



GRAVIRESPONSES IN FUNGI

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ABSTRACT

Although the orientation of mycelial hyphal growth is usually independent of the gravity vector, individual specialised hyphae can show response to gravity. This is exemplified by the sporangiophore of *Phycomyces*, but the most striking gravitropic reactions occur in mushroom fruit bodies. During the course of development of a mushroom different tropisms predominate at different times; the young fruit body primordium is positively phototropic, but negative gravitropism later predominates. The switch between tropisms has been associated with meiosis. The spore-bearing tissue is positively gravitropic and responds independently of the stem. Bracket polypores do not show tropisms but exhibit gravimorphogenetic responses: disturbance leads to renewal of growth producing an entirely new fruiting structure. Indications from both clinostat and space flown experiments are that the basic form of the mushroom (overall tissue arrangement of stem, cap, gills, hymenium, veil) is established independently of the gravity vector although maturation, and especially commitment to the meiosis-sporulation pathway, requires the normal gravity vector. The gravity perception mechanism is difficult to identify. The latest results suggest that disturbance of cytoskeletal microfilaments is involved in perception (with nuclei possibly being used as statoliths), and Ca^{2+} -mediated signal transduction may be involved in directing growth differentials.

INTRODUCTION

Fungi have been confused with plants for most of this century (and before) and this has led both to diminished effort on fungal research and to the misguided expectation that what is known about plants should be applicable to fungi. Recognition that fungi belong to a completely separate Kingdom of organisms /1, 2/ and, ironically, recent evidence that fungi may be more closely related to animals than to plants /3/, has left many aspects of fungal biology underdeveloped and under-researched. Inevitably, therefore, the problem of understanding how fungi respond to gravity is further from solution than is the analogous problem in either animals or plants.

In the fruit bodies of the higher fungi, response to gravity is crucial for proper spore liberation. The most characteristic spore distribution structures of the basidiomycete fungi are the mushrooms and toadstools. Spore release in these organisms is intolerant of water so the spore-bearing tissue must be protected from the rain. Effectively, the mushroom is an umbrella and the spore-bearing surface must be on the lower side of the cap. Spores must fall vertically between the spines (hydnums) and gills (agarics), or through the tubular pores (polypores) to escape from the cap for dispersal on air currents. All of these deceptively simple descriptive statements depend upon detection of gravity and modulation of growth processes by reference to the direction of the gravity vector. The 'umbrella' fruit body must grow *upwards*; the spore-tissue must be on the *lower* surface; and spines, gills or pores must be *vertical*. For this positional patterning to be achieved, morphogenesis must be referenced to the gravity vector.

The most easily studied response to gravity in fungi is gravitropism - directed growth towards (+ve) or away (-ve) from the stimulus - and the most obvious gravitropic reactions were

described at the end of the last century /4-7/, establishing the basic facts that agaric stems are negatively gravitropic and the gills positively gravitropic. More experimental work was done in the early years of this century /8-13/ and detailed study has now been resumed after a gap of almost 25 years. Though interesting in its own right, the gravitropic response is a simple developmental pattern-forming process. Its control demands that the organism has a gravity perception system and a means of coupling this to control differential tissue growth. Study of gravitropism is therefore a natural, non-invasive means of generating a particular morphogenetic change in a specific location. In the most recent research, gravitational biology is being used as a tool to study fundamental aspects of cell and developmental biology. Understanding the cell biology of gravity perception mechanisms could well contribute to a general understanding of sensory mechanisms, signal transduction and growth control processes.

Most of the research bears on tropic responses to gravity, rather than on morphogenetic responses, but some gravimorphogenetic responses are known, too. For example, the apple-scab pathogen, *Venturia inaequalis*, over-winters in fallen leaves and over 90% of the fruiting structures are directed towards the leaf surface that faced upward during winter /14/. In a similar phenomenon, the polypore *Phellinus contiguus* normally forms its fruiting bodies on the lower surfaces of tree branches, and in Petri dish cultures incubated mycelium-side up only disorganised masses of tissue are produced, but normal fruit bodies appear when the dishes are inverted and incubated mycelium-side down /15/.

