Autotropism in Fungal Spores and Hyphae

Author(s): P. M. Robinson

Source: *Botanical Review*, Oct. – Dec., 1973, Vol. 39, No. 4 (Oct. – Dec., 1973), pp. 367–384

Published by: Springer on behalf of New York Botanical Garden Press

Stable URL: https://www.jstor.org/stable/4353860

AUTOTROPISM IN FUNGAL SPORES AND HYPHAE

P. M. ROBINSON

Department of Botany
The Queen's University of Belfast, Belfast, N. Ireland

CONTENTS

Introduction

A tropism is a growth movement exhibited by part of an organism in response to a unidirectional stimulus. When the stimulus is due to the same organism which displays the tropism, or to a neighbouring organism of the same species, the oriented growth which occurs is an autotropism. For the purpose of this review autotropic responses are divided into two main groups. One group arises from interactions of neighbouring spores and affects the point of emergence and initial direction of growth of each germ-tube; the other group arises from interactions of neighbouring somatic hyphae and can result in the orientated growth of hyphae to give the characteristic growth pattern of a fungal colony grown on a solid medium. In the simplest sense there is an interaction of two spores or two hyphae but these responses may be modified by the size or density of the population to which the spores or hyphae belong and also by other environmental conditions. In the broadest sense autotropism can be investigated between neighbouring spore suspensions (or hyphal populations) of the same species.

Many autotropic responses in fungi have been attributed to the production of labile metabolites to which germ-tubes and mature hyphae react in a negative chemotropic manner. Whilst such metabolites may play a major part in tropic responses between two spores which are in contact or slightly separated it is intended to point out that there is

367

little evidence for the explanation of other autotropic effects in terms of self-produced, labile factors.

Techniques Used in Autotropic Studies

The techniques used for the study of autotropic responses in fungi derive indirectly from methods employed to study chemotropism in germinating spore populations. Chemotropic responses in fungi were reviewed by Ziegler (1962) and many workers have noted the effects of various chemical stimuli on the orientation of germ-tube growth. Miyoshi (1894) and Clark (1902) were the first workers to pursue the problem intensively but Clark was first to propose that the results of many chemotropic experiments could be explained in terms of fungi exhibiting a negative chemotropism to their own metabolites. He concluded that the negative chemotropism of fungi to these metabolites was greater than any positive chemotropism to food substances or oxygen. Both Fulton (1906) and Graves (1916) supported Clark's hypothesis. In this way the techniques used to assess tropic effects of applied chemicals usually resulted in the demonstration of these supposedly autotropic responses.

Three techniques have been used to demonstrate chemotropic responses: the direct method, the capillary tube method and the perforated-plate method. The direct method consisted of the application of chemicals to the surface of a solid medium seeded with spores in an attempt to influence the tropic responses of the germinating spore population (Miyoshi, 1894). Clark (1902) refined this technique; he sowed spores on a block of solid medium and pushed blocks of media containing the test chemicals towards the margins of the seeded block to make contact at the time the hyphae were approaching the limits of the seeded block. Resultant tropisms were observed and it was noted that the test hyphae always grew towards any applied unseeded block, irrespective of the chemical content of the block. This response was considered due to the negative chemotropism of the fungal hyphae to their own metabolites. In the capillary tube technique (Miyoshi, 1894; Clark, 1902; Fulton, 1906) capillaries filled with solutions of chemicals were introduced to hanging drop cultures of fungi in solid media and hyphal tropisms were recorded in the vicinity of the mouth of the capillary. This technique is based on the method used by Pfeffer (1888) to study chemotaxis in bacteria. Again, Clark and Fulton considered that most of the results were due primarily to the negative autotropism of the test fungi.

The perforated-plate technique involves the study of germ-tube orientation in the vicinity of holes in a perforated sheet which separates the spore suspension as a layer in a solid medium from an opposite layer amended with spores or various chemicals. This technique has been used by several workers and some of the variations are recorded in Table 1. The technique is versatile as it permits the study of tropic responses of germinating spore populations due to their own metabolites, to applied chemicals, or to a neighbouring spore population.

TABLE 1

PERFORATED-PLATE SYSTEMS USED BY DIFFERENT WORKERS.

Plate material	Diameter of perforations	Species tested	Author
Mica, celloidin film and strips of plant epidermis.	—	*Aspergillus niger, Botrytis cinerea, Mucor mucedo, Penicillium glaucum, Phycomyces nitens, Rhizopus stolonifer* and *Saprolegnia ferax.*	Miyoshi, 1894.
Mica	—	*Rhizopus stolonifer.*	Clark, 1902.
Mica, celloidin film and strips of plant epidermis.	100–150 μm	*Botrytis vulgaris, Cercospora apii, Monilia fructigena, Monilia sitophila, Sphaeropsis malorum* and *Sterigmatocystis nigra.*	Fulton, 1906.
Mica	8–45 μm	*Botrytis cinerea, Penicillium* sp. and *Rhizopus stolonifer.*	Graves, 1916.
Plastic	1 mm	*Aspergillus* sp., *Mucor* sp., *Penicillium* sp. and *Rhizopus stolonifer.*	Stadler, 1952.
Plastic	500 μm	*Geotrichum candidum.*	Robinson, 1973a, b.

Jaffe (1966) studied autotropic effects of conidia from *Botrytis cinerea* Pers. ex Fr. Dry spores were suspended in portions of an inert fluorochemical liquid and electrostatically precipitated in polystyrene containers; then the spores were covered with a liquid medium to permit germination. The positions of emergence of germ-tubes of spores in pairs were noted. It was found (Robinson, Park & Graham, 1968) that the autotropic behaviour of spore pairs of several species of fungi could be conveniently studied by sowing spore suspensions onto the surface of solid media. This technique minimised the risk of spores moving relative to each other as could happen on occasions when spores were studied in liquid media.

