Armillaria luteobubalina mycelium develops air pores that conduct oxygen to rhizomorph clusters

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ABSTRACT

Armillaria luteobubalina produces air pores in culture. They consist of two parts: a basal region of tissue elevated to form a mound covered with a rind continuous with that of the colony, but perforated; and an apical region of long parallel hyphae, cemented together by scattered patches of extracellular material. This forms a hydrophobic structure that is elevated above the general level of the mycelial crust and does not easily become water-logged. Air pores develop near the inoculum plug shortly after inoculation, arising directly from the mycelium, and rhizomorphs are initiated from them. The air pore contains a complex system of gas space connecting the atmosphere with the central canal of each rhizomorph. The tissue beneath the melanised colony crust also contains gas space, especially near air pores. This is also connected with the gas space of each rhizomorph and of each air pore. Measurements with oxygen electrodes show that air pores and their associated rhizomorphs conduct oxygen. The average oxygen conductance of a group of air pores with associated rhizomorphs, within agar blocks, but with rhizomorph apices cut off, was about 700 \( \mu \text{m}^3 \text{s}^{-1} \), equivalent to about 200 \( \mu \text{m}^3 \text{s}^{-1} \) for each air-pore. We conclude that the air pores conduct oxygen into the gas space below the pigmented mycelium of the colony, where the rhizomorphs - which also conduct oxygen - originate. A. luteobubalina thus has a complex aerating system which allows efficient diffusion of oxygen into rhizomorphs, and this is likely to facilitate extension of inoculum into low-oxygen environments.

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Introduction

Several observations indicate that rhizomorphs of Armillaria species need a good supply of oxygen for their initiation and growth. They range from observations that submerged mycelium must reach the substrate-air interface for rhizomorphs to be initiated and grow (Snider 1959; Smith & Griffin 1971; Pareek et al. 2001), and their abundance in the upper horizons of soil (e.g. Ono 1965, 1970; Redfern 1973; Morrison 1976) to data on the effects of restricting oxygen supply on rhizomorph growth in soil and culture (Münch 1909; Reitsma 1932; Jacques-Félix 1968; Smith & Griffin 1971; Rishbeth 1978; Worral et al. 1986). Rhizomorphs grow faster than free hyphal tips (Rishbeth 1968) and they contain a large consolidated mass of cells (Motta 1969, 1971; Motta & Peabody 1982), so it can be assumed that they have a high oxygen requirement.

Many authors have alluded to gas spaces within rhizomorphs of Armillaria (e.g. Brefeld 1877; De Bary 1887), and some have postulated a role for rhizomorphs in aeration, and indicated that the central space of the rhizomorph is a gas-channel that might be involved in conducting oxygen (vide Garraway et al. 1991; Fox 2000). For example, the
experiments of Reitsma (1932) indicated that the oxygen for growing apices in Armillaria mellea is supplied through rhizomorphs. Smith & Griffin (1971) in Armillaria luteobubalina (then known as Armillariella elegans) showed that high partial pressures of oxygen at the origin of rhizomorphs promote their growth, but oxygen at the rhizomorph surface inhibits growth, as Jacques-Félix (1968) had thought for A. mellea. Smith & Griffin (1971) reported that when oxygen becomes limiting for apical growth, rhizomorphs flatten and become lobed, and growth becomes much slower; the distance at which flattening occurred depended on the partial pressure of oxygen applied. Investigations such as these are widely quoted in support of a role for rhizomorphs in aeration (e.g.Watkinson 1979) and for oxygen in their development (e.g. Rayner et al. 1985). However, the precise pathways by which oxygen from the atmosphere may reach the tips of the first cluster of rhizomorphs produced have never been elucidated.

In the seminal paper of Brefeld (1877), A. mellea rhizomorphs developed in culture were described as arising from clusters of sclerotia, presumably the same structures that Snider (1959) termed ‘microsclerotia’. There are many reports of the presence of structures described as ‘air pores’ or ‘breathing pores’ on various parts of the mycelium, but particularly on rhizomorphs. Hartig (1874) described tufts of hyphae extending from rhizomorphs of A. mellea. Smith & Griffin (1971) showed tufts of hyphae on aborted side branches of rhizomorphs of A. luteobubalina growing into the air, and interpreted them to be ‘breathing-pores’. The central canal of the aborted branch bearing the ‘breathing-pores’ was shown to be directly connected with the central canal of the main rhizomorph, and Smith & Griffin postulated that oxygen diffused through the intertwining hyphae about 0.5 cm behind the tip of the aborted branch into the central canal of the rhizomorph. Granlund et al. (1984) and Intini (1987) used the term ‘air pores’ for structures on the surface of the rhizomorphs of A. mellea and A. obscura respectively.

