

SPIRAL GROWTH OF MYCELIAL AND REPRODUCTIVE HYPHAE

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Leading hyphae of young (24-36 h) mycelia of *Mucor hiemalis*, *Aspergillus giganteus*, *A. nidulans* and *Neurospora crassa* displayed right-handed (clockwise) curvatures whilst those of *Phycomyces blakesleeanus* displayed left-handed (counterclockwise) curvatures. Agar concentration affected both the percentage of leading hyphae of *M. hiemalis* which curved and their degree of curvature. In older, dense colonies autotropic and/or chemotropic responses obscured the intrinsic tendency of leading hyphae to curve. Stage I sporangiophores of *P. blakesleeanus* and giant conidiophores of *A. giganteus* formed coils when grown in contact with solid surfaces; these coils were formed in clockwise and counterclockwise directions respectively. This result is consistent with the hypothesis that axial rotation of the extension zone wall causes a hypha (reproductive or mycelial) to roll over the surface of a solid with the result that there is a sustained lateral displacement which imparts a curvature to the hypha. The mean radii of the curvatures formed by mycelial hyphae of *M. hiemalis*, stage I sporangiophores of *P. blakesleeanus* and giant conidiophores of *A. giganteus* were 682, 669 and 173 μm respectively.

Spirals of biological origin are relatively common (Thompson, 1942) and under certain conditions fungi (Toaze-Soulet *et al.*, 1978) and bacteria (Rogers, Ward & Burdett, 1978) grow in a helical form. In fungi the term 'spiral growth' has been used to describe the axial rotation of the extension zone wall of sporangiophores of *Phycomyces blakesleeanus* (Oort, 1931; Castle, 1942) and the curvature of mycelial hyphae growing on solid substrates (Madelin, Toomer & Ryan, 1978). The latter type of growth was first observed by Ritchie (1960) who reports that 'instead of growing radially from a point of inoculation, as a fungus mycelium ordinarily does, some hyphae grow with a definite bend'. Mycelial hyphae may curve in either a right-handed or left-handed direction, depending upon the species, giving the colony as a whole a spiral growth pattern which is in a clockwise or anticlockwise direction when viewed from above.

Madelin *et al.* (1978) found that 21 of 157 fungal isolates showed pronounced spiralling in either a clockwise (8) or counterclockwise (13) direction and a further 39 displayed a weak spiralling. Ritchie (1960) found that spiral growth was not governed by electrical or gravitational fields in the environment and was not observed in colonies grown in liquid media.

Madelin *et al.* (1978) suggest that spiral growth of a mycelial hypha is caused by the axial rotation of the extension zone wall at its tip; this rotation

causes the hypha to roll over the surface of the substrate. However, for technical reasons Madelin *et al.* (1978) were unable to demonstrate axial wall rotation at the tips of mycelial hyphae, although such rotation has been observed in stage I and IVb sporangiophores of *P. blakesleeanus* (Oort, 1931; Castle, 1942) and the giant conidiophores of *Aspergillus giganteus* (Trinci & Banbury, 1967).

We have found that spiral growth of mycelial hyphae is best observed in young mycelia and that its initiation is influenced by rigidity of the medium. We have studied the behaviour of stage I sporangiophores of *P. blakesleeanus* and conidiophores of *A. giganteus* and obtained evidence which supports the hypothesis of Madelin *et al.* (1978).

MATERIALS AND METHODS

Organisms and media

Neurospora crassa Shear & Dodge (wild type strain, SY7A) was obtained from the Fungal Genetics Stock Center, Humboldt State College Foundation, Arcata, California 95521. *Mucor hiemalis* Wehmer (QEC strain Z14), *Phycomyces blakesleeanus* Burgeff (QEC strain Z15), *Aspergillus nidulans* (Eidam) Winter (QEC strain A17) and *Aspergillus giganteus* Wehmer (QEC strain A12) were obtained from the Queen Elizabeth College culture collection. Organisms were grown on Vogel's (1956) medium except that the carbon source was 1% (w/v) glucose instead of sucrose and that the

medium was supplemented with thiamine hydrochloride (0.25 mg/l) for *M. hiemalis* and *P. blakesleeanus*. The medium was gelled with 0.5–6.0% (w/v) Davis New Zealand agar; 200 ml of medium was prepared by mixing the following ingredients which had been autoclaved (15 min at 15 lb per square inch) separately: 4 ml of Vogel's solution, 20 ml of 10% (w/v) glucose solution; 1 ml of thiamine hydrochloride solution (0.05 mg/ml) and 175 ml of the appropriate water agar.

