A pressure gradient facilitates mass flow in the oomycete Achlya bisexualis

Abishek Muralidhar,1 Emma Swadel,1 Marjolein Spiekerman,2 Sandy Suei,1† Miranda Fraser,1† Manfred Ingerfeld,1 Ayelen B. Tayagui† and Ashley Garrill1

Correspondence
Ashley Garrill
ashley.garrill@canterbury.ac.nz

1School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand
2Plant Cell Biology, Wageningen University, PO Box 633 6700 AP Wageningen, The Netherlands

We have used a single cell pressure probe and observed movement of microinjected oil droplets to investigate mass flow in the oomycete Achlya bisexualis. To facilitate these experiments, split Petri dishes that had media containing different sorbitol concentrations (and hence a different osmotic potential) on each side of the dish were inoculated with a single zoospore. An initial germ tube grew out from this and formed a mycelium that extended over both sides of the Petri dish. Hyphae growing on the 0 M sorbitol side of the dish had a mean turgor (±SEM) of 0.53 ± 0.03 MPa (n=13) and on the 0.3 M sorbitol side had a mean turgor (±SEM) of 0.3 ± 0.027 MPa (n=9). Oil droplets that had been microinjected into the hyphae moved towards the lower turgor area of the mycelia (i.e. retrograde movement when microinjected into hyphae on the 0 M sorbitol side of the split Petri dish and anterograde movement when microinjected into hyphae on the 0.3 M sorbitol side of the Petri dish). In contrast, the movement of small refractile vesicles occurred in both directions irrespective of the pressure gradient. Experiments with neutral red indicate that the dye is able to move through the mycelia from one side of a split Petri dish to the other, suggesting that there is no compartmentation. This study shows that hyphae that are part of the same mycelia can have different turgor pressures and that this pressure gradient can drive mass flow.

INTRODUCTION

Turgor pressure is often cited as the driving force for tip growth in fungi and oomycetes (Kaminskyj et al., 1992; Money, 1997). A turgid protoplast is thought to push against the cell wall which, if plastic, will yield to the pressure, increasing the cell volume. As well as driving growth, differences in turgor in hyphae may also drive the movement of material from one part of a hypha or mycelia to another, via the process of mass flow. This may play a role in the tip growth process facilitating the movement of organelles towards the tip (Ramos-Garcia et al., 2009; Lew 2011).

Turgor gradients have been measured indirectly in rhizomorphs and cords of the fungi Armillaria mellea, Serpula lacrymans, Phanerochaete velutina and Phallus impudicus (Eamus & Jennings, 1984; Thompson et al., 1985) and directly between mycelia and sclerotia of Morchella esculentum (Amir et al., 1995). In Neurospora crassa hyphae, Robertson & Rizvi (1968) indirectly estimated a turgor gradient of 5 bar between the tip and basal regions. Mass flow has been shown in N. crassa hyphae by the observation of injected oil droplets and calculations suggest that only a small pressure gradient (of the order of 0.0005–0.1 bar cm⁻¹) would be required for such movement (Lew, 2005). These gradients could be set up by ion transport in different parts of the hyphae (Lew, 2011). It has been suggested that tip growth itself could also induce mass flow across mycelia through modulation of volume (Heaton et al., 2010).

In contrast, to the best of our knowledge, there have been no reports of mass flow in the other group of hyphal organisms, the oomycetes. One oomycete, Achlya bisexualis, lacks the ability to regulate turgor as it is unable to recover turgor after a hyperosmotic shock (Lew et al., 2004). It is

1Present address: The MacDiarmid Institute for Advanced Materials and Nanotechnology, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.
2Present address: Fonterra Co-operative Group Limited, 1 George Street #15-06, Singapore 049145.

One supplementary movie is available with the online Supplementary Material.
not known whether this is the case for all oomycetes, but in this species at least, turgor is simply determined by the external osmotic potential. Pressure differentials and mass flow could thus occur if hyphae extend into new environments that have different osmotic potentials.