FUNGAL GRAVITROPISM

These above-mentioned gravimorphogenetic phenomena have never been subjected to research under microgravity conditions, nor even with clinostats. An even more remarkable 'lost opportunity' in mycological gravitational biology is that no space experiments seem to have been done with *Phycomyces* sporangiophores. Sporangioophores of *Phycomyces* are individual (though large) hyphal tips which exhibit show a number of interacting tropisms and avoidance growth-responses which have been analysed in great detail /16-20/. As early as 1961, Dennison /21/ used a centrifuge (with accelerations up to 4.35 g) to conduct experiments with sporangiophores submerged in a buoyant fluid in order to distinguish two separate sensory systems; a transient reaction extending over about 5 minutes and the long term, 'normal', gravitropic response. As the direction of the transient response was reversed in the buoyant fluid, it was concluded 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 a response to a stretch receptor /22/. Dennison /21/ concluded that the gravity sensory system of *Phycomyces* was intracellular. He suggested that 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.' Unfortunately, the cytology has been neglected. Dennison and Shropshire /23/ provide the only evidence (but only at the light microscope level) of an intracellular reorganization in response to reorientation of the sporangiophore, concluding 'The existence of protoplasmic asymmetry is a plausible first step in the gravireceptor stimulus-response chain...' and '...the gravireceptor mechanism ... must involve the rearrangement of intracellular liquid phases of differing density, specifically the protoplasm and vacuole'. Channelling of chitosomes and other peripheral vesicles into the lower part of the cell would indeed be an attractive explanation for greater expansion growth on one side of the hypha rather than the other side in relation to current models of wall growth, although it is doubtful that such a mechanism would have the sensitivity to correct small changes in orientation. However, because of the astonishing absence of cytological observation and experimentation, it remains the case that until very recently this was the only published suggestion for a gravireceptor mechanism in fungi.

Despite the fact that the above-described experiments were contemporaneous with developing space flight opportunities, *Phycomyces* has never been flown. However, there have been a variety of space flight and clinostat experiments with much larger fungal fruit bodies (for reviews see Molitoris /24/; Moore /25/; Kern and Hock /26/). Ground-based experimentation was

recently resumed after a gap of almost 25 years with studies of gravitropic bending in *Coprinus cinereus* and *Flammulina velutipes* mushroom stems /27-30/.

Experiments with *F. velutipes* were flown on the D-2 Spacelab mission /31/. Although the cultures fruited extremely well and many samples were returned for analysis, only preliminary results have so far been published /32/. However, mushroom cultures of a different sort, of the polypore *Polyporus brumalis*, have been flown on a number of Salyut missions /33/, and this species, together with *C. cinereus* and some others, has been the subject of experimentation with clinostats /25, 27, 34, 35/. The indications from both clinostat and spaceflown 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. Abnormalities of stem growth have been observed in clinostat cultures of *Panus* (= *Lentinus*) *tigrinus* and *P. brumalis* /34-36/ and the D-2 cultures of *Flammulina* /32/, but the morphogenetic event which seems most dependent on gravity is sporulation (see below).

Other fungal experiments with a bearing on morphogenesis have concerned the persistence of diurnal cycles of conidium formation in *Neurospora crassa*. The conidiation pattern generally persisted in microgravity but there were strain differences. One strain had a relatively robust rhythm though its period increased by several hours and alterations in morphology occurred. Another strain gradually lost its rhythm /37, 38/. These findings suggest that sensitivity to the gravitational field may have a genetic component which could account for cases where different responses are observed in different experiments with the same species.

GRAVIMORPHOGENETIC EFFECTS

Polarity of the primordium, the relative positions of stem, spore-bearing and cap tissues and the initial distribution of agaric gills, are all aspects of early mushroom morphogenesis which *might* be influenced by gravity, but these could all equally well be controlled by non-gravitational cues. The obvious experimental approach is to remove suspected cues and study the progress of events in their absence. Unfortunately, the influence of gravity can be escaped only in orbital laboratories, although a number of earthbound devices can help. The clinostat distributes the effect of gravity around its axis of rotation, to some extent simulating hypogravity - a reduced gravitational field (though actually being an omnilateral as opposed to the usual unilateral gravity exposure), whereas centrifuges permit increase in the apparent gravitational field (actually centripetal acceleration) - the hypergravity experience.