Inoculation and incubation

Organisms were grown in 9 cm Petri dishes containing 10 or 20 ml of medium; plates were sometimes 'dried' for 2–3 days in a 37 °C constant temperature room prior to inoculation and the medium was sometimes overlaid with sterile cellophane (325P, British Cellophane Ltd). Plates were inoculated with 0.1 ml of a spore suspension in 0.1% (v/v) Tween 80; spores were spread evenly over the surface of the medium with a glass 'hockey stick'. Each plate was inoculated with about 20 (when spiral growth was studied) or about 200 (when autotropism was studied) spores and incubated at 25° for 15–36 h. When reproductive hyphae of *A. giganteus* and *P. blakesleeanus* were studied, plate cultures were inoculated centrally with mycelium and incubated for 3–4 days at 25°; cultures of *P. blakesleeanus* were incubated in darkness by loosely wrapping them in aluminium foil, cultures of *A. giganteus* were incubated under overhead illumination to induce conidiophore growth (Trinci & Banbury, 1967).

Observation of hyphae and mycelia

A Shackman (Chesham, Bucks) 35 mm camera was used for time-lapse photomicroscopy. A Baty projector (J. E. Baty & Co. Ltd, Sussex) was used with $\times 10$ and $\times 50$ objectives to trace mycelial hypha, conidiophores and sporangiophores. The direction in which a mycelial or reproductive hypha curved was always observed with respect to the surface (agar medium or Petri dish lid) on which it was growing; when hyphae were growing on a lid observations were made from below, not through the lid. Curvature measurements on hyphae of *Mucor hiemalis* were made, as shown in Fig. 1, from the final 1 mm length of the hypha. Curvature measurements on giant conidiophores or sporangiophores were made on the type of tracings shown in Fig. 8.

RESULTS

Hyphal curvatures

Figs. 2 and 3(b) show spiral growth of leading hyphae of young mycelia of *A. nidulans*, *N. crassa* and *M. hiemalis*; hyphae of *N. crassa*, *A. nidulans*, *M. hiemalis* and *A. giganteus* curved in a clockwise direction whilst those of *P. blakesleeanus* curved in a counterclockwise direction. Madelin *et al.* (1978) classified *M. hiemalis* and *P. blakesleeanus* in groups which showed weak clockwise and counterclockwise spiralling respectively but did not observe any consistent spiralling in six species of *Aspergillus*, which included *A. giganteus*

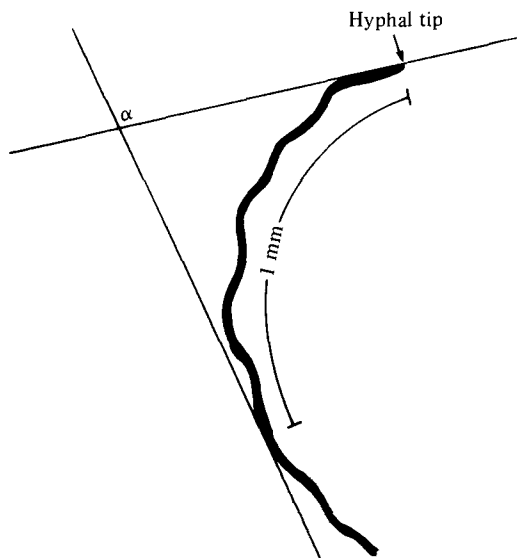


Fig. 1. Method used to measure the curvature (α) of the final 1 mm length of leading hyphae of *Mucor hiemalis*.

but not *A. nidulans*, and made no observations on *N. crassa*.

Fig. 4 shows part of a time-lapse sequence taken during growth of a mycelium of *Mucor hiemalis* on a medium containing 4% (w/v) agar; hyphae first deviated from straight growth when they were about 300 μm long. Some hyphae displayed a

more or less smooth clockwise curvature whilst others occasionally displayed an abrupt change of direction.