Like the fungi, the oomycetes form mycelia, but they differ in that the mycelia do not have septa. Therefore, all the hyphae that constitute a single mycelium should effectively be a single cell, if that mycelium has originally arisen from a single zoospore. Thus, they represent a good experimental system to study mass flow in both single hyphae and in a mycelium. As turgor is determined by the external media, we hypothesize that two interconnected oomycete hyphae that are growing on different media will have different turgor pressures and thus have a pressure gradient between them which could drive mass flow. To investigate this, we have made turgor measurements, and made observations of movements of microinjected oil droplets and of small refractile vesicles in *A. bisexualis* hyphae. These hyphae were part of a mycelium that was generated from a single zoospore and was growing on split agar plates containing media of differing osmotic potential. The data presented suggest that pressure differentials can exist in a single mycelium and that these can enable mass flow. However, there is also involvement of the cytoskeleton in movement of at least some of the organelles as the small refractile vesicles appear to move independently of the pressure gradient.

**METHODS**

**Culture maintenance and preparation.** Stock cultures of the oomycete *A. bisexualis* Coker (a female strain isolated in New Zealand from *Xenopus laevis* dung and available from the University of Canterbury culture collection) were maintained on peptone-yeast extract-glucose (PYG) containing (%, w/v) peptone (0.125), yeast extract (0.125), glucose (0.3) and agar (2) that had been overlaid with sterile cellophane, as described previously (Walker et al., 2006). The cellophane was washed in three changes of boiling water to remove manufacturing residues.

**Generating and harvesting zoospores.** To obtain zoospores, the methodology of Hardham & Suzuki (1986) was modified slightly. Hyphal plugs were cut from stock cultures and placed onto a sterilized nappy liner (that had been boiled three times) overlying PYG media. Hyphal plugs were rotated on an orbital shaker (150 r.p.m.) for 24 h at 26 °C in the dark. To induce zoospore production, the broth was replaced by a mineral salts solution containing 5 mM KNO₃, 10 mM Ca(NO₃)₂, 4 mM MgSO₄, 20 μM FeSO₄ and 20 μM di-sodium EDTA. The mineral salts solution was replaced six times over the next 2 h, after which the flask was incubated overnight on the orbital shaker (200 r.p.m.) at 26 °C in the dark.

To harvest the zoospores, the mineral salt solution was filtered through two layers of sterile Kimwipes and encysted by vortexing. The zoospores were collected by centrifugation for 10 min at 800 r.p.m. The top three-quarters of the supernatant was removed and the pellet was resuspended in the remaining mineral salt solution. These were then stored at 4 °C prior to use.

Single zoospores were obtained by pipetting 300 μl of the zoospore suspension onto cellophane overlying PYG media in a Petri dish. These cultures were incubated for 24 h at 26 °C. Single germinated zoospores that were readily discernible were individually removed by aspirating the cellophane around the individual zoospore and transferring them to split Petri dishes. Split Petri dishes were purchased commercially (Sigma Aldrich), or alternatively a welled microscope slide was used that was compartmentalized with a divider made from aluminium foil. Prior to inoculation, PYG medium containing differing amounts of sorbitol was poured onto each side of the plate. The small square of cellophane that had the single zoospore on it was placed on top of the divider, such that the zoospore was in the centre of the divider. A drop of sterile PYG broth was placed on top of the zoospore to prevent dehydration. After the transfer, the split dishes were observed through a dissection microscope to ensure the transfer of just a single zoospore. After a few hours, germination of the zoospore produced a single mycelium that grew on both sides of the split dish. Measurements with a vapour pressure osmometer (Wescor 3500; Wescor) indicated the cellophane did not act as a conduit for water during the time-course of an experiment as there was no change in the osmotic potential on either side of the split Petri dish.

**Measuring turgor.** To measure turgor, a single cell pressure probe was used. Micropipettes were pulled from borosilicate capillaries (1.2 mm outer diameter, 0.69 mm inner diameter; Harvard Apparatus) using a pipette puller (model PC-10m; Narashige) and filled with low-viscosity silicon oil (Wacker AS4; Wacker-Chemie). The micropipette was inserted into a hypha that was growing on one side of a split Petri dish. A base pressure reading was taken before the micropipette was inserted into a hypha and upon entry a meniscus appeared and shifted part way up the pipette tip; the pressure that needed to be applied to force the meniscus back down to the tip of the pipette was taken as the turgor pressure of the hyphae. Any measurement where there was evidence of a wound response in the hypha was discarded as described by Walker et al. (2006).

