# Growth-induced mass flows in fungal networks: Supplementary Information.

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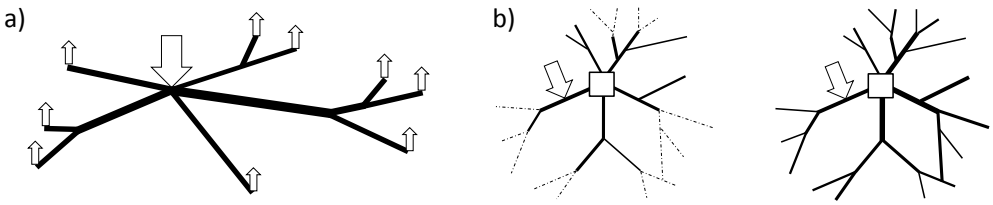
# 1 Comparison of models




| | **a) Uniform Model** | **b) Time Lapse Model** |
|---|---|---|
| **Input** | A network where each cord has a measured length, and one node is designated as the inoculum. | A pair of networks such that the 'earlier network' grows into the 'later network' over a period of time $t$. Each cord in these two networks has a measured length and volume. |
| **Assumptions** | 1) Water uptake only occurs at the inoculum.<br>2) There is a unit current in every tip.<br>3) All cords in the network have unit resistance per unit length.<br>4) Fluid follows the path of least resistance.<br>5) The total volume of fluid is conserved. | 1) Water uptake only occurs at the inoculum.<br>2) The current flowing into a cord minus the current flowing out of a cord equals the rate of change of volume for that cord.<br>3) The conductance of cords is proportional to their cross-section.<br>4) Fluid follows the path of least resistance.<br>5) The total volume of fluid is conserved. |
| **Output** | Each cord has a current. In tree-like networks, the current is equal to the number of cords 'downstream'. | Each cord has a current, and a mean velocity of mass flow. The current in the cord of interest is calculated without referring to the change in volume of that cord. |

Figure 1: **Models of growth-induced mass flow.**

# 2 Parameters and solutions of the time lapse model

**Calculating the pressure at each node**
Recall that the current in a cord is equal to the pressure drop times the conductance of the cord. It follows that at each node $i$

$$\sum_j (p_i - p_j) C_{ij} = q_i, \tag{1}$$

where $p_i$ is the pressure at node $i$, $q_i$ is the net current flowing out of node $i$ and $C_{ij}$ is the conductance of the cord between nodes $i$ and $j$. We can summarise the relationship expressed by Equation (1) by defining the following symmetric matrix **A**:

$$\begin{aligned} A_{ii} &= \sum_k C_{ik} \quad \text{and} \\ A_{ij} &= -C_{ij}. \end{aligned} \tag{2}$$

Where $\overline{p}$ is the vector form of the pressures $p_i$ and $\overline{q}$ is the vector form of the currents $q_i$, we know by Equation (1) that $\overline{p}$ satisfies the equation

$$\mathbf{A}\overline{p} = \overline{q}. \tag{3}$$

Since each row of **A** sums to zero, **A** has no inverse and we cannot uniquely determine $\overline{p}$. However, it is the pressure differences which are of interest, not the absolute value of $\overline{p}$. We are are therefore free to fix the pressure at any one node. Once we have done this, Equation (3) uniquely determines the pressure at every other node. More specifically, suppose that our network contains $N$ nodes. In that case Equation (3) represents a system of $N$ linear equations in $N$ unknowns, but as each row and column sums to zero, the $N$'th equation is a linear combination of the other $N-1$ equations. Setting $p_N = 0$ gives us a system of linear constraints on the values for $p_1, \ldots, p_{N-1}$, namely

$$\begin{pmatrix} A_{11} & \cdots & A_{1(N-1)} & A_{1N} \\ \vdots & \ddots & \vdots & \vdots \\ A_{(N-1)1} & \cdots & A_{(N-1)(N-1)} & \\ A_{N1} & \cdots & & A_{NN} \end{pmatrix} \begin{pmatrix} p_1 \\ \vdots \\ p_{N-1} \\ 0 \end{pmatrix} = \begin{pmatrix} q_1 \\ \vdots \\ q_{N-1} \\ q_N \end{pmatrix}.$$