Pareek et al. (2001) noted fluffy structures developed when the mycelium reached the substrate-air interface in tubes of liquid medium inoculated with A. luteobubalina. These were necessary for the initiation or growth of rhizomorphs, but they did not look like sclerotia except in very early stages. Closer inspection of these fluffy structures at the substrate-air interface suggested a similarity between them and the air pores developed on rhizomorphs. In this paper we report the occurrence of ‘air pores’ consistently at inoculation sites, air-pore development and internal structure, and their connection with rhizomorphs. Finally, we examined their role in aeration quantitatively by estimating oxygen conductances with an oxygen electrode. We conclude that rhizomorphs develop beneath clusters of air pores, not clusters of microsclerotia, and that the air pores and their subending rhizomorphs conduct oxygen.

Materials and methods

Fungal material

Armillaria luteobubalina Watling and Kile (isolate 930199KGS) from the culture collection of the University of Western Sydney was used throughout. This was originally isolated from fruit bodies collected from Gore Hill Park, (33.5° S, 151.15° E) North Sydney in June 1993. Stock cultures were maintained in Petri dishes on malt marmite agar (2 % malt extract from a local health-food shop), 2 % agar (Amyl Media, Scoresby, VIC) and 0.1 % Marmite (Sanitarium Health Food, Wahroonga, NSW) w/v at 23 °C, and sub-cultured every 4-8 weeks. To produce ‘air pores’ and rhizomorphs, 10 mm plugs of inoculum were cut from the edge of actively growing colonies, transferred to 2 % malt marmite agar in Petri dishes, and incubated in the dark at 23 °C.

Observations of air pores

Cultures were monitored daily for at least 10 d using a Leica dissecting microscope, and photomicrographs of air pore development were taken with a Zeiss Axiocam digital camera. For light microscopy of sections, air pores at various stages of development with associated mycelium were excised from the colony using a double-edged razor blade and rinsed in purified (reverse osmosis) water. They were then fixed in situ in a glass vial with 5 % (w/v) glutaraldehyde in 0.08 M piperazine-N-N′-bis-(2-ethanol sulphonic acid) (PIPES) buffer at pH 7.0 for 3 h, rinsed 3-4 times in 0.3 M PIPES buffer at pH 7.2 (Salema & Brando 1973), dehydrated through a graded ethanol-water series and gradually infiltrated with medium grade LR White resin (London Resin, Woking) over 5 d, all at 4 °C. Samples were polymerised at 60 °C for 10-12 h in gelatin capsules. Semi-thin sections (1.5-2 µm) were cut on a Reichert Ultracut microtome using glass knives, collected on drops of water on slides and dried on a hot plate at 35 °C for 12 h. Sections were stained either with 0.05 % toluidine blue at pH 4.4 and observed with bright-field optics, or with 0.1 % Calcofluor White M2R and observed by fluorescence microscopy (O’Brien & McCully 1981). Photomicrographs were taken on Kodak 100 ISO film.

Oxygen conductance

Conductance of air pores and other tissues to oxygen from the air was measured with gas diffusion chambers fitted with oxygen electrodes, as described by Curran (1985) except that the chamber volume was reduced to about 10 ml to increase sensitivity. The electrode output was displayed on an iMac computer (Apple Computer, Cupertino, CA) through a PowerLab/4SP analog:digital converter using Chart 3.6.6 software (ADInstruments, Castle Hill, NSW). Chambers were routinely leak-tested, and electrodes were calibrated twice daily and whenever significant drift was detected.