Autotropic responses

Madelin *et al.* (1978) stated that 'curvature is manifested only on very dilute media probably because under these conditions chemotropic

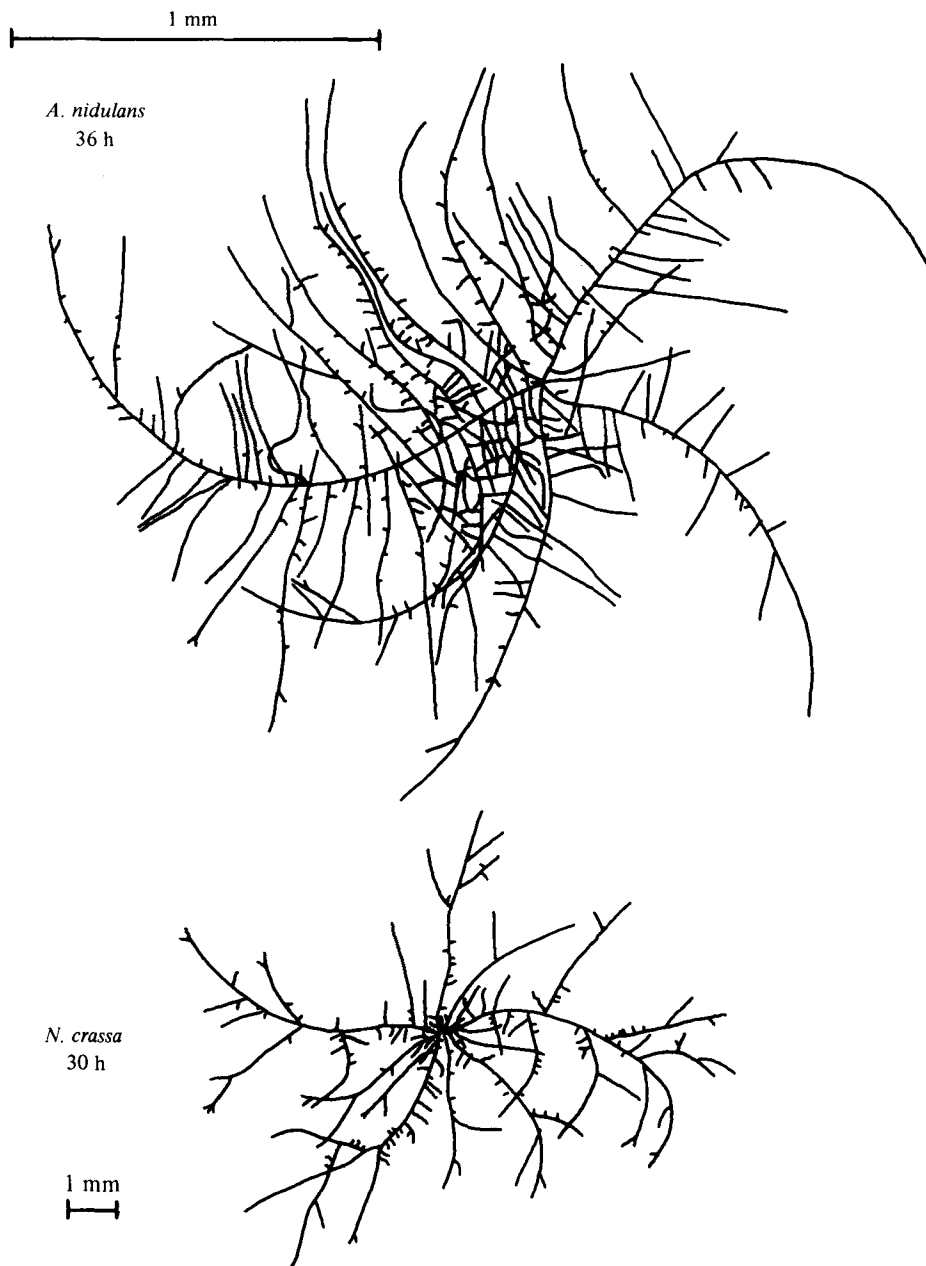


Fig. 2. Spiral growth of leading hyphae of *Neurospora crassa* and *Aspergillus nidulans* on media overlaid with cellophane. The times since inoculation are given in hours.

