus* have been selected in a number of different ways and these show that inability to complete meiosis or spore formation is not sufficient to prevent fruit-body maturation in *C. cinereus*. Both

dominant (Takemaru & Kamada, 1972; Tani, Kuroiwa & Takemaru, 1977) and recessive (Zolan, Tremel & Pukkila, 1988; Kanda *et al.*, 1989) sporeless mutants of *C. cinereus* are able to produce mature (though white) fruit bodies. However, the *Mar* mutant, which is probably defective in pre-meiotic DNA replication, does fail to fruit, producing aborted primordia (Gibbins & Lu, 1982). *Mar* primordia '...appeared to be arrested in development just prior to karyogamy ... After two or three days of arrest...the fruiting body cells...became active mitotically ... However, fresh waves of fruiting bodies developed on aborted primordia, only to abort themselves in turn' (Gibbins & Lu, 1982). The formation of clusters of rudiments on the stems of such aborted primordia is probably an aspect of the 'renewed fruiting' which is known to occur in *C. cinereus* and *C. congregatus* in response to surgical treatment (Bastouill-Descollonges & Manachère, 1984; Chiu & Moore, 1988a) and in maturation-defective developmental mutants of *C. cinereus* (Moore, 1981). On the clinostat, spore formation fails to occur because development stops prior to the onset of sporulation. It may be that some aspect of commitment to the culminating phases of fruit-body development has a requirement for the unilateral gravity vector.

The results of clinostat experiments done so far certainly suggest the possibility of interesting effects but reported studies with fungi compare poorly with the careful analytical work which has been done with plants (Larsen, 1962). The clinostat is a very useful tool which deserves sensible appreciation and proper use. Due to the rotation of the platform on which the subject is mounted the normal gravity vector constantly changes its direction with respect to the subject. At some rotation rates the organism has no time to respond to the gravitational impulse before its reaction is reversed. If the platform is pictured as a disc, facing the observer, which is rotating, clockwise, then any object mounted on the disc will experience a downward acceleration due to gravity as it traverses the right hand sector, but will experience an apparently upward acceleration as it traverses the left. The gravitational acceleration experienced by such an object will therefore be described by a sine wave in which the gravity vector changes direction every half-revolution. The faster the clinostat rotates the more equal is the distribution of natural gravitational forces but the greater is the centrifugal effect of the rotation. There is, therefore, an upper limit to the rate of rotation which is set by the sensitivity of the experimental tissue to the centrifugal forces on the clinostat. There is a lower limit to the rate of rotation, too, which is set by the presentation time. If the rate of rotation is so slow that the graviperception mechanism is activated each half revolution then the effect is not one of the absence of the gravitational stimulus but rather of

the continuous cyclical activation of the mechanism. The only attempt to measure the presentation time in higher fungi of which I am aware is Streeter's (1909) estimate of 'less than a minute' for *Amanita phalloides* and *A. crenulata*. In some plants the presentation time can be as short as 10 to 15 s, but it varies enormously between species and needs to be experimentally established for each organism used.

There is little evidence that any of these factors were appreciated in work reported prior to Gorovoj *et al.* (1989); this earlier work has little more than anecdotal value and leaves the paper of Gorovoj *et al.* (1989) as the only worthwhile report on the topic. The three fungi used by Gorovoj *et al.* (1989) responded differently to the clinostat treatment. As is implicit in the above discussion, it is logically possible to relate those differences to different phenomena – differentiation of dissepiments in *Polyporus*, establishment and maintenance of polarity and apical dominance in *Lentinus*, stepover into a culminating phase in *Coprinus* – all of which *could* be gravimorphogenetic. An additional point of significance might be extracted from the fact that the results of clinostat treatment of *Coprinus* were so different between Hasselbring (1907) and Reijnders (1963) on the one hand and Gorovoj *et al.* (1989) on the other. The former two experimenters both stressed that they grew cultures from spore germination through to maturity, whilst Gorovoj *et al.* (1989) induced fruiting and then put cultures bearing fruit body initials onto the clinostat. It may be that the higher fungi have an ability to adapt successfully to altered gravitational conditions. If this adaptation has a variable threshold it could explain why essentially normal fruit bodies developed in some cases under treatment conditions which otherwise caused regular production of abnormal or aborted structures (Gorovoj *et al.*, 1989). Unfortunately, though, the final verdict must be that the data (and particularly, information about the characteristics of the clinostats used) are too sparse for much confidence about this deduction.

The first experiments with hymenomycetes during an orbital space flight were done aboard the unmanned Soviet 'biosputnik', Cosmos 690. Zhari-kova, Rubin & Nemchinov (1977) describe an experiment in which test-tube cultures of *Polyporus brumalis* were flown as well grown mycelium so that the main phase of fruit-body development occurred during orbital flight. The Cosmos 690 cultures were in orbit for 20 d during which time they were in the dark, through irradiated twice by a <sup>137</sup>Cs source with a total dose of 800 rad. The purpose of this irradiation is not indicated in the mycological papers but was presumably a requirement of one of the other experiments in the same capsule. Because of the lack of illumination, fruit-body caps were not formed but the stems of the fruit bodies grew well, winding around the walls of the tubes in a spiral

manner, interlaced and partly accreted into balls. This experiment demonstrated that at least the early stages of fruiting could occur in zero gravity, and that the stems so formed were disoriented. Dudchenko, Nemchinov & Zarubina (1978) conducted another experiment with two *Pleurotus* species aboard the same biosputnik. Unfortunately, this report is rather confused – enzyme assays are described in detail but no data are presented, different species are referred to in text and tables, and there is no statistical treatment of numerical results. The authors claim that space flight changes a number of morphological features and the average length of fruit bodies formed by three strains of *Pleurotus ostreatus* in orbit is between 35 and 100% greater than those in earth-based control cultures which were also subjected to  $^{137}\text{Cs}$  irradiation. *Pleurotus pulmonarius* showed no such difference. Study of the 'aftermath of space flight' was attempted in this experiment by subculturing the experimental cultures after recovery in liquid medium for 17 d to measure production of mycelial biomass, mycelial protein and pectolytic enzymes. The first samples were taken after 7 d incubation and judging from the biomass yields the cultures had reached stationary phase by about this time. No worthwhile conclusion can be drawn from the data presented.