Fungal tissue was mounted in 6 mm plastic tubes, to allow its insertion into the electrode chamber. Tubes of 1.5 ml capacity cut from 3 ml plastic pipettes were used to punch pieces of agar with attached mycelium out of the cultures. Each sample was then pushed slightly out of the tube and a thin slice of agar cut off with a double-edged razor blade, to expose cut ends of the rhizomorphs. A thin layer of Vaseline petroleum jelly was applied around the circumference of the sample to prevent any leakage and it was pushed back into the tube. Extreme care was necessary to avoid any breaks in the layer of mycelium or agar, or contamination of the
exposed surface with Vaseline. The outside of the plastic pipette tube with the sample in it was then lubricated and inserted through the rubber disc of the electrode chamber, as described by Curran (1985). The layer of agar and mycelium formed a barrier between the air and the inside of the oxygen electrode chamber, and entry of oxygen depended on conduction through the fungus and agar.

To identify the oxygen conducting capacity of different parts of the fungus, the experiments were divided into several sets. Oxygen conductances of groups of air pores with an inoculum plug, pigmented mycelium, aerial rhizomorphs and plain agar were measured. To clarify whether the inoculum plug was involved in conduction of oxygen, conductivities of groups of agar were measured. To ascertain whether the oxygen conducted from the air pores travelled through the rhizomorph, conductance with uncut and cut ends of rhizomorphs was compared. Aerial rhizomorphs with one or both ends cut were also investigated. All sets of experiments were replicated 6 times, each replicate and cut ends of rhizomorphs was compared. Aerial rhizomorph tips first became apparent on day 6. The rhizomorph tips were still white and appeared as undifferentiated aggregates of hyphae. At this time several rhizomorph tips were seen in the agar, located specifically beneath the developing air pore clusters. The next stage of air-pore development did air pores resemble rhizomorph tips and there were no indications of an organised meristem.

The average number of hyphal clusters per culture counted on day 5 after inoculation was 25 (Table 1) with a range from 13-39. It was difficult among these white hyphal clusters to pick out those that were destined to develop into air pores, and it was not until the air pores were all lightly pigmented on day 7 that this distinction could be made with confidence. The number of air pores (about 21 per dish at day 7) did not change over the next three days, although some of them failed to develop fully, and remained as small rounded pigmented aggregations surrounded by a dense white mycelium (Fig 9, values in parentheses in Table 1).

In all cases, the rhizomorphs were initiated specifically beneath clusters of growing air pores (Figs 10,11). Rhizomorph tips appeared after the initiation of the hyphal clusters but at an early stage of their development into air pores: rhizomorph tips first became apparent on day 6. The rhizomorphs rapidly grew down into the agar (Fig 12) until they reached the bottom of the dish where they bent and then grew horizontally outwards (Fig 13). From an early stage the central space of the rhizomorphs was filled with gas, as indicated by its shining appearance in Fig 12.

The internal structure of air pores and their gas space

The spatial relationship between air pores and rhizomorphs and their gas spaces is best shown in vertical sections through the site of emergence of individual air pores. However, since neither the air pores nor rhizomorphs are exactly perpendicular to the agar surface, they are sectioned in many planes. The earliest stage sectioned (day 6 after inoculation) shows two rhizomorph tips located beneath the edge of an obliquely sectioned developing air pore (Fig 14). The gas space in the medulla of these rhizomorphs is continuous with the atmosphere via gas spaces between the loosely arranged hyphae of the colony surface, and extends into the base of the air pore. Fully extended but not yet mature air pores are seen to consist of very loosely intertwined approximately parallel hyphae with intercellular spaces of various sizes (Fig 15). Serial sections indicate that the air pore at this stage is a roughly cylindrical loose mass of undifferentiated hyphae. Clusters of hyphal profiles appear to be bonded together in patches by an extracellular material that stains reddish purple with toluidine blue. Fig 15, a near median section, shows two distinct regions of more closely spaced hyphae on the flanks of a central space containing few hyphae located at the base of the air pore. The medulla in the base of a rhizomorph is situated immediately beneath the air pore and is in continuity.
with a narrow gas space located beneath the surface of the colony. This region is shown better in Fig 16 where gas space continuity can be traced from the medulla of a submerged rhizomorph through the base of the air pore to the atmosphere.