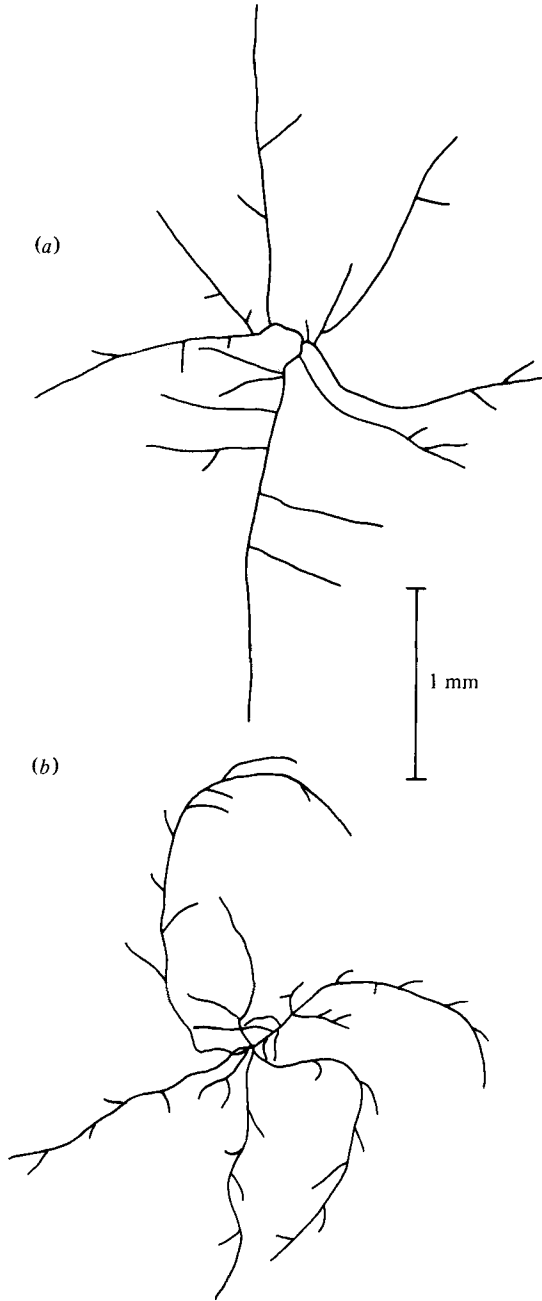


Fig. 3. Mycelia of *Mucor hiemalis* which had been grown for 24 h on media gelled with 0.5% (a) and 3.0% (b) agar.