Two experiments have been conducted, again with *Polyporus brumalis*, during flights of the manned space stations Salyut-5 and Salyut-6 (Kasatkina *et al.*, 1980, 1984; the organism was described as *Polyporus ciliatus* in the 1980 paper, but as *P. brumalis* in all subsequent publications). Salyut-5 was launched on 22 June 1976 and the recovery capsule containing material from the scientific experiments detached and landed on 26 February 1977. Salyut-6 was launched on 29 September 1977; the *P. brumalis* experiments were conducted 10 December 1977 to 16 January 1978 as part of a programme called 'IFS' (inoculation-fixation system) involving small illuminated growth chambers.

In the experiment conducted aboard Salyut-5, cultures which had already formed rudiments of fruit bodies up to 2 mm tall were taken aboard the space station. Two cultures were illuminated while four test tubes were in complete darkness during an experiment which lasted 17 d. Fruit bodies developed in all six cultures. Those developed in the dark failed to form caps, as expected, but the stems had atypical twists and bends where they touched the walls, implying that a thigmotropism became evident in the absence of both light and gravitational stimuli. Fruit bodies grown aboard Salyut-5 in the light were similar to normal and had a tubular hymenophore. However, although the hymenium extended over the walls of the tubes in control cultures, those grown in orbit had basidia at the bottom of the tubes only (Kasatkina *et al.*, 1984).

In the second experiment, on Salyut-6, cultures

were made up in such way that they were delivered to the space station bearing mycelium only. Again, cultures were incubated in weightless conditions in the light (growth chambers unilaterally illuminated) and in the dark, this experiment lasting a period of 20 d. After this time the mycelium was well grown; it covered the upper and side surfaces of the medium and a web-like mycelium extended onto the growth chamber wall. One illuminated chamber produced stroma-like mycelial structures from which fruit bodies were formed, orientated towards the light. The hymenophore was at best poorly developed and most of the fruit bodies had no hymenophore. The hymenium was hardly developed, being most pronounced in fruit bodies strongly oriented towards the light source, and therefore parallel to the surface of the medium. Hymenial paraphyses formed in orbit were smaller than in the controls and were placed in a more disorganized manner. Electron microscope examination revealed no difference in stipe anatomy between control and experimental fruit bodies, but thick-walled hyphae which were readily visible in the hymenophore of controls were absent from the hymenophores of fruit bodies formed on Salyut-5.

In the dark chamber no fruit bodies were formed, although in dark-grown controls on earth fruit-body rudiments appeared on the fifth day after the mycelium covered the medium. After recovery of the samples the dark-grown cultures from Salyut were exposed to the light and they subsequently fruited, so confirming the fertility of the mycelium.

These experiments demonstrated the ability of *Polyporus brumalis* to form fruit bodies without gravity. They suggest that gravity may be required for initiation of fruiting (rudiment formation) in the dark but that this requirement can be satisfied by illumination in the absence of gravity. The experiments in orbit also seem to support the conclusion from clinostat experiments that it is hymenophore development which is most dependent on the gravity vector in this organism. Fruit bodies which were initiated in orbit generally failed to form the hymenophore; rudiments which were on cultures when they went into orbit continued development and produced some hymenophore tissue though hymenium development was limited. Seemingly, they had received sufficient impulse prior to launch to at least initiate hymenophore development though this could not sustain an equal extent of hymenium development. There is a parallel here with the clinostat experiments of Gorovoj *et al.* (1989) which showed that hymenophore development stopped on the clinostat at later stages the further the tissue had developed prior to the start of clinostat treatment.

Comparing results obtained from experiments with clinostats with those from the few experiments which have been done on orbital space craft (Table 1) it seems that a clinostat of the design used by

**Table 1.** Comparison of the main results from experiments conducted on clinostats and those conducted in orbital space craft

Organism	Developmental behaviour	Ref.*
(a) Clinostat experiments		
<i>Pycnoporus cinnabarinus</i>	Tubular hymenophore formed over whole surface of fruit body	1
<i>Schizophyllum commune</i>	Gills formed on dorsal surface of fruit body	1
<i>Coprinus cinereus</i>	Normal in every way... fruit body grew perpendicularly to the substratum.	1
<i>Coprinus narcoticus</i>	No effect on development	2
<i>Polyporus brumalis</i>	Extremely shallow hymenial tubes	3
<i>P. brumalis</i>	Tubular hymenophore not formed; hymenium aberrant, karyogamy rare, spores infrequent	4
<i>Lentinus tigrinus</i> (as <i>Panus tigrinus</i> )	Stem often branched, many without caps When formed, caps always morphologically abnormal, though did have gilled hymenophore on ventral surface	4
<i>Coprinus cinereus</i>	Primordium development initially normal but then aborted prior to spore formation	4
(b) Orbital experiments		
<i>Polyporus brumalis</i>	<i>Cosmos 690</i> . Grown in the dark; stems twisted around walls of the test-tube culture vessel	5
<i>Pleurotus ostreatus</i>	<i>Cosmos 690</i> . Fruit body formed in orbit generally much longer than the control	6
<i>Pleurotus pulmonarius</i>	<i>Cosmos 690</i> . No size difference between experiment and control	6
<i>Polyporus brumalis</i>	<i>Salyut-5</i> . Cultures flown with pre-formed fruit-body rudiments. Tubular hymenophore formed in orbit but hymenium limited to base of tube. Cap-less stems formed in the dark, bent and twisted with unusual swellings. <i>Salyut-6</i> . Cultures flown as mycelium only. No fruit body initials formed in the dark. Fruit bodies formed in the light lacked tubular hymenophore	8