Types of Autotropic Behaviour Observed in Fungi

Three main types of autotropic behaviour may occur between neighbouring spores or between neighbouring spore populations. Two neighbouring spores can exhibit positive, negative or neutral autotropic behaviour and large populations of spores can be influenced similarly by a neighbouring population of the same species. Hyphae can react similarly.

Jaffe (1966) has demonstrated that germinating spores of *B. cinerea*

show pronounced positive autotropism when spores in pairs are examined in liquid media. On the other hand, it was found (Robinson *et al.* 1968) that spores of *B. cinerea* show neutral autotropism when spore pairs seeded on the surface of solid media are examined. Sporangiospores of *Mucor plumbeus* Bon. were found (Robinson *et al.* 1968) to exhibit marked negative autotropism; sporangiospores of *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind and conidia of *Trichoderma viride* Pers. ex Fr. also showed negative autotropism but this was not so pronounced as in *M. plumbeus*. The patterns of germ-tube emergence exhibited by spore pairs of the different species of fungi examined indicate that a range of autotropic behaviour is likely to be found. Variation in autotropic behaviour of one species can be marked under different experimental conditions but is very slight under standard conditions.

Observations on autotropic behaviour within a single spore population and between spore populations have been made by several workers using the perforated-plate assay system. Fulton (1906) demonstrated that germling hyphae showed a lower percentage of turning towards a layer containing hyphae than towards a sterile layer. Graves (1916) demonstrated that when approximately equal amounts of mycelium of *R. stolonifer* occur in two similar layers of medium the hyphae do not turn either towards or away from the opposite layer. Stadler (1952) reported that a germinating spore population of *R. stolonifer* exhibited a pronounced negative autotropism such that the hyphae turned towards a hole separating the spores from an unseeded opposite layer and away from a hole separating the spores from a more densely seeded opposite layer. It was found (Robinson, 1973a) that germinating arthrospores of *Geotrichum candidum* Link ex Pers. also behave in this manner with respect to other arthrospore populations.

Stadler (1952) distinguished two main tropic responses of germinating spores, the tropism relating to the position on the spore from which the germ-tube emerged and the tropism relating to any subsequent direction of growth of this germ-tube. He noted in his experiments that the initial direction of growth of a germ-tube was due mainly to the position on the spore at which it arose. Subsequent deviations from this initial direction of growth were slight. This observation would be true for Stadler's experiments in which spores were sown at relatively high densities in a rich culture medium but would probably not hold for a low-density population for reasons which will be outlined later. There is a shortage of observations on the autotropic responses of neighbouring hyphae and the apparent negative autotropism of hyphae as it affects the familiar pattern of colony development.

Metabolites Responsible for Autotropic Effects of Spores

Autotropic effects exhibited by neighbouring spores or neighbouring populations of spores have been attributed to the production of labile metabolites by germinating spores. Graves (1916) suggested that staling

substances were responsible for the various autotropic effects he noted with spore populations in perforated-plate systems. He stated that 'The staling substances appear to be either thermolabile or volatile.' Stadler (1952) also concluded that a staling substance was responsible for the effects that he observed in perforated-plate systems and that this substance was 'an unstable chemical material.'

Jaffe (1966) worked with spore pairs of *B. cinerea* and concluded from earlier rheotropic studies (Müller & Jaffe, 1965) with the same species that each spore emits 'a diffusible, unstable, locally effective, macromolecular growth stimulator.' This growth stimulator was considered to have a half-life of approximately ten seconds and a diffusion constant of the order of 10^{-7} cm^2/sec. Such a macromolecule would have a radius of action of approximately one spore diameter in a static medium and Jaffe (1966) noted that the autotropic influence became less marked as the distance between spores increased. The positive autotropism could be reversed by the presence of 0.3 percent (v/v) carbon dioxide in the air above the experimental system. He concluded that under these conditions the autotropic behaviour of the spore pairs is dominated by production of an inhibitor of germ-tube emergence. Presumably this inhibitor would have similar physical properties to the growth stimulator already mentioned. It was found (Robinson *et al.* 1968) that a decline in negative autotropism occurred as spores in pairs of *R. stolonifer, M. plumbeus* and *T. viride* were separated by distances of 1–40 μm and it was concluded that a diffusible inhibitor of germ-tube emergence might be responsible for the negative autotropic effects described.

The factors considered responsible for autotropic effects in spore pairs are only effective over a short range and it is unlikely that these metabolites could cause the long-range autotropic effects noted for neighbouring spore populations by Stadler (1952). Stadler described a perforated-plate experiment in which a dense suspension of spores of *R. stolonifer* seeded in agar induced a negative chemotropic response in a less dense suspension separated by 2.5 mm (at least 150 spore diameters). He demonstrated that the metabolites postulated to explain this effect were probably labile since cell-free filtrates from dense spore suspensions were not effective in eliciting a chemotropic response when incorporated into an agar medium and placed opposite a layer of medium which contained a germinating spore suspension. Stadler's experiments, like those of earlier workers, did not eliminate the chemotropic effects of sporangiospores on each other within the same layer of agar. It might be expected that some spores would be more affected by the autotropic factors of their immediate neighbours than those produced by a more distant spore population.