**Studying mass flow.** To study mass flow, hyphae growing on split Petri dishes were mounted on a Zeiss IM35 inverted microscope (Carl Zeiss) and impaled by the pressure probe. The meniscus that formed upon impalement of a hypha was initially pushed to the tip of the micropipette by applying pressure, before increasing it carefully to microinject a single droplet of silicone oil into the hypha. A Nikon E5400 camera with a 10 × objective lens was used to obtain images of the hyphae at 30 s intervals to track the movement of this droplet. These images were aligned using the stack function of the MTrackJ plugin (Meijering et al., 2012) in ImageJ. Movement of the oil droplet was measured using the ruler tool in Adobe Photoshop CS6 (version 13.0).

**Tracking vesicle movements.** To study vesicle movements, split plates were mounted on a Leica L5S confocal microscope and imaged with a 50 × objective lens (numerical aperture 0.5). Time-lapse videos were recorded at 2 s intervals between frames for 2 min. Videos were then exported to Image J (version 1.47t; Schneider et al., 2012) and converted to image sequences. The speed and direction of movement of individual vesicles from each image sequence were measured and tracked manually using the ‘Add’ and ‘Measure’ features of the MTrackJ plugin of ImageJ.

**Neutral red staining.** To image dye movement through the mycelia, neutral red dye at a concentration of 0.1 mM was added to the agar media on one side of a split Petri dish or welled slide, prior to the media being poured onto the dish/slide. A single zoospore was then
transferred to the divider on cellophane as described above. The plates were then incubated at 20°C for 24, 48 and 72 h prior to imaging with a Leica MZ10F stereofluorescence microscope using bright-field and the green filter set (Green fluorescence G 546/10 nm 590 nm) for fluorescence images.

RESULTS

Growth and pressure differences in a single mycelium

We measured growth and turgor of hyphae that were part of a single mycelium that was growing on a split Petri dish with PYG media containing different concentrations of sorbitol (and hence with different osmotic potentials) on each side of the dish. To facilitate this, a single zoospore on a small thin strip of cellophane was inoculated onto the centre of the Petri dish. Upon germination, a germ tube emerged from the zoospore (Fig. 1 inset) that subsequently branched and formed mycelia that grew on both sides of the Petri dish (Fig. 1). At 24–45 h post-germination, hyphae were impaled with the pressure probe and pressure was measured. Hyphae on the 0 M side of the dish had a mean turgor pressure \((\pm \text{SEM})\) of \(0.53 \pm 0.03\ \text{MPa}\) \((n=9)\), which was significantly higher \((P<0.05, \ t\text{-test})\) than that in hyphae growing on the 0.3 M sorbitol side of the Petri dish, which had a mean turgor pressure \((\pm \text{SEM})\) of \(0.33 \pm 0.027\ \text{MPa}\) \((n=13)\) (Fig. 2). The respective turgor values were not significantly different (both \(P>0.05, \ t\text{-test}\)) from those in hyphae that were growing on standard (i.e. non-split) Petri dishes containing media with either 0 or 0.3 M sorbitol. The turgor values for these were \(0.49 \pm 0.13\ \text{MPa}\) \((0\ M\ \text{sorbitol})\) \((n=5)\) and \(0.3 \pm 0.12\ \text{MPa}\) \((0.3\ M\ \text{sorbitol})\) \((n=5)\) (Fig. 2). Upon impalement, some hyphae reacted with a rapid wound response, resulting in the formation of a plug of cellular material. Pressure readings from hyphae exhibiting a wound response or that gave any indication of a plugged or partially plugged micropipette were discarded (as described by Walker et al., 2006).