\* References: 1, Hasselbring (1907); 2, Reijnders (1963); 3, Plunkett (1961); 4, Gorovoj *et al.* (1989); 5, Zharikova *et al.* (1977); 6, Dudchenko *et al.* (1978); 7, Kasatkina *et al.* (1980); 8, Kasatkina *et al.* (1984).

Gorovoj *et al.* (1989), with a rotation speed of 2 rpm, is a good analogue of the 'zero' gravity state. This is a crucial point, which really needs further proof in the form of at least a few more experiments in orbit, because it means that many of the questions for which answers are most urgently required can be approached experimentally at relatively low cost on the ground rather than demanding the high cost of orbital flight. However, one essential question which is not answerable without orbital flight is the relative positions taken up by the cell organelles in zero *g*. To determine this 'rest distribution' requires fixation of the material in space and this does not seem to have been attempted in any of the orbital experiments so far done with fungi. It is essential to establish this rest distribution, though, in order to ascertain

relationships between organelles which might be involved in gravity perception in the true absence of the normal gravity vector.

*Hypergravity-enhanced gravitational fields.* With reference to gravitropisms in the sporangiophore of *Phycomyces*, Dennison & Shropshire (1984) comment on 'the great variability in the geotropic response at 1 *g*' and variability and inconsistency seems also to characterise research on the higher fungi. Dennison (1961) improved the reproducibility by use of a centrifuge to increase the effective 'gravitational' field (up to 4.35 *g*). Gorovoj *et al.* (1989) used a sophisticated centrifuge to study the influence of hypergravity on morphogenesis at applied centrifugal accelerations of 1 and 4.5 *g*.

Their device not only provided centrifugal force to imitate hypergravity by fast rotation in the horizontal plane, but at the same time the object was slowly rotated in the vertical plane (i.e. on a clinostat) to eliminate the effect of natural gravitation. Furthermore, they were able to place the experimental subject in the horizontal plane in such a way that the growth of the mushroom occurred either with its cap pointing towards the axis of rotation, that is in the direction opposite to that of the centrifugal force (centrifugal placement, providing hypergravitation acting in the same direction as the normal gravity vector) or in the same direction as the centrifugal force (centripetal placement, providing hypergravitation of the opposite polarity to the normal vector). In every experiment Gorovoj *et al.* (1989) rotated four cultures bearing fruit body rudiments placed centrifugally and four placed centripetally. Neither treatment greatly affected fruit body morphogenesis either macroscopically or microscopically and the normal gravitropic reactions occurred with the stems of centripetally placed fruit bodies bending through 180° so that the caps assumed their normal position with respect to the gravity vector imitated by the centrifugal force. More extensive experimentation with this apparatus is eagerly awaited.