The pseudoparenchyma forming the cortex of the rhizomorph is easily distinguishable. The hyphae at the colony surface adjacent to the air pore are loosely arranged at this stage but pigmentation begins around the air pores and the surface hyphae

Figs 1-8 – Stages of air-pore development. Fig 1 - Loose clusters of non-pigmented hyphae (two examples indicated by arrows) at 4 d. Bar = 1 mm. Fig 2 - Day 7: numerous air-pore initials (dense clusters of hyphae). Bar = 10 mm. Fig 3 - Day 7: lightly pigmented air pores with associated white mycelium. Bar = 1 mm. Fig 4 - Day 8: denser white mycelium over a lightly pigmented developing air pore. Bar = 1 mm. Fig 5 - Extending cylindrical air pore after removal of the overlying white mycelium. Bar = 1 mm. Fig 6 - Day 9: loose mycelium at the tip of air pore emerging from the covering mycelium (viewed from above). Bar = 1 mm. Fig 7 - Day 10: fully extended air pores. Colony surface is pigmented. Bar = 5 mm. Fig 8 - A cluster of mature air pores 30 d after inoculation. Bar = 10 mm.
Table 1 – Initiation and growth of air pores and rhizomorphs in Armillaria luteobubalina cultures

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>Description of air pores</th>
<th>Number of air pores (mean ± S.E.M.)</th>
<th>Number of rhizomorph tips (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>mycelium</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>white hyphal aggregates</td>
<td>25.0 ± 4.0</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>white hyphal aggregates</td>
<td>24.9 ± 3.0</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>7</td>
<td>lightly pigmented</td>
<td>21.0 ± 3.0</td>
<td>9.4 ± 0.6</td>
</tr>
<tr>
<td>9</td>
<td>lightly pigmented, emerging</td>
<td>21.6 ± 2.4</td>
<td>26.2 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>fully extended including (air pores that failed to develop fully)</td>
<td>21.8 ± 2.2</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Five culture plates were examined daily for 10 days after inoculation, and the numbers of air pores and rhizomorph tips were counted (n.d. = not determined). Greater numbers of air pores and variability on days 5 and 6 after inoculation indicate the difficulty in establishing whether an aggregation of hyphae would develop into an air pore.

Fig 9 – Day 21: air pore that failed to develop fully: apparently arrested at an intermediate stage of development. Bar = 0.25 mm.

Figs 10-11 - Relationship between emergent air pores and submerged rhizomorphs in Petri dish cultures.

Fig 10 - Surface view of colony 30 d after inoculation, showing clusters of air pores in three distinct locations (circles A, B and C). Bar = 10 mm. Fig 11 - Underside of the same Petri dish on day 30, with the image digitally reversed to allow direct comparison, showing three sites of rhizomorph initiation beneath air pore clusters. Rhizomorphs emerging from position A are short, while those from B and C have reached the edge of the plate. Bar = 10 mm. Fig 12 - Vertical section through the agar in the centre of a group of developing air pores showing a cluster of rhizomorphs. Bar = 1 mm. Fig 13 - Dish of well-grown rhizomorphs viewed from the underside. Bar = 5 mm.
Oxygen conduction in Armillaria
rapidly become consolidated into a pseudoparenchymatous crust with a developing rind at its surface (Fig 17). The outermost cortical cells that will form the rind are small in diameter (5-18 µm) and surrounded by extracellular lightly pigmented material, while inner cortical cells are larger (12-37 µm diam) and without pigmentation. A wide gas space containing scattered fine hyphae is present beneath this crust and separates it from the agar surface (Figs 17, 18).

The overall relationship between fully differentiated air pores, submerged rhizomorphs, and the radially spreading mycelium of the colony is shown in Fig 18. At higher resolution (Fig 19) the rind outside the air pores is seen to consist of tightly packed cells embedded in pigmented extracellular material, with no obvious gas spaces. Several fine hyphae branch from wider hyphae in the underlying cortex and traverse the rind. Some emerge into the atmosphere while others appear cut off or broken. Below the consolidated pseudoparenchyma layer of the crust there is a large gas space containing loosely arranged hyphae (Fig 18). It is widest beneath each air pore and tapers off with distance from air pore clusters. The medullary gas space in the base of rhizomorphs, which is also filled with loosely arranged hyphae, is continuous with this gas space underlying the colony crust (Figs 18, 22).