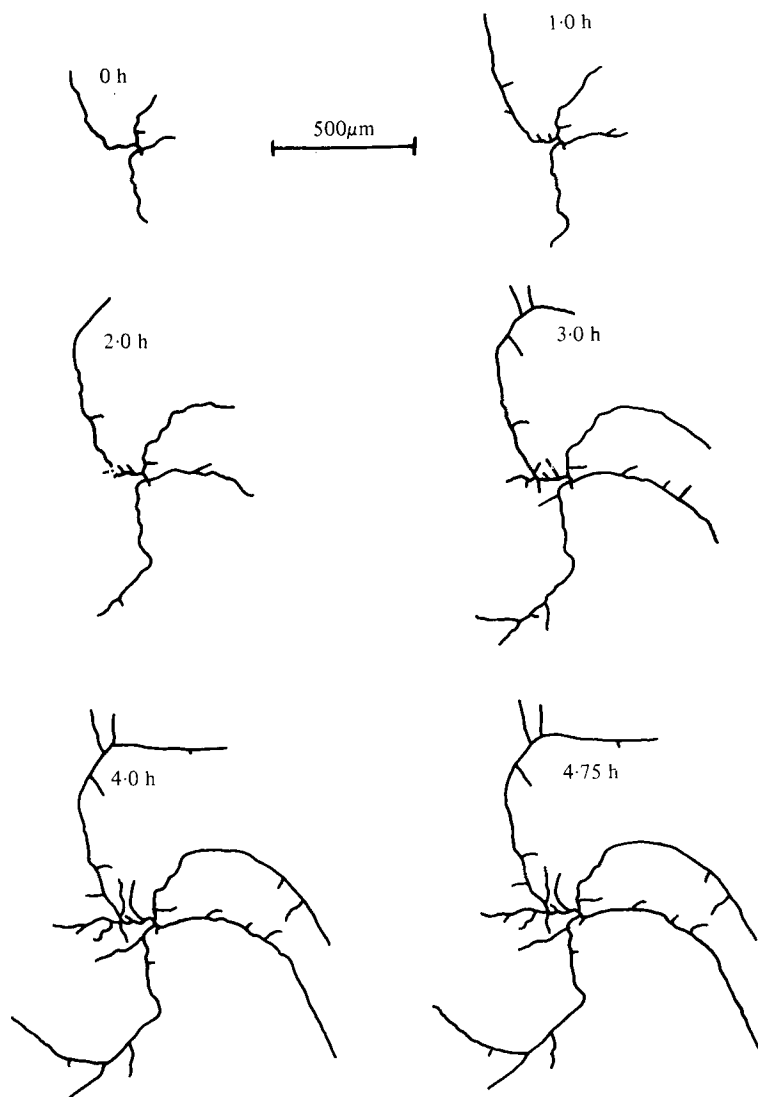


Fig. 4. Time-lapse sequence of the growth of a mycelium of *Mucor hiemalis* on a medium gelled with 4% agar.

responses, which otherwise override and obscure intrinsic tendency to curvature, are reduced'. We observed that hyphal curvatures were often displayed in sparse, young mycelia but not in dense, older colonies. In addition, autotropic or 'avoiding' reactions were observed when germ tube or branch hyphae approached one another in a particular way (Fig. 5); Robinson (1973*a, b*) defines autotropism as a tropic response where the uni-directional stimulus originates from the same

organism or from a neighbouring organism of the same species and has suggested that oxygen gradients established in the substrate as a result of the organism's metabolism may be the stimulus which induces fungal autotropism. We observed that in an autotropic response the responding hypha turned away from the hypha to which it was reacting whilst the hypha which stimulated the response did not usually deviate from its original direction of growth. The mean distances which

Spiral growth

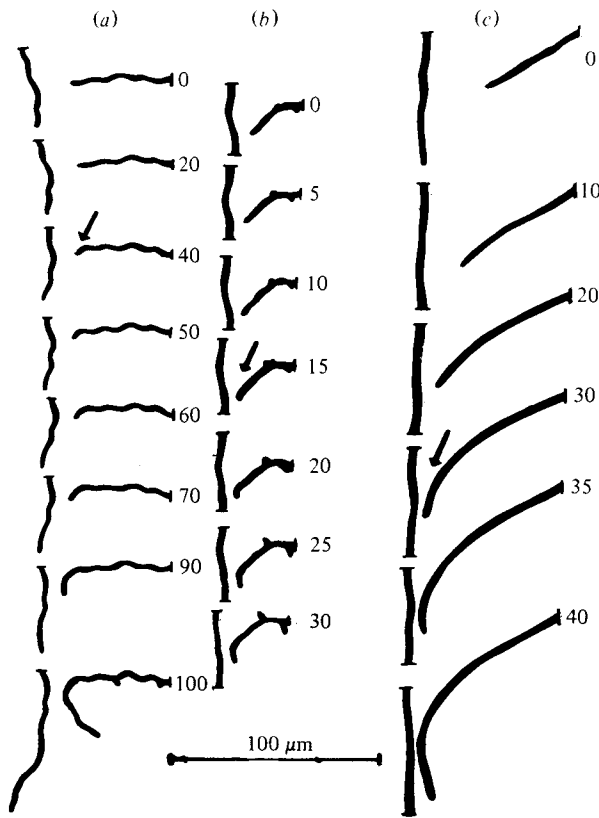


Fig. 5. Negative autotropic responses of pairs of hyphae of *Aspergillus nidulans* (a), *Mucor hiemalis* (b) and *Neurospora crassa* (c). The point where the avoiding reaction was first observed is indicated by the arrow. The figures give times in minutes. The plates were overlaid with cellophane prior to inoculation.