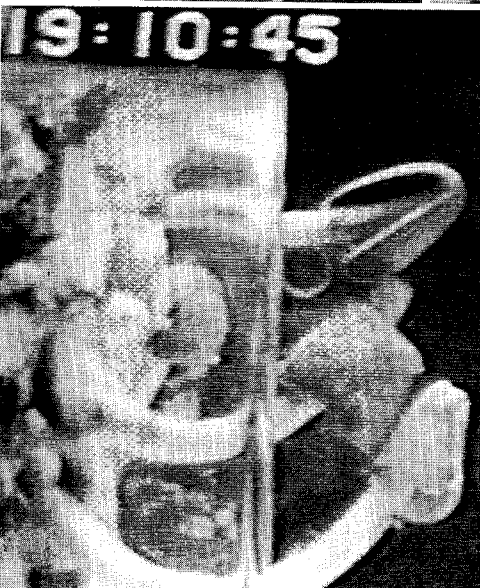
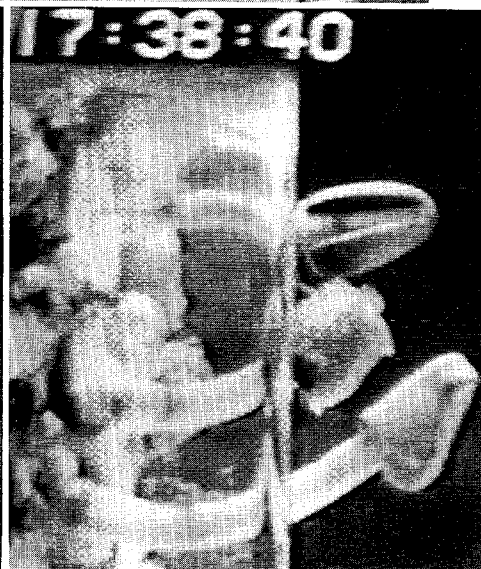
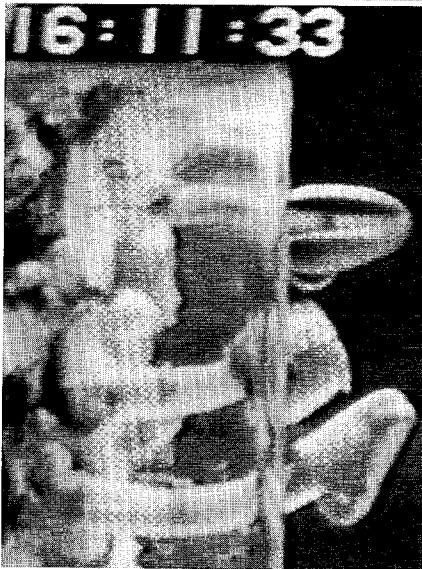
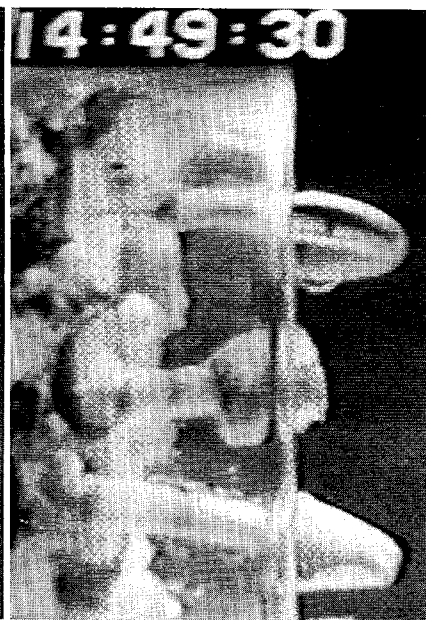
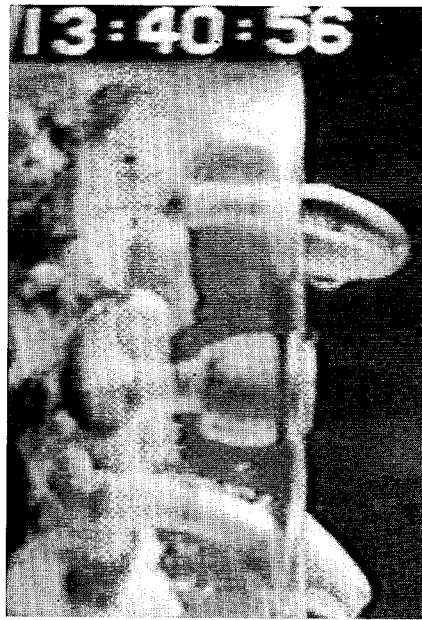
#### *Perception of the gravitational impulse*

The questions of the localization and nature of the structures responsible for perception of the gravitational impulse in hymenomycete fruit bodies have hardly been investigated. Borriss (1934), who worked on *Coprinus lagopus* (= *C. cinereus*?), dismissed the possibility that glycogen granules might serve an analogous function to the statolith starch grains of plants even though glycogen often accumulates at the base of the cell. This polar arrangement is most obvious in the base of the stem, the distribution being more uniform in cells of the apical parts where the growth zone that expresses the gravitropic response is located. Moreover, he states that the glycogen is not displaced even by inverting for several hours. Sections were treated with iodine+potassium iodide to stain the glycogen for these observations and Borriss (1934) describes other structures in these sections, which only become apparent on the addition of I/KI, that he claimed could be involved in graviperception. 'From about the 3rd stage on, there appear in the apical stipe cells particles of plasma, which are situated in about the centre of the cell, and are connected with the marginal protoplasm layer by threads of plasma on all sides. This central accumulation of plasma often contains abundant glycogen...' and 'could exert pressure and tractive force by the agency of the plasma threads, and thereby bring about an orientation. Of course, with this hypothesis we are dealing

purely with a supposition, but the picture is so striking that one is led directly to ideas of this kind' (Borriss, 1934).

The other account of a cytological examination of gravitropism in higher fungi seems to be the following, which appears in Gooday (1985): 'The cytology of geotropism of stipes of *Coprinus cinereus* was investigated by the late G. H. Banbury (personal communication). Excised stipes of 10–50 mm that had been laid on their side in a moist atmosphere raised their apices as they elongated. Examination of these cells by light and electron microscopy of transverse thin sections showed that when horizontal, the distribution of cell contents was displaced so that the vacuole occupied most of the upper part, and the cytoplasm was concentrated in the lower part'.

These two accounts appear to be *the only* reports of any cytological examination of gravitropism in hymenomycetes. Although more attention has been devoted to the sporangiophores of *Phycomyces*, the cytology has also been neglected and it remains the case that no sensory apparatus has been identified. The most likely suggestions are those made by Dennison (1961) on the basis of his experiments with sporangiophores of the phycomycete *Phycomyces*. Dennison conducted centrifuge experiments, with sporangiophores submerged in a buoyant fluid, identified two separate sensory systems; a transient response extending over about 5 min and the long term response – the 'normal' geotropic response. As the direction of the transient response was reversed in the buoyant fluid it was concluded to have no direct connection with gravity but to be a by-product of mechanical forces, probably exerted on the cell wall, arising from the action of gravity on the sporangium (such forces are reversed in direction between experiments in air and in a dense buoyant fluid). This was subsequently shown to be response to a stretch receptor (Dennison & Roth, 1967). The long term geotropic response was not changed in direction in experiments in the fluid, which Dennison concluded to mean that the sensory system was intracellular. He suggests the sensory mechanism may involve particles or inclusions within the cell which differ in density from their surrounding medium. Noting that protoplasm and vacuole constitute two liquid phases differing in density, he suggested that the denser protoplasm should occupy the 'lower' side of the cell and that '...thickening of the protoplasmic layer causes a more rapid elongation of the adjacent wall'. Despite its age, this remains the only suggestion for a gravireceptor mechanism in fungi. Dennison & Shropshire (1984) provided the first evidence (at the light microscope level) of an intracellular reorganization in response to reorientation with respect to the normal gravity vector, concluding 'The existence of protoplasmic asymmetry is a plausible first step in the gravi-