Germinating spores produce stable metabolites which can retard or inhibit germination of spores of the same species and it is reasonable to consider the possibility that some of these metabolites may function as autotropic factors or augment the autotropic factors already men-

tioned. Most of the information which relates to these stable metabolites comes from studies on the failure of spores of many fungal species to germinate when sown at high densities. Three main factors are possibly at work here: limitation of oxygen (especially under static conditions of experimentation in liquid media), limitations of nutrients (particularly those containing carbon and nitrogen) and the production by the spores of metabolites inhibitory to germination. In very few instances have these self-inhibitors been characterized. The phenomenon of self-inhibition is particularly well established for spores of many pathogenic fungi and recently Macko, Staples, Allen & Renwick (1971) have iden-tified the self-inhibitors produced by uredospores of *Puccinia graminis* Pers. var *tritici* Eriks. & E. Henn. as the *cis*- and *trans*-isomers of methyl ferulate. It was later found that the *cis*- but not the *trans*-isomer of methyl ferulate inhibited germination (Allen, 1972). This contrasts with the work of Forsyth (1955) who concluded that the inhibitor might be trimethyl-ethylene. Forsyth collected the inhibitor by floating a con-tainer of acetone in the spore suspension and it could be argued that the acetone vapour might affect the spores so as to produce metabolites which were not typical of normal metabolism. The research on *P. graminis* is a good example of the frustrations involved in characterizing some biologically active metabolites since the problem was first tackled in 1955 by Allen (and Forsyth). Lingappa & Lingappa (1965, 1966) worked on self-inhibition in *Glomerella cingulata* (Stonem.) Spauld. & Schrenk but whilst evidence was produced which indicated that a stable fraction in conidial exudates was inhibitory to germination the active principle was not identified. Trinci & Whittaker (1968) described self-inhibition for *Aspergillus nidulans* (Eidam) Wint. and conclude that this is due to an insufficient supply of carbon dioxide during the early stages of germina-tion. However, the fact that increasing the carbon dioxide level in a medium relieves self-inhibition does not necessarily mean that the self-inhibition is a result of a lack of carbon dioxide in the first instance. Blakeman (1969) described self-inhibition in *Mycosphaerella ligulicola* Baker, Dimock & Davis. An unidentified inhibitory principle produced by suspensions of pycnidiospores (formed on cultures incubated at 15°C) was not readily volatile nor affected by high temperature.

Spores also produce metabolites which stimulate germination and these can be demonstrated in germinating spore suspensions of many fungi. If the time-course of germination is plotted for a range of spore concentrations there is frequently found an optimal density for rapid spore germination (T. A. Graham, unpublished data). It was found (Robinson *et al.* 1968; Robinson, 1973a) that germination stimulators can be demonstrated in *R. stolonifer, M. plumbeus* and *G. candidum* by seeding spore suspensions onto the surface of solid media and recording the time-course of germination of spores in pairs and of isolated single spores. The spore pairs germinate faster than the isolated spores. In other experiments it was found (Robinson *et al.* 1968) that culture fil-

trates from young spore suspensions of *R. stolonifer* (i.e. prior to germ-tube emergence) stimulate germination of spores of *R. stolonifer* but nothing can be said concerning the nature of the metabolites except that they appear to be relatively stable at room temperature. Stadler (1952) described experiments in which germinating spore suspensions of *R. stolonifer* produced stable promoters of hyphal extension-growth. He considered the possibility that these growth promoters might cause the hyphal tropisms away from dense spore suspensions in perforated-plate experiments. However, experiments in which filtrates from dense, germinating spore suspensions were applied opposite to seeded test layers did not induce any chemotropic response.

Whilst there appears to be sound evidence for the production of stable or relatively stable inhibitors and promoters of germination by germinating spores, there is no clear evidence that such metabolites may be involved in the autotropic responses of spores or spore populations. It was found (Robinson *et al.* 1968; Robinson, 1973a) that spores in pairs frequently display negative autotropism and a mutual stimulation of germination. It seems from this evidence that the autotropic factors which result in negative autotropism do not retard or inhibit spore germination but primarily affect the position of germ-tube emergence. Also, spores of *G. candidum* in pairs display marked negative autotropism yet the central spore(s) in chains of three or more spores germinate as rapidly as the terminal spores.

Metabolites Responsible for Autotropic Effects of Hyphae

The rather specialised hyphal tropisms associated with sexual processes in some fungi have been reviewed by Machlis (1966). Many of these are attributed to hormones and volatile metabolites. Apart from this work and the tropisms induced by unilateral illumination of sporangiophores (Castle, 1966) there has been little research on tropisms of hyphae. There are reports of tropisms induced by the direct application (or application in capillary tubes) of various substances to hyphae growing on solid media. These early experiments are reviewed by Fulton (1906) and it seems that many of these so-called tropic responses could have been due to local growth stimulation in the region of some of the test substances, the increased hyphal density being misinterpreted as a tropism. Trinci (1970) described a tendency for some lateral branches of *Aspergillus nidulans* (Eidam) Wint. to grow parallel to and close to the parent hyphae from which they originated. Strands of three to four hyphae growing parallel to one another were observed quite frequently. This effect could be interpreted as positive autotropism but there was no evidence to show that a chemotropic response was involved in the process. A similar effect has been found with hyphae of *G. candidum* (P. M. Robinson, unpublished data) when colonies were grown on the surface of a 'Cellophane' film placed on a solid medium. The mechanism of this effect is obscure. A negative autotropic response has also been

described by Trinci (1970) in which the tip of a germ-tube of *A. nidulans* turned away from a more proximal portion of the same germ-tube. The length of the germ-tube tip involved in this movement was 37 μm and evidence was presented that the region of the germ-tube apparently involved in the autotropic movement may correspond to the extension zone of the hypha.