Table 1. Distances (μm) separating pairs of hyphae when autotropic responses were first observed (see Fig. 5)

	Mean distance between pairs of hyphae when autotropic responses were observed*	Minimum and maximum distances between pairs of hyphae when autotropic responses were first observed	
		Minimum	Maximum
<i>Neurospora crassa</i>	30 ± 11	15	51
<i>Aspergillus nidulans</i>	27 ± 16	4	68
<i>Mucor hiemalis</i>	24 ± 10	11	46

* Standard deviation of the sample; each result is the mean of 10–24 observations. The plates had been incubated for 18–24 h at 25°.

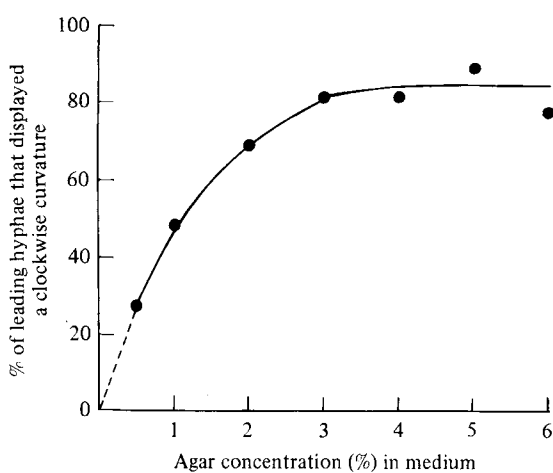


Fig. 6

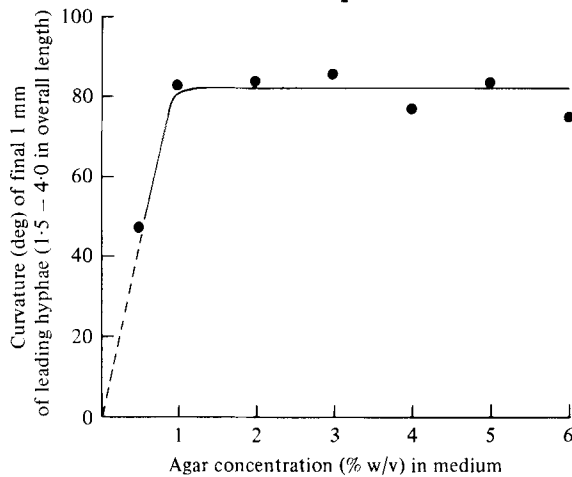


Fig. 7

Fig. 6. Effect of agar concentration on the percentage of leading hyphae (hyphae which were at least 1–2 mm long) which showed a distinct clockwise curvature. The graph is extrapolated to zero because no curvature is observed in liquid media.

Fig. 7. Effect of agar concentration on the degree of bending of the final 1 mm length of hyphae which showed clockwise curvatures. The graph is extrapolated to zero because no curvature is observed in liquid media. The medium was not overlaid with cellophane.

separated pairs of hyphae when autotropic responses were first observed and the maximum range of the response were similar for the three species studied (Table 1). Autotropic responses were sometimes not observed when one hypha approached a second at an angle of about 90°. We conclude from these observations that in dense colonies autotropic and/or chemotropic responses obscure the intrinsic tendency of hyphae to display curvatures. Thus leading hyphae at the margin of mature colonies tend to grow radially outwards from the centre of the colony.

Effect of agar concentration on hyphal curvature

Figs. 3 and 6 show the effect of medium rigidity (agar concentration) on the curvature of leading hyphae of 24 h old mycelia of *M. hiemalis*. The percentage of leading hyphae (defined for this purpose as hyphae which were at least 1–2 mm long) which showed a distinct clockwise curvature increased with agar concentration up to 3.0% (w/v) but then remained constant (at about 80%) at higher concentrations (Fig. 6). An essentially similar result was obtained when media were overlaid with cellophane prior to inoculation. Mature colonies of *M. hiemalis* had a mean radial growth rate of about 515 $\mu\text{m h}^{-1}$ and this rate was not affected by agar concentration. Curvatures were not observed when hyphae grew at the interface

between two thin layers of medium having either the same or different agar concentrations.