receptor stimulus-response chain...' and '...the gravireceptor mechanism...must involve the rearrangement of intracellular liquid phases of differing density, specifically the protoplasm and vacuole'. In relation to current models of wall growth this would be an attractive explanation for greater expansion growth on one side of the hypha rather than the other side. If the vacuole floats upwards it could in consequence so restrict the volume of protoplasm adjacent to the upper wall surface as to channel a greater concentration of chitosomes and other peripheral vesicles into the lower part of the cell. Thus, there would be a greater potential for wall expansion in the lower regions of the cell. This is good explanation of a response to severe change in orientation and it is also attractive in that it does not require any special mechanisms for either perception or response to gravity and could consequently apply to any fungal cell which (a) has some capacity for wall growth and (b) has a vacuole. Formation of a vacuole and, indeed, control of its density through, perhaps, regulation of water flow across its membrane could then be part of the regulatory mechanism which determines that fungal cells (of different sorts or of different ages) differ in their response to gravity. It is more doubtful whether such a mechanism would have the sensitivity to direct correction of small changes in orientation, and how it could be linked to morphogenetic control.

#### COMMUNICATING SIGNALS IN MUSHROOM FRUIT BODIES

Tropisms result from differential growth and it seems to be generally assumed that tropic bending of such structures as the mushroom stems are conditioned by uneven distribution of growth regulators, an idea which was probably introduced by Buller (1934) and Borriss (1934). Comparison of the upper and lower parts of the gravitropically bending mushroom stem seems to be the best and experimentally most accessible candidate for investigation of a true growth hormone and is a simpler comparison to make than the comparison between cap and stem which has been the principle experimental system used in studies aimed at detecting fungal growth hormones. Over the years there have been frequent claims of experimental evidence for growth hormones in mushroom fungi. Many reports

lack numerical data and adequate controls, but the failure to establish any consistent mechanistic model from even the best documented of these accounts is disturbing, especially in comparison with the success achieved in the parallel (and contemporary) search for, isolation, characterisation and commercial exploitation of plant growth hormones. Many of the experimental approaches have relied on the observation that partial removal of the cap often results in curvature of the stipe with the greatest amount of stipe growth occurring under the remaining cap. Indeed, the one consistent 'article of faith' which has derived from all of this work is that growth of the stipe depends upon the continued presence of the cap. This has always been taken to imply that cap tissues produced growth hormone(s). Much of the literature has been reviewed by Gruen (1982).

Hagimoto & Konishi (1959) found an association between gill segments remaining on a surgically modified cap and the bends of the stem in *Agaricus bisporus*. Gruen (1963) removed halves of caps of young fruit bodies of *A. bisporus* and found the stems bent by 135° in 8 d, the bend was always directed towards the removed part of the cap. Gruen concluded that gills are the centre of regulation of the growth of the stem, possibly producing a 'growth factor' which is not formed in other parts of fruit bodies. In more extensive experiments of the same type with the same species, Hagimoto (1963) showed that there is a reliable correlation between the position of the gills and bending of the stem and claimed that there are two independent centres of regulation of the gravitropic bends of the stem; one in the gills, the other in the stem.

The literature divides hymenomycetes into two groups according to their dependence on the cap for growth of the stem. In *A. bisporus* and *Flammulina velutipes* the influence of the cap extends through the whole period of development. Removal of the cap or gills stops growth of the stem, and its gravitropic reactions are weakly expressed (Gruen, 1963, 1969, 1982; Hagimoto, 1963). Experiments with *Coprinus* spp. (Borriss, 1934; Eilers, 1974; Gooday, 1974; Cox & Niederpruem, 1975) indicate that the cap is necessary only at the initial stages of the development. After the stem reaches  $\frac{1}{8}$  to  $\frac{1}{4}$  of its normal length, it can continue growth and show gravitropic responses without the cap. A zone of active growth is in its middle part which can expand to 3–4 times of its initial length even after excision; neither the top

**Figure 1.** Some basic characteristics of the gravitropic response of the basidiomycete *Coprinus cinereus*. The illustration shows a series of stills from a time-lapse video recording of events which follow after a culture is placed on its side. Each image is identified with the time of day (h:min:s). This culture bore three fruit bodies at about the same stage of development when the experiment was started just before 13.41 h. A segment of cap tissue was removed from the upper one to reveal the stem, the entire apex (cap + stem) was removed from the central one and from the lower fruit body the entire cap was removed, to be replaced by an 'artificial cap' made from an equal weight of 'Blu-Tack' (this specimen was dislodged at 20.49). Note that the first response to reorientation is that the stem sags (uniformly over its length) under the weight of the cap. A positive gravitropic response is evident within 1 h; this does not require the fruit body apex or the cap and the resultant growth curvature extends over a considerable length of stem.

nor base of an isolated stem have such an ability (Cox & Niederpruem, 1975). Cox & Niederpruem (1975) incubated excised stems vertically upside down, and found they bent around in a U-shape so that the stem apex again pointed upwards. Cutting off the apex made no difference to the response and when three separated segments were so treated, only the middle part of the stem exhibited a gravitropic reaction. Clearly, neither the cap nor the stem apex is required for gravitropic bending of the stem (Fig. 1).