Growth of the mycelia, measured by the distance from the central divider to the furthest edge of the mycelia, was significantly greater \((P<0.05, \ t\text{-test})\) on the 0 M side of the split Petri dish than the 0.3 M sorbitol side. Distances after 48 h of growth were \((\text{mean} \pm \text{SEM}, n=5)\) 24 ± 2 and 19.2 ± 1.3 mm, respectively.

Microinjected oil droplets move down the pressure gradient

We next investigated whether this pressure gradient could drive mass flow. If oil were injected into a hypha on the 0 M side of the split Petri dish it would be expected to move in a retrograde direction (away from the tips of the hyphae) and towards the 0.3 M sorbitol side of the dish. Conversely, if oil were injected into hyphae on the 0.3 M sorbitol side of the split Petri dish it would be expected to move in an anterograde direction, towards the tips of those hyphae and away from the 0 M sorbitol side of the dish. We thus used the pressure probe to microinject

---

**Fig. 1.** A single mycelium growing on both sides of a split Petri dish. A rectangular piece of cellophane, which had a single zoospore on it, was placed over the central divider of the split dish. The two sides of the Petri dish contained PYG media that had differing sorbitol concentrations: 0 M (left side of the dish) and 0.3 M (right side of the dish). Upon germination the zoospore put out a germ tube (inset), which formed branching hyphae that grew on both sides of the dish. Bars, 0.2 μm and (inset) 10 μm.

**Fig. 2.** Turgor pressure in hyphae that were growing on media containing different concentrations of sorbitol. On the left the bars represent data from hyphae that were growing on split Petri dishes with 0 M sorbitol on one side of the dish and 0.3 M sorbitol on the other. The bars on the right represent data from mycelia that were grown on regular Petri dishes that had PYG media containing either 0 or 0.3 M sorbitol. Values represent mean \(\pm \text{SEM}\).
silicon oil into hyphae and tracked the speed and direction of the movement of the oil droplets (Fig. 3). The data presented in Table 1 show that in 71% of hyphae (10 of 14 hyphae) on the 0 M sorbitol side of a split Petri dish, the droplets moved in a retrograde direction as would be predicted by the pressure gradient. In 14% of hyphae tested (two of 14), the droplets showed no movement with no discernible wound response and in the remaining 14% of hyphae tested (two of 14), the droplets moved in the opposite direction to what was expected (i.e. in an anterograde manner). Conversely, in 77% of hyphae (10 of 13) growing on the 0.3 M side of a split Petri dish, microinjected oil moved in an anterograde direction as would be predicted by the pressure gradient. Of the remaining hyphae, 15% (two of 13) showed oil movement in a retrograde direction and 8% (one of 13) showed no movement, again with no obvious wound response. The speed of oil droplet movement was not significantly different on the two sides of the Petri dish with mean speeds of 0.07 \( \text{m s}^{-1} \) (0 M sorbitol side) and 0.05 \( \text{m s}^{-1} \) (0.3 M sorbitol side). In some hyphae, the movement of oil droplets was slowed and then eventually stopped by an accumulation of vesicles and a wound response (Fig. 4). These hyphae were excluded from the dataset that is presented in Table 2, as although the direction of mass flow could be determined, the rate of mass flow could not be accurately measured.

In contrast to the movement of oil droplets, the movement of small refractile vesicles was random, irrespective of which side of the Petri dish the hyphae were growing (Fig. 5, Movie S1 [available in the online Supplementary Material], Table 2). These organelles were chosen for observation due to the relative ease with which they could be seen and tracked with the microscope. On the 0 M sorbitol side of the Petri dish, 51.5% (696 of 1352) of vesicles that were tracked moved in an anterograde direction and 48.5% (656 of 1352) moved in a retrograde direction. Similarly, on the 0.3 M sorbitol side of the Petri dish, 47.3% of vesicles (639 of 1351) moved in an anterograde direction and 52.7% (712 of 1351) moved in a retrograde direction. The speed of these movements was approximately an order of magnitude faster than the movement of the injected oil droplets (Table 2).