The relationship between the tissues of individual air pores and underlying rhizomorphs at maturity is shown most clearly in sections that are near the median longitudinal plane of the air pore (Fig 22). The medullary gas space of an obliquely sectioned rhizomorph lies immediately below the air pore. The rhizomorph is surrounded by a pseudoparenchymatous cortex except at its base. A mound of heterogeneous tissue has now differentiated at the base of the air pore (Fig 22). This consists of an outer pigmented rind and a narrow loosely arranged and discontinuous cortex surrounding a central region of fine sparsely arranged hyphae, with a large amount of extracellular space (Figs 20, 22). Groups of more or less parallel hyphae that appear to have their origin at the rhizomorph cortex (as inferred from serial sections) have grown across the gas space and entered the basal region of the air pore in a plane oblique to the colony surface (Figs 22-23). Many are cemented together in groups and anastomoses are frequent. This results in a complex arrangement of individual and aggregated hyphae bridging the gas space between the rhizomorph into the basal region of the air pore. Most of these appear to enter the air pore at its periphery, leaving a central region with a large amount of extracellular space containing loosely arranged fine hyphae, resembling the other gas spaces (Figs 20, 22, 23). Above the rind of the differentiated basal region the air pore comprises a cylinder of loosely arranged more or less parallel hyphae with a large amount of extracellular space that forms a continuum with the atmosphere (Figs 21, 22), as also shown at the earlier stage (Fig 15). Many of the hyphae are pigmented and/or have granular deposits on their walls, and patches where hyphae are cemented together by extracellular material are still apparent (Fig 21). The main differences between fully extended and fully mature air pores are the presence in fully mature air pores of many more pigmented hyphae throughout, and the differentiated basal region.

Continuity of gas space between the rhizomorph medulla and the atmosphere via the air pore depends crucially on the nature and amount of extracellular space in the rind specifically in the basal region of the air pore (Fig 22). Figure 24 shows that the tissue of the rind and cortex in the basal mound of the air pore is not consolidated, in contrast to that over the colony crust. There are many areas where there is a clear continuity between the gas space within the basal mound and the atmosphere via gaps in the rind (Fig 24). In many of these areas the cortex is poorly developed. Many of the hyphae that extend into the air pore from the rhizomorph cortex have grown through this unconsolidated rind and contribute to the hyphal mass of the cylinder of loosely arranged hyphae comprising the main body of the air pore. Many of these emergent hyphae have pigmented walls.

A schematic summary from sectioning data of the structural evidence (Fig 25) illustrates the spatial relationships of the various regions of the air pore at maturity, its relationships with the rhizomorphs, melanised crust and surrounding mycelium, and the distribution of gas spaces in these various regions and their interconnections.

**Oxygen conductance**

There was essentially no conductance to oxygen (average $4 \times 10^{-12} m^2 s^{-1}$) unless the rhizomorphs originating from the region of the base of the air pore were sliced, exposing their cut ends inside the electrode chamber (Table 2a); this was done in all subsequent measurements. Conductances to oxygen of plain agar blocks without mycelium, and agar blocks covered with pigmented mycelium, were very low.
(Table 2b, set 2), presumably representing the rate of oxygen leakage around the agar in the plastic tubes. Groups of air pores showed very much greater conductance to oxygen (Table 2b, set 2). Using cultures inoculated from a loop rather than an inoculum plug, conductance of groups of air pores again was significantly higher than pigmented mycelium or aerial rhizomorphs (Table 2b, set 3). Across all observations the conductance to oxygen per air pore was $198 \pm 27 \times 10^{-15}$ m$^3$ s$^{-1}$ (mean $\pm$ S.E. of the mean, $n = 25$, Table 2d). Aerial rhizomorph conductances were very variable, and were significantly greater than those of agar and pigmented mycelium in set 2, but not in set 3 (Table 2b). The increase in conductance from cutting off the tops of aerial rhizomorphs was not statistically significant (Table 2c).

**Discussion**

This paper reports the development of air pores at the substrate-air interface in pure cultures of *Armillaria luteobubalina* and confirms their role in conducting oxygen to rhizomorphs. These air pores are not borne on aborted side branches of rhizomorphs as were the ‘breathing pores’ of Smith & Griffin (1971), but are produced on recently inoculated mycelium and initiated before rhizomorphs. Nevertheless, there is a very clear spatial relationship between air pores and rhizomorphs. Of the names previously used, ‘breathing pores’ (Smith & Griffin 1971) and ‘air pores’ (Granlund et al. 1984; Intini 1987), we chose ‘air pores’ because gas exchange is presumably by diffusion rather than mechanical ventilation.