Numerous studies seem to suggest that extracts or diffusates of the cap can have growth stimulatory effects on the stipe. Disappointingly, the only chemical candidates which have been extracted are various amino acids and ammonium salts (Konishi & Hagimoto, 1962; Konishi, 1967). Although such compounds have been shown to have specific inhibitory effects on sporulation (Chiu & Moore, 1988*a, b*), they divert growth between different routes of differentiation rather than enhancing or inhibiting it. It is also difficult to believe that such simple components of primary metabolism would be employed as *specific* growth hormones by a group of organisms which exhibit enormously versatile and varied ability to synthesise secondary metabolites in other areas of their activity (Claydon, 1985) extending even to the production of plant growth hormones.

The evidence concerning hormones in higher basidiomycetes is confused, conflicting and fragmentary. Lilian Hawker wrote in 1950: 'It is desirable that research should be directed towards an interpretation of tropisms in fungi based on the study of growth-regulators. At present nothing is known of any mechanism in fungi comparable to the redistribution of auxins in the higher plants.' Sadly, this is still true 40 years later.

However, Jeffreys & Greulich (1956) reached the conclusion from their experiments that 'The results suggest, not only that auxin is not involved, *but also that no other hormone is involved*. It seems likely that each hyphal strand responds individually to environmental factors. Because the strands are aggregated, this results in a unit action by the stipe' (the stress is mine). There are two other papers (Banbury, 1962; Gorovoj *et al.*, 1989) which contain statements which seem to reveal the author's conviction that the apparently coordinated expression of gravitropic response is in truth a common but independent response by the individual component hyphae of the structure concerned. I would not go so far as to say that there are no growth hormones in fungi but I do suspect that by concentrating attention on growth hormones, we are not necessarily looking at the right mechanism of growth co-ordination in fungi. It has been remarked before that lateral communication does not seem to be provided for in fungal tissues (Reijnders & Moore, 1985), there being no direct lateral connections between cells as there are in

animals and plants. Yet it is abundantly clear that cell and tissue patterns are established during development of fungal multicellular structures. Many of these patterns are akin to others found in animals and plants, and like them, tissue patterns in the mushroom fruit body are probably best interpreted as arising through the activity of 'morphogens' acting at a distance (Moore, 1984, 1988; Reijnders & Moore, 1985), which may therefore be viewed as short-distance growth hormones. The origin of organizing centres for the gill plates in *Coprinus cinereus* has been shown to depend on their spatial arrangement (Rosin & Moore, 1985; Moore, 1987) in such a way that two neighbouring centres are able to inhibit the formation of a third centre between them until their radial growth into the expanding fruit body separates them by a distance which releases undifferentiated tissue between them from the inhibitory influence emanating from the existing organizing centres. There is, in other words, a morphogenetic field around the gill organizing centre within which new centres cannot form. Similarly, the distribution of the specialized cystidial cells in the spore-bearing hymenial tissues of *C. cinereus* has been shown to be non-random. Differentiation of a cystidium being influenced by pre-existing cells of the same type (Horner & Moore, 1987). Again, a morphogenetic field seems to exist around cystidia, extending to a radius of about 5 cell diameters. Similar considerations probably also apply to the non-random distribution of the hyphae which give rise to sphaerocysts in the stem of *Lactarius rufus* (Watling & Nicoll, 1980).

These observations imply that localised patterning of tissues might be *explained* in terms of the asymmetric distribution of some chemical (the morphogen or inducer) which thereby extends the influence of the morphogenetic centre over the surrounding hyphae. In the specific cases referred to above the morphogenetic fields extend only over short distances – in the 20–100  $\mu\text{m}$  range. It is important to appreciate that this is only a plausible explanation. In no case has a morphogen been extracted and characterized. At the other end of the developmental scale there is evidence for a variety of chemicals and extracts which have the ability to induce or enhance fruiting in higher fungi. These include zeralenone (Nelson, 1971; Wolf & Mirocha, 1973), extracts from *Agaricus* (Rusmin & Leonard, 1978) and cerebrosides (Kawai & Ikeda, 1982) which induce fruiting in *Schizophyllum commune*, phenolic lactones extracted from sexually deficient strains of *Aspergillus nidulans* (Champe & El-Zyat, 1989), and extracts of *Pyrenopeziza brassicae* which influence ascocarp development in that organism (Siddiq *et al.*, 1989). The mode of action of such compounds as these is unknown and, indeed, their true significance must remain in doubt until their chemistry is properly established. Application of even simple



nutrients like ammonium salts can induce fruiting in some species (Morimoto, Suda & Sagara, 1981) so it is important to know the effect exerted by fruit-inducing extracts on the medium as well as their primary effect on the organism. Another example of a morphogenetic effect which might be chemically mediated is the interaction between *Armillaria mellea* and *Entoloma abortivum* in which the former disturbs the developmental pattern of the latter, resulting in arrest in various stages in development of the agaricoid fruit bodies of *E. abortivum* and production of carpophoroids, which are aborted, hypertrophied or otherwise abnormal fruit bodies (Watling, 1974). Presumably *A. mellea* either produces or destroys some extracellular chemical signal which is required for normal development of *E. abortivum* to proceed.