**A. bisexualis mycelia lack compartmentalization**

The above results suggest that it is possible to have a pressure differential across a mycelium. However, if this were to drive mass flow then that mycelium would need to be a continuum and there would be no compartmentation of the hyphae on one side of the dish from those on the other. The fact that oomycetes are coenocytic would suggest that this is the case. Nevertheless, the accumulation of vesicles as depicted in Fig. 4, and the previous

---

**Table 1. Direction of oil movement driven by the pressure gradient in the majority of hyphae**

<table>
<thead>
<tr>
<th>Oil movement</th>
<th>Side of the split Petri dish where the oil was microinjected into the hypha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted direction of oil movement if mass flow is occurring</td>
<td>0 M sorbitol side</td>
</tr>
<tr>
<td>Number of hyphae showing anterograde oil movement</td>
<td>Retrograde</td>
</tr>
<tr>
<td>Mean speed of anterograde movement (( \mu \text{m s}^{-1} ))</td>
<td>2 of 14 (14%)</td>
</tr>
<tr>
<td>Mean speed of retrograde movement (( \mu \text{m s}^{-1} ))</td>
<td>0.07</td>
</tr>
<tr>
<td>Number of hyphae showing retrograde oil movement</td>
<td>10 of 14 (71%)</td>
</tr>
<tr>
<td>Mean speed of retrograde movement (( \mu \text{m s}^{-1} ))</td>
<td>0.02 ( \pm ) 0.01</td>
</tr>
<tr>
<td>Number of hyphae showing no oil movement</td>
<td>2 of 14 (14%)</td>
</tr>
</tbody>
</table>
description of deposition of wall material by a retracting protoplast in a plasmolysed hypha (Chitcholtan et al., 2012), suggest that it may be possible for individual hyphae to become compartmentalized and therefore not be part of the mycelial continuum. To investigate whether the mycelia on the split Petri dishes were truly coenocytic, we tracked the movement of the dye neutral red. Initial attempts at placing a drop of neutral red solution over hyphae on one side of the plate or alternatively at microinjecting the dye proved unsuccessful as it appeared that insufficient dye was taken up to track movement. The inclusion of neutral red in the agar media on the 0 M side of the Petri dish did enable sufficient dye uptake to track movement. Subsequent inoculation of a split dish in the manner described above gave a mycelium that grew on both sides of the dish. The dye was taken up into hyphae and this then was visible in hyphae on the other side of the plate (Fig. 6).

**DISCUSSION**

Turgor pressure is often described as the driving force for tip growth in hyphae of fungi and oomycetes. As the tip yields to pressure, the volume of the hypha increases. In fungi, in addition to forcing the tip to yield, turgor may play an additional role in that if there are pressure gradients along the hypha, then mass flow may move the cytoplasm in an anterograde direction. Mass flow, as indicated by the movement of injected oil droplets, has previously been shown in *N. crassa* hyphae and the velocity of these movements was dependent on the pressure (Lew, 2005). Calculations suggest that mass flow could be facilitated by only a small pressure gradient (of the order of 0.0005–0.1 bar cm\(^{-1}\)) (Lew, 2011). Such flow has been suggested to play a role in positioning of nuclei, mitochondria and vacuoles in *N. crassa* (Ramos-García et al., 2009; Abadeh & Lew, 2013). Pressure gradients could be set up by the activity of membrane transport proteins at different locations along the hyphae, either through differential distribution or through differential activation of ion channels in the plasma membrane (Silverman-Gavrila & Lew, 2002).

In the present study, we have used a pressure probe to demonstrate that mycelia of a single oomycete can have a pressure gradient between their hyphae, if those hyphae are growing on different media. The movement of microinjected oil suggests that this pressure gradient can drive mass flow. This raises the question of whether pressure gradients and mass flow could play a role in tip growth in oomycetes, driving the forward movement of the cytoplasm. In nature, a pressure gradient could be set up if the hyphae were to extend into an area with a more negative osmotic potential. This would essentially be analogous to the experimental conditions that we have set up with the split Petri dishes. In addition, a pressure gradient could be generated, as per the fungi, through asymmetric distributions of membrane transport proteins (Garrill et al., 1992). Lew (2011) has suggested that hyphae at the edge of the mycelia could extend into new areas, while further back maximal nutrient uptake occurs, leading to water influx that would then drive mass flow of the cytoplasm.