From observations of spores streaked diametrically across the surface of a solid medium it was found (P. M. Robinson, unpublished data) that the early growth orientation of germling hyphae produced by sparsely sown spores is very erratic and tortuous. Germlings of *Aspergillus niger* van Tiegh. are good examples of this phenomenon. As the hyphal density increases so branches and main hyphae near the perimeter of the streaked area turn and grow at right angles to this area and the hyphae become regularly spaced. New hyphae arise as branches and become orientated away from the seeded area to give the characteristic appearance of the colony. Since most species of fungi examined have spores which exhibit negative autotropism, this raises the question as to whether or not the factor(s) which may determine the centrifugal growth of hyphae from an inoculum is the same as that responsible for autotropism at the germling stage. The simplest approach would be to postulate the production of a single chemotropic factor by spores, germlings and mature hyphae, the concentration of this factor being proportional to the density of the fungus material. There is no evidence, however, that the hyphal orientation associated with the production of a circular colony is influenced by the production of one or more chemotropic factors by the fungus material. Under certain conditions of culture the availability of oxygen may be important in the determination of some of the tropic responses which hyphae show during colony development. It was found (Robinson, 1973a) that in hyphae of *G. candidum* (grown in a perforated-plate system used to demonstrate chemotropic effects) the position of branch initiation was correlated with the oxygen concentration gradient in the region of the hole separating the layers. In assay systems containing a high-density inoculum of spores and hyphae in one layer and an unseeded opposite layer there was a marked tendency for the branches to originate from the side of the cell nearer the hole. The branches did not arise from the distal ends of the hyphal cells, as happens in intact colonies grown on the surface of solid media, but could occur at either end or even at the middle. The experiment indicates that any region of the hyphal cell of *G. candidum* can produce a branch but that under normal conditions of culture the distal region is favoured. It is possible that the position of branch initiation could be influenced by the oxygen concentration gradient under normal cultural conditions on solid media and that the mechanism of spacing of the leader and branch hyphae is also influenced by this gradient.

The hyphal fusions which occur in many fungi are somewhat specialised examples of positive autotropism. Burnett (1968) has described

these fusions and discussed in detail the hypotheses presented by Buller (1931, 1933), Raper (1952) and Park & Robinson (1966) to explain their formation.

Mechanism of Autotropic Effects

It seems logical to regard the labile autotropic factors produced by germinating spores as stimulators or inhibitors of one or more processes which culminate in the emergence of a germ-tube. Jaffe (1966) concluded that in *B. cinerea* the stimulator responsible for positive autotropism was initially emitted uniformly by each spore but then came to be emitted most rapidly by the presumptive growing point it favoured. This would favour the *cis* configuration discussed in the next section. Similarly, negative autotropic effects can be explained if an inhibitor of germ-tube emergence is produced uniformly by each spore; the maximum concentration would be in the region where the spores are in contact and germ-tube initiation would be most inhibited in this region so favouring negative autotropism.

The situation as outlined seems simple but there are complications. Rarely do populations of spores in pairs exhibit total positive or negative autotropism. It may be, as suggested by Jaffe (1966), that spores of *B. cinerea* produce positive and negative autotropic factors concurrently and the resultant behaviour is due to an interaction. It was found (Robinson *et al.* 1968) that spore pairs of *R. stolonifer* and *M. plumbeus* showed marked negative autotropism. In those spore pairs (called + −) in which one germinating spore was positive (+) and its neighbour negative (−) in autotropic behaviour the first germ-tube to emerge was usually +. The results indicate that the early stages of germination in some species which are predominantly negative in autotropic behaviour may be characterized by the production of positive autotropic factors. It is of interest that in + − spore pairs of *T. viride* (which is slightly negative to almost neutral in autotropic behaviour) there was no tendency for either the + or − germ-tube to emerge first.

Other factors must be postulated to explain autotropism between relatively widely-spaced spore populations. If it is accepted that some fungi show negative chemotropism to their own metabolites it is necessary to explain such long-range effects in terms of chemotropic factors which are less labile and/or of lower molecular weight than the factors already discussed in relation to autotropism of pairs of spores. Stadler (1952) put forward a hypothesis to explain both orientated germination and hyphal tropisms as observed in germinating spore populations in perforated-plate experiments. He concluded that these effects were due to the formation of a concentration gradient of a staling substance which inhibited spore germination and hyphal growth. The substance was thought to act as a cell wall-strengthening agent which retarded (or prevented) germination or growth on the side of the spore or hypha exposed to the higher concentration. In an attempt to detect such a

substance he partially embedded spores of *R. stolonifer* in the floors of two channels cut from blocks of water-agar. He filtered a two-day-old culture of *R. stolonifer* and let the filtrate stand for several hours to let the concentration of unstable metabolites decline. Some of the filtrate was allowed to flow directly over spores in one of the channels (the control). Some of the filtrate was passed over the original mycelium in a filter-lined funnel prior to flowing over spores in the second channel. The control population showed 35–40 percent germination after ten hours whereas the other series showed 10–15 percent germination at this time. He concluded that the unstable products of metabolism did in fact suppress growth. Stadler did not record how many spores were observed in each series and the results would only be marginally significant on a count of 100 spores for each series. The low percentage germination of the control population would appear to indicate the presence of stable germination-retarding metabolites or possibly the depletion of nutrients in the filtrate used as spores of *R. stolonifer* require exogenous nutrients for germination.

Cis and *Trans* Germination Patterns

Jaffe (1966) noted that when spores of *B. cinerea* were touching in pairs or slightly separated there was a marked tendency for the germ-tubes produced to form from the same side of a line joining the centres of the spores. This tendency was called *cis*; when germ-tubes formed from opposite sides of a line joining the centres of the spores the pattern was called *trans*. The tendency towards the *cis* configuration was found (Robinson *et al.* 1968) in spore pairs of *R. stolonifer, M. plumbeus* and *T. viride* and, as noted by Jaffe, the degree of the *cis* tendency declined with increasing distance between spores.