There is clear reason to believe that cell and tissue patterns in hymenomycetes may depend on locally-active, diffusible morphogens. Yet the search for fruit-inducing substances and for growth hormones is considerably complicated by the extensive 'metabolic commerce' which must take place in the one case between the mycelium and its substratum and in the other case between the cap and its supporting stem. Whatever might be believed about the presence of growth hormones, the wide range of experiments which have been published clearly imply that there is some level of co-ordination in the response which fruit bodies make to reorientation in the gravitational field and to surgical removal of part or all the cap. Interplay between cap and stem must involve such extensive exchange of 'general purpose' metabolites that some are bound to have quite fortuitous effects on growth patterns if extracted. Distinguishing these from true growth hormones is a task which has not yet been accomplished. A gravitropically-responding stem seems to be a far better candidate for a 'morphogen hunt'. If the difference in growth pattern between the upper and lower halves of the stem really is due to lateral diffusion of a morphogen/hormone it should be easier to distinguish this by comparison of two halves of an otherwise identical structure than it is to distinguish active agents in, for example, extracts of cap tissues.

Another point worth making is that no alternative to the hormonal interpretation seems to have been entertained, even as a minor element of the phenomenon of control of stem growth. In particular, no attention has been given to the potential of the mass of the cap as a formative feature. One reason why this might be considered significant is the demonstration by Magnus (1906) that restriction of fruit body expansion leads to additional stem elongation. Buller (1931) loaded weights on to elongating fruit bodies of *Coprinus sterquilinus* and found that one, with a stem cross sectional area of only 29 mm<sup>2</sup>, lifted over 200 g. The ecological significance of this is ably (if rather extremely) illustrated by the ability of *C. atra-*

*mentarius* to erupt through an asphalt path (Gooday, 1985) and other fruit bodies to lift paving stones (discussion in Buller, 1931, p. 120). In other words, since the fruit body is likely to emerge beneath leaves, twigs and other litter, or beneath surface pans of caked mud and soil then there would be selective advantage in linking compression of the stem with enhanced growth of that organ.

Significantly, Dennison & Roth (1967) have shown that the sporangiophore of *Phycomyces* responds in just this fashion; stretching causing a five-minute interval of slower growth and compression (experimentally achieved as a partial release from stretch) a similar interval of faster growth. Although *Phycomyces* sporangiophores adapted to changing load so that the growth rate had returned to its previous steady-state value within about 50 min, these experiments show that the hyphal growth mechanism can respond to applied forces. If each of the hyphae making up the stem of a mushroom fruit body behave similarly then considerable growth co-ordination could be achieved simply through the mechanical links between its different parts. In gilled fungi this could have a counterpart if a 'pendulum' effect of gills in disturbed fruit bodies caused differential growth or expansion of the 'upper' hymenial layer in order to bring them back to the vertical. The implication is that experimental treatments involving complete or partial removal of the fruit-body cap (the class test for 'hormonal effects') will inevitably promote lessened growth of the stem because by lessening the weight the stem has to bear its hyphae will be relieved of some of the compression they would otherwise experience. If only half the cap is removed, then stem hyphae beneath the half which remains will still be under compression and will grow relatively more than those in the neighbouring part of the stem. Consequently the stem will curve towards the side from which the cap has been removed.

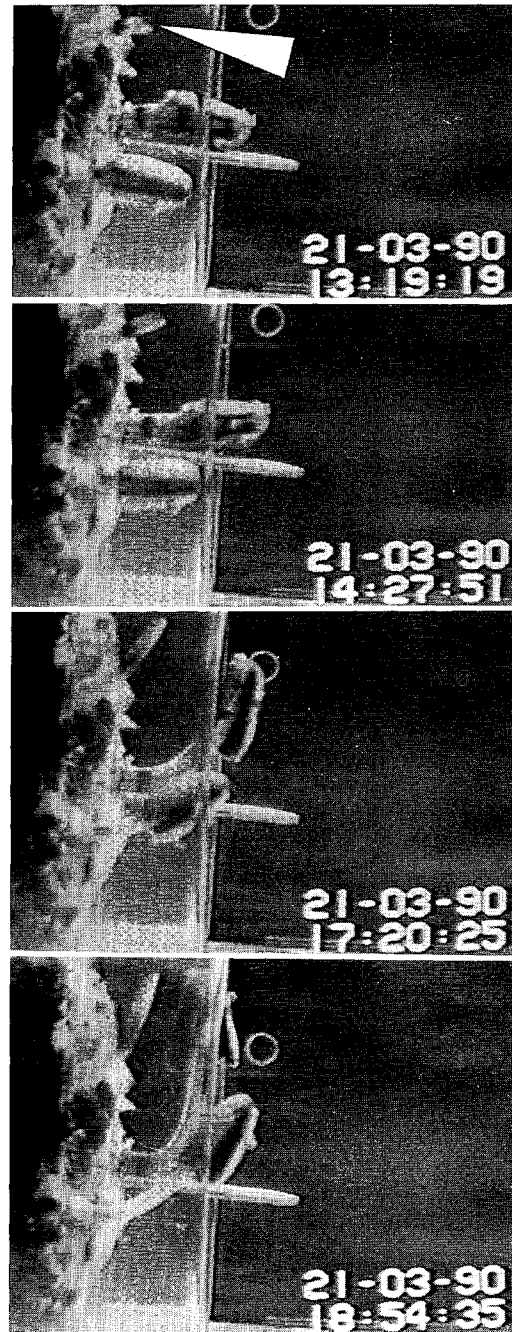
Unlike the individual cells of animals and plants, the basic building block in the fungi is the continuous fibrous tube of the hypha which is ideally suited to service as a tension tie, as a hydraulic conduit and as a means of communicating tension and compression stresses through a structure. If the hyphae making up the mushroom behave like this considerable growth co-ordination could be achieved simply through the mechanical links between its different parts. Recent studies have revealed developmental processes in different mushrooms which seem to rely on hydraulic inflation of tissues on the one hand (in *Volvariella bombycina*; Chiu & Moore, 1990a) and on tensioning tissues into place on the other (in *Coprinus cinereus*; Chiu & Moore, 1990b).