**Table 2. Direction of movement of small refractile vesicles independent of the pressure gradient**

<table>
<thead>
<tr>
<th>Vesicle movement</th>
<th>Side of split Petri dish where the vesicle was observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 M sorbitol side</td>
</tr>
<tr>
<td>Anterograde movement</td>
<td>696 of 1352 organelles (51.5 %)</td>
</tr>
<tr>
<td>Mean speed (μm s(^{-1}))</td>
<td>1.8 ± 0.46</td>
</tr>
<tr>
<td>Retrograde movement</td>
<td>656 of 1352 organelles (48.5 %)</td>
</tr>
<tr>
<td>Mean speed (μm s(^{-1}))</td>
<td>1.6 ± 0.65</td>
</tr>
</tbody>
</table>
toward those leading hyphae. As such, mass flow could create macropolarity in the mycelia (Lew, 2011).

The ability to set up a pressure gradient would be consistent with an ability to regulate turgor. In our earlier work studying turgor regulation in *A. bisexualis*, we exposed hyphae to both a hyper- and a hypo-osmotic shock (Lew et al., 2004). As hyphae were unable to recover from the hyperosmotic shock, it was concluded that they were unable to regulate turgor. However, it is possible that the hyperosmotic shock (media supplemented with 0.5 M sorbitol) was too great for the hyphae to recover from, given more recent work on other Stramenopile organisms. Studies on the yellow green algae *Vaucheria erythrospora* and *Vaucheria repens*, which phylogenetically share a relatively recent common ancestor to *A. bisexualis*, suggest that they are able to recover turgor after a hyperosmotic shock (Muralidhar et al., 2013).

---

Fig. 5. Micrographs showing organelle movements that were tracked using the track and measure feature of MTrackJ plugin in ImageJ. Tracking of organelle movement revealed no difference in the direction or speed of cytoplasmic streaming irrespective of which side of the split Petri dishes the hyphae were growing. The red and blue plots indicate the movements of four representative organelles, two that displayed anterograde movement (blue) and two that displayed retrograde movement (red). Bar, 20 μm.

---

Fig. 6. Mycelia that were generated from a single zoospore were coenocytic, as indicated by the movement of the dye neutral red through hyphae from one side of the split Petri dish to the other. In the example shown (a, b), the left side of the split Petri dish contains PYG supplemented with neutral red and 0 M sorbitol and the right side contains PYG supplemented with 0.3 M sorbitol, but with no neutral red. A single zoospore was inoculated onto the divider (the central translucent area) and after 72 h had formed the mycelia with hyphae on both sides of the dish (a). A fluorescence image of the mycelia showed neutral red in hyphae on both sides of the dish (b). Bar, 0.5 mm.
The magnitude of the shock from which they are able to recover differs between the two species, which is consistent with the environments in which these species are found: V. erythropora is present in estuaries and V. repens grows in freshwater. Thus, it is possible that the hyperosmotic shock that was given to A. bisexualis was too great for it to recover its turgor. It is also worth noting that with respect to a hypo-osmotic shock, turgor did not change in A. bisexualis, although this was put down to experimental difficulties in accurately administering a hypo-osmotic shock. A hypo-osmotic shock administered to V. erythropora produced an initial increase in turgor and then a return to the original turgor level (S. Seizova and A. Garrill, unpublished data). Clearly, more work is needed to better determine whether the oomycetes are capable of turgor regulation and thus be able to generate pressure gradients that can drive mass flow. Certainly as hyphae extend there is an increase in hyphal volume, and so nutrient uptake must occur for turgor to remain constant. This may be due to some form of turgor regulation (for example, a HOG-like or os-like mitogen-activated protein kinase cascade) or a passive outcome of continued cytoplasmic synthesis.

The direction of oil movement was as would be predicted by the pressure gradient in the majority of hyphae [71 % (0 M sorbitol side) and 77 % (0.3 M sorbitol side)]. We are unsure why, in some hyphae, there was movement in the opposite direction to that predicted [14 % (0 M sorbitol side) and 15 % (0.3 M sorbitol side)]. One possible explanation is that, if the hyphae are able to set up pressure gradients themselves, then in some hyphae this was set up sufficiently to overcome the gradient that was imposed by the experimental conditions on the split Petri dishes. In those hyphae in which there was no movement, there may have been a wound response and accumulation of vesicles that we could not discern through the microscope that prevented movement of the droplets.