The *cis* configuration is not favoured by any particular type of autotropic behaviour neither is the magnitude of the *cis* effect related to the degree of the autotropic response observed. It was found (Robinson *et al.* 1968) for several species that by seeding spores on 'Cellophane' placed over solid media the negative autotropic response of spores in pairs was increased but the *cis* tendency was not altered significantly. This leads to the supposition that if *cis* and *trans* germination patterns are mediated via labile metabolites then these factors may be different from the short-range autotropic factors already discussed.

The *cis* effect can be explained for species displaying positive autotropism if the first germ-tube to emerge produces the same positive autotropic factor as is already being produced by each spore. The part of the neighbouring (non-germinated) spore surface to be affected would be on the same side of a line joining the spore centres as the first germ-tube. This would favour the *cis* configuration. In species which show negative autotropism the *cis* configuration could result if the first emergent germ-tube in some way negates the action of the negative autotropic factor (or factor inhibiting germ-tube emergence) produced by

each spore. This factor would decline more on the non-germinated spore surface on the same side of a line joining the spore centres as the germ-tube originated. Germ-tube initiation would be less inhibited on this side resulting in the *cis* formation.

There is another explanation for the *cis* configuration exhibited by spore pairs from many different species of fungi. It is now well established that spores in groups of two germinate more rapidly than isolated spores. If each spore produces a germination stimulator during germination and this diffuses from the whole germling surface then the surface of the neighbouring (non-germinated spore) nearest the emergent germ-tube would receive more of the stimulator than the surface furthest away. This could tend to reflect in the *cis* configuration. All these hypotheses depend on non-synchronous germination of the spores in each pair and this has been shown to be the case for most species so far examined.

The Role of Oxygen and Carbon Dioxide

A hypothesis has been proposed (Robinson, 1973b) to explain the results obtained in different assay systems, particularly the perforated-plate system, used by other workers to assess the chemotropic activity of various substances and the autotropic responses of spore populations. It is thought that spores and hyphae may become positively chemotropic to oxygen in an environment where oxygen is at a low concentration and is the factor most likely to limit germination or hyphal growth. In the perforated-plate assay the tropic responses have been observed in spores which germinate just outside the perimeter of the hole separating the layers and as near as possible to the plane of the hole, i.e. beneath the surface of the medium. The spore suspensions used are often dense and because of the slow rate of diffusion of oxygen in agar (Smith & Griffin, 1972) lack of oxygen could begin to limit germination of the test spores. If less dense spore suspensions were employed then the oxygen concentration would limit the growth of the emergent germ-tubes at a later stage of development. This was found (Robinson, 1973a, b) in perforated-plate experiments with *G. candidum* in which the position of germ-tube emergence on the spores is related to the density of the spore suspension. The higher the sowing density of the almost cylindrical spores the more pronounced the emergence of the germ-tubes from the regions of the spores nearest the hole separating the spores from an unseeded opposite layer or layer containing a lower density of spores. If spores were seeded at a low density no tropic response was noted with regard to the position of germ-tube emergence but with increasing growth of the germ-tubes there was an increasing tendency for the germ-tubes to turn towards the hole. Graves (1916) showed an increase in tropic response of germlings of *R. stolonifer* as hyphal length increased and Stadler (1952) mentioned briefly an enhanced tropism with increasing spore density in *R. stolonifer* in tests with non-spore opposite layers. In experiments with *G. candidum*, oxygen was the most likely factor to be

limiting germination at the higher spore densities and it is considered that many of the tropic responses described previously in perforated-plate systems could be correlated with a deficiency of oxygen. Substances considered by earlier workers to function as positive chemotropic agents could have acted by stimulating respiration of the spores in the test layer which would result in a lower oxygen concentration in this layer and a steeper oxygen concentration gradient between the two layers in the region of the hole. This idea could explain the apparent positive chemotropic response of germinating spores to turnip juice described by Graves (1916) and Stadler (1952, 1953). Graves (1916) recorded that cultures of R. *stolonifer* grew more vigorously in turnip juice than any other media tried and this observation would assume an enhanced oxygen consumption in the presence of the juice. Stadler (1952) noted that the positive tropism to a staled turnip juice was less than to the unstaled juice. The unstaled juice would, presumably, be a richer growth medium and enhance respiratory activity more than the staled juice.

On the proposed hypothesis oxygen may function as a positive chemotropic factor when the concentration gradient of oxygen in the medium surrounding the spore suspension begins to decrease the respiratory activity of one region of the surface of a spore or hypha relative to the neighbouring or opposite surface regions. It is of interest to consider how oxygen might exert such a positive chemotropic response. It is also important to differentiate the two tropisms considered in this review: a tropism reflected in the position at which a germ-tube arises (i.e. the initiation of a growth centre), and a tropism reflected in an alteration of direction of growth in a hypha (i.e. the diversion of a pre-existing growth centre). Germ-tube initiation and hyphal extension-growth are energy-requiring processes and a gradient in respiratory activity across a spore or hyphal tip could condition an orientation response in several ways. It is known that one or more inner walls may form inside a spore during germination (Hawker & Abbott, 1963; Bracker, 1966; Border & Trinci, 1970; Gull & Trinci, 1971). The area on the innermost wall from which the germ-tube arises could be determined by the synthesis of wall components in response to a gradient in respiratory activity across a spore. A gradient in respiratory activity across a hypha could be ultimately reflected in the distribution and numbers of apical vesicles associated with wall synthesis at the growing apical region. These vesicles have been observed by several workers (Brenner & Carroll, 1968; Mc-Clure, Park & Robinson, 1968; Girbardt, 1969; Hemmes & Hohl, 1969; Grove & Bracker, 1970; Grove, Bracker & Morré, 1970). When oxygen concentration begins to limit growth fewer apical vesicles may be produced on the side of the hyphal tip region facing the lower oxygen concentration so resulting in reduced wall synthesis on that side. Observations on the vesicles in hyphal apices are consistent with the idea that they may contribute new plasma membrane, wall precursors and materials to maintain the apical wall in an extensible form (Grove *et al.*

1970). The hypothesis fits in with the suggestion that hyphae turn by 'bulging' (Stadler, 1952; Carlile, 1966) in a particular direction as it is reasonable to suppose that a decrease in incorporation of wall material via apical vesicles on one side of a hypha could lower extensibility in that region. The hypha would bulge from the more extensible side, i.e. towards the higher oxygen concentration.