Air pores are distinct from sclerota or microsclerotia, despite initially being very similar. Many sclerotia and microsclerotia arise, like air pores, as more or less spherical loose hyphal aggregates, and later become pseudoparenchymatous and brown-pigmented in the outer part (Willetts 1972, 1978; Willetts & Bullock 1992). However, air pores continue their development to become elongate structures with a totally different internal structure from microsclerotia. Initiation of air pores early in mycelial development, before rhizomorphs, explains why rhizomorphs are not initiated in culture unless there is an interface between the mycelium and air: air pores must develop first. It explains Rishbeth’s (1968) observations that from their inception rhizomorphs grow much faster than hyphal tips. The timing of air pore development and their position suggest that they play a strategic role in gas exchange and aeration of *Armillaria* rhizomorphs, and perhaps associated mycelium as well.

There is no apparent barrier to oxygen diffusion among the hyphae of the air pore region above the rind, and oxygen would be expected to diffuse from the air throughout the intercellular spaces without impediment. The spaces in the rind at the basal region of the air pore would be expected to allow oxygen to diffuse readily into the mound of tissue beneath, which has large intercellular spaces. This gas space is continuous with that underlying the melanised crust of the colony, and is in direct continuum with the central canal of all the rhizomorphs via the gas space in the loose mycelium on the agar surface beneath the air pore. Its oxygen is replenished via the air pore, supplying the mycelium under the melanised crust and providing an extensive reservoir of oxygen for the rhizomorphs to draw upon. As oxygen diffuses through this gas space system as a whole it will be utilised by hyphae. The gas space will be depleted of oxygen, and gradients will be set up. Regions with the most hyphae relative to the volume of gas space they draw on will be the most depleted, as also will be those furthest from the atmosphere, and the depletion gradients will automatically direct the diffusion of oxygen to where it is needed. All this depends on the extracellular space being filled with gas and not liquid. This is difficult to assess from sections but it is obvious in fresh material. The silvery appearance of the rhizomorph canals in the agar indicates that they are filled with gas, and the melanised crust can be peeled away from the agar surface to reveal that the hyphae beneath are free and not consolidated by extracellular mucilage. There is gas space in rhizomorphs of *A. luteobubalina* (Smith & Griffin 1971; Pareek et al. 2001), and in *A. mellea* even at maturity when the medulla contains much hyphal tissue (Cairney et al. 1988).

The ultimate test of whether the system works is whether it conducts oxygen. Some aerial rhizomorphs, and all samples of air pores (attached to rhizomorphs that were cut below, near their apex) did so, indicating that air pores and rhizomorphs both conduct oxygen, and are connected. The difference between samples with and without cut rhizomorphs, however, does not allow us to conclude that the tip, or rind and cortex along the flanks of rhizomorphs, are impermeable to oxygen, since we know the agar in controls is not permeable. For similar reasons, we cannot assess from these data the oxygen permeability of the melanised rind. The closely packed hyphae of...
Figs 22-24 – Longitudinal sections through fully differentiated air pores fixed at 30d after inoculation. Fig 22 - Median longitudinal section: central region (a) of basal mound; rind of the colony (r). Occasional cemented patches (p) and variously sized intercellular spaces among more or less parallel loosely arranged hyphae above. Rhizomorph medullary gas space (m). Bar = 100 μm. Fig 23 - Detail from an adjacent section showing hyphal connections traversing the gas space from the rhizomorph cortex into the base of the air pore (a). Rind (r) at colony surface continues into base of air pore. Gas space with loose hyphae beneath rind and cortex: g. Medulla of base of rhizomorph: m. Bar = 100 μm. Fig 24 - LS showing detail of the basal region from an adjacent section. Large gaps in rind (arrows) and discontinuity of cortex allow connection from atmosphere to internal gas space. Bar = 50 μm.
Fig 25 – Diagram showing the spatial relationship between air pore, rhizomorph (R) and mycelial crust, comprising a rind (r) and cortex (c). Gas space in the rhizomorph medulla (m). Parallel air pore hyphae cemented in small patches (p) by extracellular material. Gas space (g) surrounds the hyphae growing on the agar surface under the mycelial crust. Mycelium also grows submerged in the agar (my). Bar = 0.5 mm.