Tip growth rates in fungi and oomycetes can be variable but are usually between 1 and 10 μm min⁻¹ (McKerracher & Heath, 1987; Jackson, 2001). This is comparable to the speed that the oil droplets were moving at in the hyphae and is consistent with a role for mass flow in moving the cytoplasm forward as the tip extends. Within this moving cytoplasm individual organelles can still move via active transport, given our observations of the small refractive vesicles. The small refractile vesicles moved in both anterograde and retrograde directions, irrespective of the pressure gradient and thus, for these organelles at least, molecular motors are likely to move them along cytoskeletal tracks. The movement of these vesicles occurs around an order of magnitude faster than the movement of the injected silicon oil. These rates are comparable to what has previously been reported for organelle movement along microtubules in N. crassa where the mean velocities for mitochondria and particles were 1.4 and 2.0 μm s⁻¹, respectively (Steinberg & Schliwa, 1993). Thus, our data suggest that irrespective of any mass flow, active transport of organelles is also likely to be important with respect to their position along the hypha.

If we consider the aseptate hyphae of A. bisexualis as tubes, it is possible to try to predict the rate of movement of an oil droplet given our measured pressure gradient and compare this with the observed rates that we present in Table 1. To do this we use the Hagen–Poiseuille equation:

\[ v = \frac{\pi R^4 |\Delta P|}{8 \eta L} \]

where \( R = \) radius of the hyphae, \( \Delta P = \) pressure gradient, \( \eta = \) viscosity and \( L = \) hyphal length. If we assume \( R = 1.3 \times 10^{-5} \) m (taken from the radius of the hypha in Fig. 3), \( \Delta P = 0.2 \times 10^6 \) Pa (from the pressure gradient in Fig. 2), \( \eta = 1 \times 10^{-3} \) PAs (see below) and \( L = 1 \times 10^{-3} \) m (taken from the width of the mycelium in Fig. 1), this would give a rate of \( 2.2 \times 10^{-8} \) m s⁻¹. This is within the range of our measured rates of \( 2 \times 10^{-8} \) to \( 8 \times 10^{-8} \) m s⁻¹ (Table 1). While the predicted and measured rates of oil movement are comparable, we do advise some caution. The value of viscosity that we used was based on that reported by Fushimi & Verkman (1991) for the fluid cytoplasm of fibroblasts. This value was also used by Lew (2005) for calculating the pressure gradients that would be needed to sustain mass flow rates in N. crassa hyphae. However, the oomycete hyphae are likely to be more viscous. Furthermore, given their level of vacuolation and the presence of cytoskeleton-anchored organelles, there are additional factors that might impede the movement of oil droplets. It is also possible that the mycelia do not behave as simple tubes and that the branched network evident in Fig. 1 has an impact on the rate of flow. More pertinent than the use of the simple Hagen–Poiseuille equation might be modelling such as that used to describe blood flow in vascular networks (McDougall et al., 2002).

In summary, we present data that suggest that a pressure gradient can exist in an oomycete mycelium and that this can drive the movement of microinjected oil droplets from the area of high pressure to the area of low pressure. This would suggest that mass flow can occur in an oomycete mycelium but that this might require fine tuning of hyphal turgor. While previous studies have suggested that oomycetes cannot regulate turgor, it is possible that the methodologies used in previous work (Lew et al., 2004) may have missed the finer elements of turgor regulation. Clearly, the topic of turgor regulation in these organisms is one that warrants further investigation. Despite the possible mass movement of cytoplasm it is likely that organelle movements also occur by active transport along cytoskeletal tracks.

ACKNOWLEDGEMENTS

This work was supported by a University of Canterbury Internal Research Grant. We thank Dave Collings, Tijs Ketelaar and Tim

...
REFERENCES


Edited by: A. Herrera-Estrella