The hypothesis offers an explanation for Stadler's long-range chemotropic effect. Oxygen diffuses far more rapidly than the autotropic factor described by Müller and Jaffe (1965) and one population of spores could, by utilizing oxygen more rapidly by virtue of its high density, influence the tropic response of a less dense population separated by 2.5 mm or so via a hole in a plate. Less oxygen would be available to diffuse towards the less dense population and oxygen concentration would become rate limiting for growth more rapidly than if the denser suspension was replaced by an unseeded layer. The hypothesis is compatible with the observations of Stadler (1952) that species of *Mucor*, *Aspergillus* or *Penicillium* elicit a negative tropic response in germinating spores of *R. stolonifer*.

The possible role of oxygen in determining the position of branch initiation and the centrifugal growth of hyphae in a colony has already been discussed. It is not intended to give the impression that chemotropism to oxygen is the explanation for most autotropic effects but to offer this hypothesis as an alternative explanation for some autotropic effects, particularly long-range effects, that have been previously explained in terms of labile metabolites produced by spores. There is also some evidence that the autotropic behaviour of spore pairs can be modified by exposure to a steep oxygen concentration gradient and it was found (Robinson, 1973a, b) that even the usually marked negative autotropism of pairs of spores of *G. candidum* was reduced under these conditions. Thus, under some conditions, oxygen may be an important factor in certain short-range autotropic effects.

It was found (Robinson, 1973a) that when a dense spore suspension of *G. candidum* was seeded opposite to an unseeded layer of medium in a perforated-plate system the first spores to germinate were within the perimeter of the hole. There was a gradient in germination such that spores took longer to germinate as their distance from the hole increased. Whereas the spores within the perimeter of the hole did not exhibit tropisms in a particular direction, the spores beyond the perimeter germinated towards the hole. Even the last spores able to germinate showed a marked tropic response to the hole in spite of the intervening zone of germlings. This observation supports the idea that the point of origin of germ-tubes, and their subsequent direction of growth, may not be primarily due to a negative chemotropism to metabolites produced by the germinating spore population in a perforated-plate system.

Fulton (1906) considered the possibility 'that fungi might show an aerotropic sensibility, either as a positive reaction to oxygen, or as a
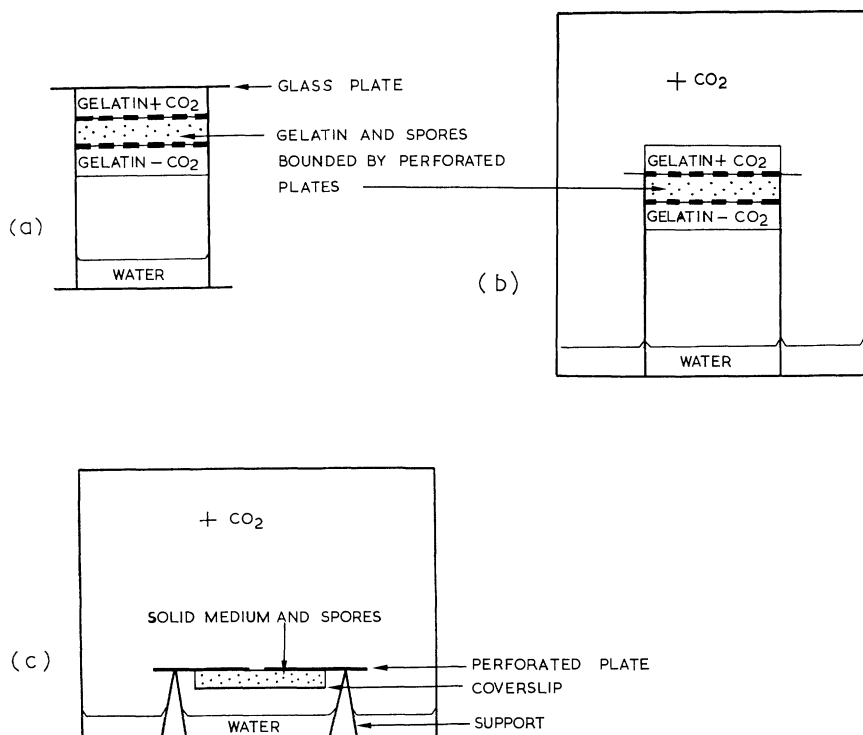
FIG. 1. Experiments with carbon dioxide. (a) Fulton (1906); a layer of gelatin containing spores was separated by perforated plates from gelatin saturated with carbon dioxide on one side and normal gelatin on the other. A glass cover was placed over the exposed surface of the gelatin saturated with carbon dioxide and the assembly fitted into a cell containing water. In no case was the percentage turning towards the carbon dioxide gelatin greatly different from that toward the normal gelatin. (b) Fulton (1906); the experiment was set up as in (a) except that a glass cover was not placed over the exposed surface of the gelatin saturated with carbon dioxide. The whole was placed under a bell jar 'practically filled with carbon dioxide.' The turning toward the gelatin containing carbon dioxide and exposed to an atmosphere of carbon dioxide was as great as towards normal gelatin. (c) Robinson (1973a); spores were seeded in a solid medium on one side of a perforated plate. The other side was left blank and the assembly placed in an anaerobic jar containing on various occasions different percentages of carbon dioxide in air. So long as the carbon dioxide concentration permitted germination of the spores in the test region the tropic response to the hole was as marked as to air from which the carbon dioxide had been removed.

negative reaction to carbon dioxide.' He experimented with perforated-plate systems to determine the roles of oxygen and carbon dioxide and concluded that the observed hyphal turning was not due to oxygen consumption or carbon dioxide production. In fact, his experiments did provide some evidence that carbon dioxide was not likely to function as

a chemotropic factor but they did not eliminate the possibility that oxygen might function as a chemotropic factor. It was found (Robinson, 1973a) that carbon dioxide is not likely to induce positive or negative chemotropic responses in *G. candidum*. The experiments with carbon dioxide are summarized in Fig. 1.