Clinostat experiments

Hasselbring /12/ investigated the effect of growth on a clinostat on development of *Polystictus* (= *Pycnoporus*) *cinnabarinus*, *Schizophyllum commune* and *Coprinus* spp. and Reijnders /39 [p. 316]/, Plunkett /40/ and Badham /41/ also report clinostat experiments. Unfortunately, most of these are of very limited value because the authors report only selected results ('... only a few gave results ...' /12/; 'The results given relate to cases where no obvious arrest to development occurred' /40/. However, Hasselbring reports formation of spore-bearing tissue over the whole surface of clinostated fruit bodies of *Pycnoporus cinnabarinus* and *S. commune* and Plunkett /40/ states: 'In the two fruit-bodies studied extremely shallow hymenial tubes, 1 mm. deep, were produced but developed no further.' These are the first reports of morphogenesis being disturbed by absence of a unilateral gravity vector.

The carefully executed clinostat experiments of Gorovoj *et al.* /34/ used *Polyporus brumalis*, *Panus* (= *Lentinus*) *tigrinus* and *Coprinus cinereus* and showed a consistent absence of the tubular spore-bearing tissues in fruit bodies of *Polyporus brumalis* arising on cultures grown on the clinostat. A series of five experiments were performed, each with 4 replicate cultures, but in no case was any sign observed of the typical tubular spore-bearing tissue, or even of the network of 'dissepiments' (which are the primordial rims of the tubular spore-bearing tissue) which initiates it. In some experiments, Gorovoj *et al.* /34/ placed samples on the clinostat with fruit bodies in different stages of development, clinostating being continued until mature fruit bodies were obtained. In those in which cultures consisted only of mycelium at the start, the

mature fruit bodies had a smooth spore-bearing surface; in the second series of cultures which bore fruit body rudiments up to 5 mm in size when transferred to the clinostat, the mature 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, comprising cultures which initially bore fruit bodies whose caps had begun to differentiate, the mature fruit bodies had a network of dissepiments on the lower surface of the cap. However, formation of the normal tubular spore-bearing tissue did not occur in any of the samples. These results parallel those obtained by the earlier workers and imply that the unilateral gravity vector is required for formation of the tubular spore-bearing tissue. Microscopically, there were no major anatomical differences in the structure of fruit bodies grown on the clinostat compared with control, but the spore-bearing tissue in clinostat cultures stopped development at the dikaryon stage. Karyogamy was rarely observed and basidia with spores were infrequently seen.

The situation was quite similar in *C. cinereus* grown on the clinostat, as no normal, spore-yielding, fruit bodies were obtained. However, in *C. cinereus*, clinostat growth did not impair progress through the initial stages of development: all the main parts of the fruit body - cap, stem, spore-bearing tissue, veil - were formed, but all remained underdeveloped. As in *Polyporus*, it seems that the unilateral gravity vector is required for normal progress through meiosis and sporulation; in its absence *Coprinus* fruit body development stops prior to spore formation.

In contrast, the greatest influence of the clinostat on *Lentinus tigrinus* was on morphogenesis of the stem. This formed numerous branches rather than a cap. Thus, it seems that in this organism absence of a unilateral gravity stimulus results in loss of polarity and apical dominance. The device used by Gorovoj *et al.* /34/ incorporated a centrifuge able to apply centrifugal accelerations of 1 and 4.5 g. Exposure to hypergravity, though, did not greatly affect fruit body morphogenesis either macroscopically or microscopically and the normal gravitropic reactions occurred.