Fig. 7 shows the effect of agar concentration on the curvature of leading hyphae displaying spiral growth. The mean hyphal curvature was more or less constant except when the medium was gelled with 0.5% (w/v) agar. Thus the percentage of hyphae induced to curve increased with agar concentration up to 3% (w/v) but hyphae which 'spiralled' displayed a more or less constant mean curvature on all agar concentrations except the lowest employed (0.5%).

Curvatures formed by conidiophores and sporangiophores grown in contact with a solid surface

Madelin *et al.* (1978) suggested that mycelial hyphae curve when grown on solid substrates because of the axial rotation of the extension zone wall at their tips, but for technical reasons they were unable to demonstrate tip rotation. However, it is known (Trinci & Banbury, 1967; Castle, 1942) that walls at the tips of giant conidiophores of *A. giganteus* and stage I sporangiophores of *P. blakesleeianus* spiral in a left-handed direction (left-hand spiralling follows the direction of a left-handed screw, i.e. looking down on top of the hypha, rotation is in a clockwise direction). If the hypothesis of Madelin *et al.* (1978) is correct these reproductive hyphae should display counterclock-

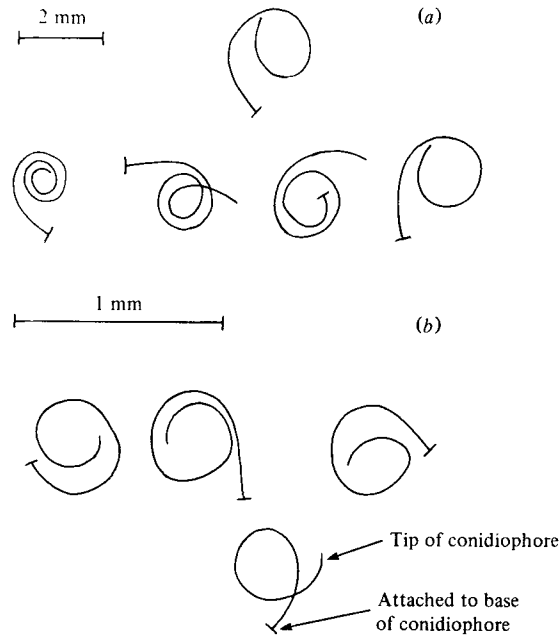


Fig. 8. Tracings of 'coils' formed by (a) stage I sporangiophores of *Phycomyces blakesleeanus* and (b) conidiophores of *Aspergillus giganteus* growing in contact with the Petri dish lid.

wise curvatures when grown in contact with solid surfaces. To test this prediction, stage I (lacking sporangia) and IVb (supporting sporangia) sporangiophores of *P. blakesleeanus* were grown in darkness so that eventually, as a result of negative geotropism, they came in contact with the Petri dish lid. When this happened stage I sporangiophores, which were then about 10 mm tall and had a mean diameter of about $80 \mu\text{m}$, formed coils on the underside of the lid (Fig. 8). The coils were formed in a clockwise direction with respect to the supporting surface and had a mean (of 14 stage I sporangiophores) curvature of $86 \pm 10^\circ$ per 1 mm length of sporangiophore. Stage I sporangiophores also formed coils when grown in contact with layers of 0.5 or 3.0% water agar. However, stage IVb sporangiophores did not form coils under any circumstances, possibly because the sporangium prevented contact between the extension zone wall and the supporting surface. In addition some sporangia 'burst' when they came in contact with solid surfaces.

Giant conidiophores of *A. giganteus* also formed coils when grown in contact with the lid of a Petri dish (Fig. 8); the conidiophores studied had a mean diameter of about $27 \mu\text{m}$. As predicted, the coils produced by conidiophores of *A. giganteus* were formed in a counterclockwise direction with

respect to the supporting surface; there was a mean (of 24 conidiophores) curvature of $334 \pm 53^\circ$ per 1 mm length of conidiophore. Madelin *et al.* (1978) reported that the radii of growth curvatures of hyphae of *Sordaria fimicola* (Rob.) Ces. & De Not. were commonly between 200 and $800 \mu\text{m}$, and mostly about $400 \mu\text{m}$. The mean radii of the curvatures formed by mycelial hyphae of *M. hiemalis*, stage I sporangiophores of *P. blakesleeanus* and giant conidiophores of *A. giganteus* were 682, 669 and $173 \mu\text{m}$ respectively, i.e. approximately within the range observed for *S. fimicola*.