The physical continuity of hyphal elements is a feature unique to the fungi and it is arguable that selective advantage would accrue from its being used in various ways. It may well be that the best

explanation for the communication of growth co-ordinating signals along the length of the stem (or indeed in any fungal tissue in a direction which is in the long axis of the constituent hyphae) is in the physical continuity of the hyphal elements being able to transmit mechanical forces which promote changes in growth kinetics in regions of special stress.

Even some of the experiments which are usually interpreted as providing the best evidence for hormone activity are open to reinterpretation in this light. For example, experiments purporting the interrupt the flow of growth hormones from the producing tissue (cap) to the reacting tissue (one side of the stem) by insertion of mica barriers (Hagimoto & Konishi, 1959, 1960) also break the chain of connected elements which would communicate mechanical forces. So in the same way that geological faults dissipate strain as movement at the fault line, such treatment would convert the compression force which might enhance growth into disruption at the site of the injury.

A general interpretation of graviperception in plants along these mechanistic lines was recently experimentally rejected by Sliwinski & Salisbury (1984) who concluded that 'Stretching or compressing [plant] tissue apparently does not influence its growth rate much if at all'. Many years before, when the same problem was discussed by Darwin (1907) the 'mechanical forces' proposition was discarded on the grounds that plant organs supported along their entire length still respond to gravity. Obviously, the experiments of Dennison & Roth (1967) show that growth rate of the fungal hypha *can* be correlated with stretch and compression forces. On the other hand, there seem to be few experiments in which reoriented fungal fruit bodies have been supported in any way. Certainly, in the most extensive 'classic' experiments of Buller (1909) with '*Psalliota campestris*', *Coprinus plicatilis* and *C. plicatilooides*, Streeter (1909) with *Amanita phalloides* and *A. crenulata*, Plunkett (1961) with *Polyporus brumalis*, and Badham (1982) with *Psilocybe cubensis*, cultures were simply placed on their sides and the illustrations show fruit bodies cantilevered horizontally from the growth medium. In such a situation it is easy to see how the lower side would be under compression and the upper in tension so that the distribution of mechanical forces within the stipes could co-ordinate alterations of growth rate so as to effect 'gravitropic curvature'. The only experiments in which some support was provided seem to be those of Street (1909) in which she inserted *Amanita* stems into glass tubes 'held firmly in a horizontal position by wire'; this treatment led to the stem showing an upward curvature beginning at the end of the tube. When support is provided, it seems to accentuate the angle of bending (Fig. 2) as though



**Figure 2.** Effect of mechanical support on the gravitropic response of *Coprinus cinereus*. As in Figure 1, a culture bearing three fruit bodies at about the same stage of development was placed on its side. The cap was removed from the upper fruit body (arrow), the central fruit body was provided with a mechanical support; the lower fruit body was left as a control. Note that growth curvature was far more acute in the supported fruit body, and that the cap-less stipe shows an intermediate level of curvature. This implies that mechanical stresses caused by the weight of the fruit body modulate the gravitropic response. However, note especially that the supported fruit body had lifted clear of its support within the first hour. This suggests that gravity *perception* may be integrated with mechanical information at the time of first exposure so as to adjust a growth response which is not fully expressed until 5 h later.

the presence of the support has an immediate and long lasting effect on the initial perception process.

It is unlikely (indeed, one is tempted to say impossible) that the published data relating to gravitropism in mushroom fungi can be explained on the basis of mechanical effects alone, but mechanical effects seem never to have been seriously entertained, even as a contributing factor. It is extremely important to distinguish between aspects of development which are mechanical consequences of other events and those which are due to control by chemical signalling. Yet, the only aspect of what might be called the engineering principles used in construction of the mushroom which has been examined is the relationship between fruit body height, cap diameter and diameter of the stem. Ingold (1946) and Bond (1952) used published illustrations of a wide range of agarics to extract graphical relationships between these features and arrived at the conclusion that smaller fruit bodies have proportionately longer and more slender stems. Watling (1975) arrived at a different graphical representation for the Bolbitiaceae and indicated that more detailed analysis could not be done using published collections of illustrations which were selective in their inclusion of species and in their descriptive boundaries of species. Watling (1975) took measurements from fresh specimens and pointed out that divergences from any general formula reflect habit characters and habitat preferences. Such analyses are a useful start. They indicate that meaningful information can be extracted from relatively simple measurements, but they also indicate some pitfalls. Only facile generalizations can be expected if measurements of different species are combined; the analyses cannot be divorced from developmental observations; and such dimensional measurements have very limited value unless they are accompanied by measurements of the structural characteristics of the tissues and their components.

Current techniques should allow analysis of mushroom fruit body structures both as static and as developing entities using light and electron microscopy, time-lapse film and video and computer-aided image analysis to simplify microscope and video images statistically to produce representative drawings of each part of the fruit body at each stage of development. It should be possible to show how the structure responds to conditions (like re-orientation in the normal gravitational field, etc) outside its 'design criteria'—i.e. the stresses of altered gravitational and other forces. The mushroom structure could well be a convenient model to investigate engineering problems on the millimetre and sub-millimetre scale ranges.

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