Many metabolites produced by fungi could presumably effect tropic responses on germinating spores or actively growing hyphae if respiratory activity was affected in the manner proposed to explain the possible action of oxygen as a chemotropic factor. One requirement would be that a concentration gradient of the metabolite across the spore or hyphae would reduce or stimulate respiratory activity on one side of the structure relative to the other. To assay these stable metabolites would be difficult since many interactions would have to be considered. Any effect these metabolites had on oxygen uptake could be important as stimulation or inhibition of oxygen uptake would affect the oxygen concentration gradient in the immediate environment. Whereas a germinating spore suspension in a perforated-plate system would react rapidly or gradually (depending on sowing density) to a self-imposed gradient of oxygen concentration, a metabolite applied to an opposite layer might only produce a tropic response over a critical concentration range. Too high a concentration might inhibit growth completely; too low a concentration might have no effect. Only a narrow zone of spores or hyphae might be expected to react to such a gradient. The problem becomes immense if one considers labile metabolites which may be unable to be˙ assayed (due to their short half-life) in the absence of their source.

Conclusions and Summary

1. Three types of autotropic response can be demonstrated for spores in pairs. Depending on the species, autotropic behaviour may be positive, negative or neutral but negative autotropism occurs in the majority of species examined to date.

2. The autotropic behaviour of spores in pairs is constant for a single species under a given set of experimental conditions but can be modified experimentally.

3. The autotropic responses of neighbouring spores are considered due to the production of positive and negative autotropic factors which are labile and operative over a short range of approximately 50 μm. Spores may produce both types of factor and the resultant response could be due to an interaction of the two factors.

4. The short-range positive and negative autotropic factors are most likely to act by stimulating or retarding one or more of the processes which culminate in germ-tube emergence. There is no evidence that these factors promote or retard germination.

5. Germinating spores produce stable or relatively stable promoters and inhibitors of germination but there is no direct evidence that the autotropic responses of spores can be attributed to these metabolites.

6. The autotropic effects observed between neighbouring (but relatively widely-spaced) spore populations in perforated-plate systems are not considered to be due to the short-range autotropic factors (mainly negative) postulated to explain autotropic effects between spores in pairs. These long-range effects have been explained in terms of hypothetical labile metabolites to which the germlings are negatively chemotropic but these metabolites have never been demonstrated convincingly.

7. The autotropic responses shown by a germinating spore population in perforated-plate systems towards unseeded medium or a neighbouring spore population are related to the density of the spore population. In these systems spores are usually sown at high densities and the responses are observed beneath the surface of the medium in the plane of the hole separating the two layers. Under these conditions it is proposed that orientated germination or hyphal turning may occur as responses to the oxygen concentration gradient in the immediate environment of the spores or hyphae.

8. Oxygen may function as a chemotropic factor in perforated-plate systems when the concentration falls to a level which retards germination or hyphal-extension growth. It is considered that under such conditions the chemotropic responses occur as a result of a gradient in respiratory activity across a spore or hyphal tip. The position on the inner wall of the spore from which the germ-tube arises may be determined by the synthesis of wall components in response to this concentration gradient. In hyphae, this gradient could affect the distribution and numbers of apical vesicles associated with wall synthesis at the apical region. If the extensibility of the cell wall is related to the degree of incorporation of new wall material via these vesicles then a hypha would 'bulge' from the most extensible region which would be towards the higher oxygen concentration.

9. The short-range autotropic responses of pairs of spores of G. candidum can be modified by and correlated with exposure to a steep oxygen concentration gradient, each spore germinating from the end nearer the higher oxygen concentration.

10. The position of branch initiation in cells of G. candidum in a perforated-plate system is also correlated with the oxygen concentration gradient of the system. Branches originate from the sides of cells facing the higher oxygen concentration.

11. It is considered that many positive and negative chemotropic effects attributed to applied chemicals by earlier workers may have arisen

indirectly from the effects of these substances on the respiratory activity of the test spores or germlings which in turn would affect the oxygen concentration in the assay system.

References

ALLEN, P. J. 1955. The role of a self-inhibitor in the germination of rust uredospores. Phytopathology **45**: 259–266.

————. 1972. Specificity of the *cis-* isomers of inhibitors of uredospore germination in the rust fungi. Proc. Nat. Acad. Sci. U.S.A. **69**: 3497–3500.

BLAKEMAN, J. P. 1969. Self-inhibition of germination of pycnidiospores of *Mycosphaerella ligulicola* in relation to the temperature of their formation. J. gen. Microbiol. **57**: 159–167.

BORDER, D. J., & A. P. J. TRINCI. 1970. Fine structure of the germination of *Aspergillus nidulans* conidia. Trans. Br. mycol. Soc. **54**: 143–146.

BRACKER, C. E. 1966. Ultrastructural aspects of sporangiospore formation in *Gilbertella persicaria.* In *The Fungus Spore* (ed. M. F. Madelin), pp. 39–58. London: Butterworths.

BRENNER, D. M., & G. C. CARROLL. 1968. Fine-structural correlates of growth in hyphae of *Ascodesmis sphaerospora.* J. Bact. **95**: 658–671.