Experiments in orbit

Test-tube cultures of *Polyporus brumalis* were flown during an orbital space flight aboard the unmanned Soviet 'biosputnik', Cosmos 690 /42/. The Cosmos 690 cultures were in orbit for 20 days during which time they were in the dark. In the absence of illumination, fruit body caps were not formed but the stems of the fruit bodies grew well so this experiment demonstrated that at least the early stages of fruiting could occur in zero gravity. Further experiments were conducted, again with *Polyporus brumalis*, during flights (in 1977-78) of the manned space stations Salyut-5 and Salyut-6 /43, 44/. In the experiment conducted aboard Salyut-5 cultures bearing fruit body rudiments up to 2 mm tall were taken onto the space station and some were incubated with illumination while others were in darkness. All formed fruit bodies, those in the dark failed to form caps, as expected; those grown in the light were similar to normal and had a tubular spore-bearing tissue, although the hymenium was restricted to the bottom of the tubes /44/. In the experiment on Salyut-6, cultures were delivered to the space station bearing mycelium only. Again, cultures were incubated in weightless conditions either in the light or dark. Fruit bodies were formed in the light, but most of the fruit bodies had no spore-bearing tissue and little hymenium. No fruit bodies were formed in the dark although in dark-grown controls on earth fruit body rudiments were formed in the dark. The Salyut dark-grown cultures were shown to be able to fruit when illuminated after recovery, so it seems that some aspect of the fruit body initiation process in the dark requires the gravity vector.

The limited experiments done in orbit seem to support the conclusion from clinostat experiments that it is development of the spore-bearing tissue which is most dependent on the gravity vector. No such effect was seen in the most recent D-2 experiments with *Flammulina velutipes* /32/ which may reflect differences in sensitivity between different species and/or differences in sensitivity according to the stage of development attained at the time of microgravity exposure (see the account of the clinostat experiments of Gorovoj *et al.*, /34/ described above).

GRAVITROPISM IN AGARIC MUSHROOMS

Different groups of hymenomycetes exhibit different gravitropic responses /36/. In most agarics and polypores the spore-bearing tissue may be the main centre of gravitropic growth, with its own orientation mechanism assuring positively gravitropic growth, and possibly exerting some (hormonal?) influence on growth of the stem. Species of *Coprinus* exhibit a different pattern - the gills are not gravitropic and the stem alone is responsible for spatial orientation. Finally, bracket fungi are probably best considered to show gravimorphogenetic rather than gravitropic reactions. After their position is changed the direction of growth changes in the meristemoid zones and only the newly formed parts of the fruit body are vertical. The fruit body as a whole, though, is not gravitropic.

Gravitropism develops as the hymenomycete fruit body grows. The very youngest fruit body initials grow perpendicularly away from the surface on which they arise independently of the direction of light or gravitational signals /5, 8, 9, 40/, a reaction which may be analogous to the avoidance reactions of *Phycomyces* sporangiophores /17-19/. As they develop further, the stems of field mushrooms are generally non-phototropic but show a marked negative gravitropism, whereas the wood-inhabiting and coprophilic species are often both phototropic and gravitropic /40/. Usually, an initial period of light-seeking growth in the earliest stages of development is followed by negative gravitropism. Gravitropism of the stem provides the 'coarse' adjustment of fruit body position, the 'fine' adjustment is done by the positively gravitropic spore-bearing tissue.

Buller /9, [pp. 51-52]/ showed that all the gills of the common Field Mushroom *Agaricus campestris* could readjust to the vertical providing the cap was not tilted beyond 30°. These responses can be quite rapid. Buller /9, 10/ detected the gravitropic adjustment of the gills of *Agaricus* within an hour and states: 'If one turns a [*Coprinus*] fruit-body from a vertical to a horizontal position, the stem begins to turn up the pileus within about three minutes after first receiving the geotropic stimulus' /9, [pp. 69-70]/. At the other end of the spectrum, the responses can be maintained for very long periods. In bracket polypores the tubes often extend vertically downwards for considerable distances and as many of these fruit bodies are perennial, this gravitropic growth may continue for many years /10, [p. 112]/.