Table 2 – Oxygen conductances in cultures of Armillaria luteobubalina

(a) Effects of cutting rhizomorphs beneath the agar

<table>
<thead>
<tr>
<th>Set no.</th>
<th>n</th>
<th>Air pores (with uncut rhizomorphs below): conductance</th>
<th>Number of air pores</th>
<th>Conductance per air-pore</th>
<th>Air pores (with cut rhizomorphs below): conductance</th>
<th>Conductance per air pore</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>6* ± 3</td>
<td>3 ± 0.3</td>
<td>2 ± 1</td>
<td>532± 132</td>
<td>182 ± 55</td>
</tr>
</tbody>
</table>

(b) Conductances of parts of cultures

<table>
<thead>
<tr>
<th>Set no.</th>
<th>n</th>
<th>Plain agar</th>
<th>Air with pigmented mycelium</th>
<th>Air pores</th>
<th>Number of air pores</th>
<th>Conductance per air pore</th>
<th>Aerial rhizomorph</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6</td>
<td>18* ± 3</td>
<td>21* ± 7</td>
<td>987± 86</td>
<td>4 ± 0.7</td>
<td>291 ± 83</td>
<td>187* ± 131</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>n.d.</td>
<td>7* ± 12</td>
<td>619± 81</td>
<td>5 ± 0.7</td>
<td>132 ± 27</td>
<td>34± 26</td>
</tr>
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</table>

(c) Effects of cutting on aerial rhizomorph conductance

<table>
<thead>
<tr>
<th>Set no.</th>
<th>n</th>
<th>Aerial rhizomorph, agar and rhizomorph cut below</th>
<th>Aerial rhizomorph, cut top</th>
<th>Aerial rhizomorph, cut top and below</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>6</td>
<td>29* ± 30</td>
<td>21* ± 7</td>
<td>212* ± 94</td>
</tr>
</tbody>
</table>

(d) Summary of oxygen conductances over all observations

<table>
<thead>
<tr>
<th></th>
<th>Agar conductance</th>
<th>Pigmented mycelium conductance</th>
<th>Aerial rhizomorph conductance</th>
<th>Air pores conductance</th>
<th>Number of air pores</th>
<th>Conductance per air pore</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 ± 3 (6)</td>
<td>12 ± 6 (18)</td>
<td>56 ± 28 (31)</td>
<td>679 ± 68 (25)</td>
<td>4 ± 0.3 (25)</td>
<td>198 ± 27 (25)</td>
<td></td>
</tr>
</tbody>
</table>

A difference in superscript letter indicates a statistically significant difference (p < 0.05) within a row, for the measured conductances only. All conductances are in 10⁻¹² m² s⁻¹; mean ± standard error of the mean are shown (n.d. = not determined).
tufts of hyphae bursting through the rind of rhizomorphs at the substrate air interface. The mature structure that we have found here is more complex, but nevertheless contains hyphae that have grown through a rind layer. Those hyphae which cross the sub-air-pore space into the basal tissue mound and thence through the spaces in the rind to form the apical cylinder of hyphae appear to have arisen from consolidated pseudoparenchyma around the site of origin of the developing rhizomorphs. The mature structure satisfies the requirement for longevity and survival and, since the crust and the air pore are both hydrophobic, should prevent waterlogging of the air pores even when it is sufficiently wet to cause pooling on the crust surface.

It appears that we have here an elaborate, sophisticated aeration system that extends and incorporates all the mycelial thallus. It meets the following requirements for a good aeration system: abundant gas space relative to tissue volume; good connectivity with all parts that need aeration (including longitudinal channels in conducting organs); and protected entry points that have longevity and survive adverse conditions, that resist waterlogging and yet also survive desiccation, and that have good support so that they do not collapse. The possession of a more elaborate gas space system than was formerly thought perhaps explains the success of some Armillaria spp. as pathogens in waterlogged situations. It may also be an important factor in pathogenesis in allowing a mycelium to grow on a wet root surface and to send hyphae or rhizomorphs as inoculum into live roots or cut stumps, in places where conditions may be hypoxic. It would seem that oxygen conduction in

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References