BULLER, A. H. R. 1931. *Researches on Fungi.* Vol. IV. London: Longmans, Green & Co. Ltd.

————. 1933. *Researches on Fungi.* Vol. V. London: Longmans, Green & Co. Ltd.

BURNETT, J. H. 1968. *Fundamentals of Mycology.* London: Edward Arnold Ltd.

CARLILE, M. J. 1966. The orientation of zoospores and germ-tubes. In *The Fungus Spore* (ed. M. F. Madelin), pp. 175–186. London: Butterworths.

CASTLE, E. S. 1966. Light responses of *Phycomyces.* Science, N.Y. **154**: 1416–1420.

CLARK, J. F. 1902. On the toxic properties of some copper compounds with special reference to Bordeaux mixture. Bot. Gaz. **33**: 26–48.

FORSYTH, F. R. 1955. The nature of the inhibiting substance emitted by germinating urediospores of *Puccinia graminis* var. *tritici.* Can. J. Bot. **33**: 363–373.

FULTON, H. R. 1906. Chemotropism of fungi. Bot. Gaz. **41**: 81–108.

GIRBARDT, M. 1969. Die Ultrastruktur der Apikalregion von Pilzhyphen. Protoplasma **67**: 413–441.

GRAVES, A. H. 1916. Chemotropism in *Rhizopus nigricans.* Bot. Gaz. **62**: 337–369.

GROVE, S. N., & C. E. BRACKER. 1970. Protoplasmic organization of hyphal tips among fungi: vesicles and spitzenkörper. J. Bact. **104**: 989–1009.

————, ————, & D. J. MORRÉ. 1970. An ultrastructural basis for hyphal tip growth in *Pythium ultimum.* Am. J. Bot. **57**: 245–266.

GULL, K., & A. P. J. TRINCI. 1971. Fine structure of spore germination in *Botrytis cinerea.* J. Gen. Microbiol. **68**: 207–220.

HAWKER, L. E., & P. McV. ABBOTT. 1963. An electron microscope study of maturation and germination of sporangiospores of two species of *Rhizopus.* J. gen. Microbiol. **32**: 295–298.

HEMMES, D. E., & H. R. HOHL. 1969. Ultrastructural changes in directly germinating sporangia of *Phytophthora parasitica.* Am. J. Bot. **56**: 300–313.

JAFFE, L. F. 1966. On autotropism in *Botrytis*: measurement technique and control by CO_2. Pl. Physiol. **41**: 303–306.

LINGAPPA, B. T., & Y. LINGAPPA. 1965. Effects of nutrients on self-inhibition of germination of conidia of *Glomerella cingulata.* J. gen. Microbiol. **41**: 67–75.

————, & ————. 1966. The nature of self-inhibition of germination of conidia of *Glomerella cingulata*. J. gen. Microbiol. **43**: 91–100.

McCLURE, W. K., D. PARK, & P. M. ROBINSON. 1968. Apical organization in the somatic hyphae of fungi. J. gen. Microbiol. **50**: 177–182.

MACHLIS, L. 1966. Sex hormones in fungi. In *The Fungi*. Vol. II. (eds, G. C. Ainsworth & A. S. Sussman), pp. 415–433. New York: Academic Press.

MACKO, V., R. C. STAPLES, P. J. ALLEN, & J. A. A. RENWICK. 1971. Identification of the germination self-inhibitor from wheat stem uredospores. Science, N.Y. **173**: 835–836.

MIYOSHI, M. 1894. Über chemotropismus der Pilze. Bot. Ztg. **52**: 1–28.

MÜLLER, D., & L. F. JAFFE. 1965. A quantitative study of cellular rheotropism. Biophys. J. **5**: 317–335.

PARK, D., & P. M. ROBINSON. 1966. Aspects of hyphal morphogenesis in fungi. In *Trends in Plant Morphogenesis* (ed. E. C. Cutter), pp. 27–44. London: Longmans, Green & Co. Ltd.

PFEFFER, W. 1888. Über chemotactische Bewegungen von Bakterien, Flagellaten und Volvocineen. Unters. bot. Inst. Tübingen **2**: 582–661.

RAPER, J. R. 1952. Chemical regulation of sexual processes in the Thallophytes. Bot. Rev. **18**: 447–545.

ROBINSON, P. M. 1973a. Chemotropism in fungi. Trans. Br. mycol. Soc. **61**: 303–313.

————. 1973b. Oxygen—positive chemotropic factor for fungi? New Phytol. **73**: 1349–1356.

————, D. PARK, & T. A. GRAHAM. 1968. Autotropism in fungi. J. Exp. Bot. **19**: 125–134.

SMITH, A. M., & D. M. GRIFFIN. 1972. Partial pressures of oxygen in agar media. Trans. Br. mycol. Soc. **58**: 167–169.

STADLER, D. R. 1952. Chemotropism in *Rhizopus nigricans*: the staling reaction. J. cell. comp. Physiol. **39**: 449–474.

————. 1953. Chemotropism in *Rhizopus nigricans*: II. The action of plant juices. Biol. Bull. **104**: 100–108.

TRINCI, A. P. J. 1970. Kinetics of apical and lateral branching in *Aspergillus nidulans* and *Geotrichum lactis*. Trans. Br. mycol. Soc. **55**: 17–28.

————, & C. WHITTAKER. 1968. Self-inhibition of spore germination in *Aspergillus nidulans*. Trans. Br. mycol. Soc. **51**: 594–596.

ZIEGLER, H. 1962. Chemotropismus der Pilze. In *Handbuch der Pflanzenphysiologie*. Vol. XVII/2. (ed. W. Ruhland), pp. 398–411. Berlin: Springer-Verlag.