Recent research

We have used video recording and computer-based video-image analysis to complete the first kinetic analysis of gravitropism of the stems of the mushroom fruit bodies of *Coprinus cinereus* /28/. Completion of meiosis in the cap coincides with the stem becoming competent to react gravitropically. The gravitropic response in this species is rapid, being evident within 30 min of reorientation. The bend is initiated near the apex of the stem, it then becomes more acute and progresses basally, traversing 40% of the initial length of the stem. Gravitropic bending is most likely the result of asymmetric distribution of growth /45/, as represented by cell expansion, being stimulated by a diffusing, extracellular growth factor produced by the apical region of the stem. Bending raises the apex, and as this approaches an angle of about 35° to the horizontal another process, which we call curvature compensation, begins to adjust the degree of bending so that the apex can be brought exactly vertical. Approximately 90% of bending is compensated (i.e. effectively reversed) to bring the stem to the vertical. This curvature compensation requires that the apical region is free to move towards the vertical (when the apex is restrained to the horizontal, the stem can bend into a complete circle). Bending and curvature compensation appear to be quite separate processes and the mechanism involved in curvature compensation is uncertain, but it may entail a second diffusing signal. The gravitropic responses of *Flammulina velutipes* are quite similar /30/, though the rate of response of this species is very much slower than *Coprinus*, and bending is restricted to a very narrow reaction zone just beneath the junction between cap and stem. As in *Coprinus*, though, free movement of the stem apex is essential for its adjustment back to the vertical; when the apex is secured at right angles to the gravity vector the stem bends well beyond 90° /30/.

What is 'the apex'? Large segments of the apical part of the stem can be removed without affecting either the ability of the remaining stem to show gravitropic bending or its ability to

compensate the curvature so induced and adjust to the vertical. Apical segments 10, 20 and 30 mm long have been removed from 50-60 mm stems prior to their being laid horizontal, and even when more than half of the stem was removed the remaining segment usually responded gravitropically and usually adjusted to the vertical; i.e. curvature compensation, as well as gravitropic bending, occurred normally in these specimens /29/. The only consistent influence of apex removal was on the timing of the first visible gravitropic response (= reaction time): the greater the portion of stem removed, the longer it took for a response to gravity to be observed. One way of accounting for this might be to suppose that although the whole of the upper half may contain hyphal cells able to produce the gravitropic 'reaction', the proportion of such gravitropically competent cells is successively reduced in zones further from the true apex.

What makes stems bend? Work is in progress at the moment on a morphometric analysis of the cell patterning which achieves the gravitropic curvature /45/. *Coprinus* stems contain two populations of hyphae: narrow and inflated /46/. During normal vertical growth of *Coprinus* stems, there is evidence that inflated hyphae inflate further and that the proportion of narrow hyphae declines as the stem grows from 45 to 70 mm, indicating that normal vertical stem extension involves both an increase in cross-sectional area of inflated hyphae and recruitment of narrow hyphae into the inflated population. Similar analyses of the upper and lower regions of the bent zones in gravitropically-responding stems show convincingly that neither cell cross-sectional area nor cell-size population structure changes during bending, there is a change only in cell length /45/. This suggests strongly that the growth mechanism which causes gravistimulated stems to bend is different from that which generates extension growth. The observations also provide a contrast with plant stems where increases have been recorded in both length and diameter during gravitropic bending /47/. In *Flammulina velutipes* the upper half of a bending stem extends at a lesser rate than normal /30/, this amplifying the bending moment caused by the enhanced growth rate of the lower half.

Sensitivity to gravitational stimulation. Although research on fungal gravitropism has been in progress for more than a century, the first report of the use of a clinostat to estimate the presentation time (the minimum gravistimulation time needed to evoke a response) for any fungal gravitropism was published in 1992 /27/. Continuation of this research has shown the presentation time to be 7 minutes. The extent of the gravitropic response, measured as the angle of the stem apex at maximum curvature, was dependent upon the gravitational exposure time (Hatton, 1993). The reaction time did not depend on exposure time and exposures were not additive. An unexpected feature of clinostat experiments with *C. cinereus* fruit bodies is that stems placed on the clinostat after various gravity exposure times 'relaxed' by 5° immediately after reaching maximum curvature /48/. This relaxation process has parallels in the 'tropic reversal' of *Phycomyces* sporangiophores /49/, 'springback' of plant roots /50/, 'spatial memory' of maize coleoptiles /51/ and 'autotropisms' of seedlings /52, 53/. Such similarities suggest that in all these otherwise very different cases, gravitropic bending has an initial, reversible, phase of plastic bending which is followed by a 'fixation' phase if the tropic stimulus has been maintained but which relaxes if the tropic stimulus has been removed. Overall, these clinostat experiments suggest (i) that the gravitropic impulse is an 'all-or-nothing' signal in *Coprinus cinereus*, (ii) that sustained exposure to the unidirectional gravity vector is necessary for the normal gravitropic response, (iii) that perception and response probably occur in the same tissue regions, and (iv) that gravitropic bending is a two-stage process with an initial, reversible, phase of plastic bending.