DISCUSSION

Our observations indicated that the intrinsic tendency of hyphae to curve is easier to detect in sparse, young mycelia than in dense, mature colonies; Madelin *et al.* (1978) reduced hyphal density by growing the organisms on dilute nutrient medium.

Madelin *et al.* (1978) propose that curvature of mycelial hyphae on solid surfaces is a consequence of the axial rotation of their extension zone walls. They suggest that if the extension zone wall spirals in a left-handed direction the hyphal tip will be displaced to the left by a lateral rolling action over the supporting surface and that this sustained lateral displacement will impart a

curvature to the hypha; the colony as a whole will thus have a spiral growth pattern which is in an anticlockwise direction when viewed from above. However, if the extension zone wall spirals in a right-handed direction the hyphal tip will be displaced to the right and the colony will have a clockwise spiral growth pattern.

The results obtained with reproductive hyphae of *P. blakesleeanus* and *A. giganteus* are consistent with the hypothesis of Madelin *et al.* (1978). However, only the conidiophores of *A. giganteus* were displaced in the predicted direction. Mycelial hyphae of *P. blakesleeanus* and *A. giganteus* curved in the opposite direction to their respective reproductive hyphae. However, Castle (1942) observed a reversal in the direction of tip spiralling during differentiation of sporangiophores of *P. blakesleeanus*.

The observed reduction in curvature (Fig. 3) when hyphae of *M. hiemalis* are grown on media gelled with 0.5% agar and its absence in liquid culture is presumably caused by 'slippage' between the surface of the hypha and the supporting medium, i.e. hyphae 'bore' through the surface of soft agar. However, under certain conditions hyphae may grow in a helical form even in liquid media (Toaze-Soulet *et al.*, 1978).

Our observations on reproductive hyphae, particularly those of *A. giganteus*, together with the fact that the curvature of hyphae of *M. hiemalis* was less on less rigid surfaces, strongly supports the suggestion of Madelin *et al.* (1978) that spiral growth of colonies is due to rotation of the extension zone wall at the tips of hyphae. This is, however, really only a partial explanation of the phenomenon, as we do not know what causes the rotation. And we can carry the question of causation one step further back, since while the rotation of the extension zone is almost certainly related to the spiral structure of the hyphal wall we know neither the nature of this connection nor the origin of the spiral structure.

It follows that all these problems ought to be studied together, so that we can profit from the light each can throw on the others. Madelin *et al.* (1978) derived their expression for the curvature of hyphae on the assumption that the rotation could be considered to be confined to a small region quite close to the apex. Ortega, Harris & Gamow (1974) showed that rotation is greatest in the upper part of the extension zone of sporangiophores of *P. blakesleeanus*, although it does occur in the lower part, even at the base of the extension zone where they found no measurable elongation. If most or all of the rotation occurs close to the apex, this suggests the need for a reappraisal of the attempt by Preston (1948) to explain the rotation

of the wall as analogous to the rotation of a loaded spiral spring (also see Middlebrook & Preston, 1952). His model was criticized by Castle (1953), but one of the chief arguments against it – the observation that the sense of the rotation can be reversibly altered by change of temperature – is weakened if almost all the rotation occurs near the apex. It should be noted that Preston's model predicts that most of the rotation will occur near the apex if we combine it with results of Saunders & Trinci (1979) which indicate that the elastic moduli of the 'spring' increase rapidly away from the apex.

This study provides further evidence of the value of the stage I sporangiophore of *P. blakesleeanus* as a model system to study hyphal growth. It is perhaps unfortunate that most of the many studies on this organism have been made with the stage IVb sporangiophores. These sporangiophores have an intercalary extension zone and are thus less comparable with mycelial hyphae.

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(Accepted for publication 18 January 1979)