THE PROBLEM OF PERCEPTION

Perception of gravity is potentially quite different from perception of other stimuli because there is no gravitational gradient. The perception system must depend on gravity establishing an asymmetric distribution of matter within the organism. Gravity-induced asymmetries might 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 tissue.

Little work has been done to establish the location and nature of the structures responsible for perception of gravity in hymenomycetes. Borriss /54/, described '... particles of plasma, ... situated in about the centre of the cell ...' in *Coprinus lagopus* (= *C. cinereus*?). Gooday /55/ states that 'The cytology of geotropism of stems of *Coprinus cinereus* was investigated by the late G. H. Banbury (personal communication) ... light and electron microscopy ... showed that when horizontal, the distribution of cell contents was displaced so that the vacuole occupied most of the upper part, and the cytoplasm ... the lower part.' These two accounts appear to be **the only** reports of any cytological examination of gravitropism in mushrooms prior to 1990.

Control of cell calcium accumulation has been implicated in the gravity perception mechanism of plants. Using concentrations and treatments known to eliminate gravitropism and other tropisms in plant organs, the role of Ca^{2+} in the gravitropic mechanism of *Coprinus* was examined by exposing stems to a Ca^{2+} channel blocker, verapamil; a Ca^{2+} ionophore, A23187; a Ca^{2+} chelator, BAPTA; or calmidazolium, an inhibitor of calmodulin-mediated Ca^{2+} uptake /56/. These inhibitors had no effect on gravity perception but the ionophore (which enhanced stem extension), the chelator and calmidazolium (which had no effect on stem extension) all significantly diminished the gravitropic response. It is noteworthy that the ionophore, A23187, enhanced stem extension growth rate (by 30%) but decreased the rate of response, i.e. rate of bending, by 43%. This suggests that tropic bending may not result from a simple redistribution of the normal growth potential of the stem (see above also). It is concluded that, Ca^{2+} is not involved in gravity perception, but may regulate gravitropic growth differentials via accumulation of Ca^{2+} within a membrane-bound compartment. Treatment of stems with agents disrupting H^+ -gradients, stretch-activated ion channels or microtubule polymerisation had no effect on gravitropism. Treatment with cytochalasin B caused diminished gravitropic bending **and** significantly delayed the response, a result not observed with other inhibitors. This suggests that the cytoskeletal microfilaments may be involved directly in gravity perception.

A similar conclusion has been reached by Monzer and Haindl /57/ who analysed the effects of cytoskeletal inhibitors in the graviresponse of *Flammulina*. Both cytochalasin D and ethyl-N-phenylcarbamate caused partial loss of graviresponse at concentrations of 10^{-6} molar and above. Actin microfilaments in the gravisensitive tissue were positionally correlated with the nuclei, whereas microtubules were mainly oriented longitudinally and located at the periphery of the cell. Nuclear motility in living hyphae was reduced by treatment with 10^{-4} molar cytochalasin D. Kern and Hock /32/ postulate that gravitational displacement is transmitted to the plasma membranes by actin microfilaments of the cytoskeleton, most likely as a result of the nuclei acting as statoliths.

It is interesting that there is now a growing unity of opinion that actin microfilaments are involved in sensing the gravitational vector in individual cells of animals, plants and fungi. Proof of this notion for the fungal system will depend on further basic research on fungal cell biology. We need to know the intimate details of relationships between all parts of the cytoskeleton, the plasma membrane and all the organelles, including the most recently identified highly motile tubular vacuole system /58-60/. This requires a major effort, but study of gravitational responses can make a significant contribution to improved understanding of the workings of the fungal cell.

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