This is equivalent to the following system of linear constraints on the values for $p_1, \ldots, p_{N-1}$,

$$\begin{pmatrix} A_{11} & \cdots & A_{1(N-1)} \\ \vdots & \ddots & \vdots \\ A_{(N-1)1} & \cdots & A_{(N-1)(N-1)} \end{pmatrix} \begin{pmatrix} p_1 \\ \vdots \\ p_{N-1} \end{pmatrix} = \begin{pmatrix} q_1 \\ \vdots \\ q_{N-1} \end{pmatrix} \tag{4}$$

plus an additional equation

$$A_{(N-1)1}p_1 + A_{(N-1)2}p_2 + \ldots + A_{(N-1)(N-1)}p_{N-1} = q_N. \tag{5}$$

Because the rate of water uptake is equal to the rate of growth, the total in-current is equal to the total out-current. In other words, $\sum q_i = 0$. We therefore know that $q_N = -q_1 - \ldots - q_{N-1}$. We can use Equation (4) to eliminate each of the $q_i$ (as $q_i = A_{i1}p_1 + A_{i2}p_2 + \ldots + A_{i(N-1)}p_{N-1}$), and this shows that Equation (5) is redundant. In other words, given that the total in-current is equal to the total out-current, Equation (4) is sufficient to determine our solution. It contains $N-1$ independent linear equations in $N-1$ unknowns, which uniquely determines the values $p_1, \ldots, p_{N-1}$.

**The parameters of the time lapse model**

We require the parameter $\delta$ because newly forming cords must have some conductance, or else they would not be connected to the rest of the network, and their growth could not induce currents. The precise value of $\delta$ is not significant, but the calculation of currents requires that all cords have a non-zero conductance. Alternatively, we could remove the parameter $\delta$ by supposing that the conductance of cord $ij$ is $\frac{\sigma(u_{ij}+v_{ij})}{2l_{ij}}$. Since an extension of an cord can be represented as a new cord, the length of cords does not change. Hence this formula is equivalent to supposing that the cross-sectional area of cord $ij$ is half way between the cord's cross-section in the initial network and its cross -section in the resulting network. However, in that case cords that increase in size would carry more current than cords that do not, precisely because in this alternate model, cords that become large are assigned a larger conductance. This is undesirable (and not the method we employ), because we want an unbiased estimate of current to correlate with changes in area. We could also have avoided the parameter $\delta$ by saying that newly forming cords are not part of the network, but in that case we would have to devise an additional algorithm for assigning out-current to the cords from which the new cords grow: something we 'get for free' by using our model with $\delta$.

The parameter $\sigma$ specifies the conductance per unit area for each cord in the network. The value of $\sigma$ does not affect the calculated currents, as it is the relative conductance of cords that determines the distribution of currents. However, the pressure gradients predicted by this model will be inversely proportional to $\sigma$.

Varying the parameter values $\sigma$ and $\delta$ does not significantly affect our calculation of the currents induced by growth. However, we do need to make a significant assumption concerning the relationship between conductance and cross-sectional area. We assume that conductance is proportional to cross-sectional area, but this assumption is only of consequence when the network provides a number of alternate routes between the site of water uptake and the site of growth. Because our boundary conditions are fixed in terms of currents, the current in part of a branching tree will not depend on the conductance of the network. For example, if there is a certain current flowing out of the tips of a branching tree, where there is only route to each tip, the specified current has to flow through a given sequence of cords regardless of their conductance. In practice, the fungal networks we are studying are composed of branching trees connected to a net-like core. Within the netlike structure more current will tend to flow through the larger cords, as fluids naturally follow the 'path of least resistance'.

# 3 Pressure gradients and wall shear stress

**Viscosity, velocity and laminar flow**

To assess whether the flows in fungi are laminar, we first estimate the Reynolds number. This is defined as the ratio of inertial to viscous forces (Nobel, 1991), and

$$\mathrm{Re} = \frac{\rho v d}{\eta}, \tag{6}$$

where $\rho$ is the density of the fluid (approximately equal to that of water: $1\mathrm{gml}^{-1}$), $v$ is the mean velocity of the fluid, $d$ is the diameter of the hypha or transport vessel, and $\eta$ is the dynamic viscosity of the fluid. The viscosity of cytoplasm is reported to be similar to that of water, $1\mathrm{gs}^{-1}\mathrm{m}^{-1}$ (Fushimi & Verkman, 1991), but the fluids within fungi could plausibly be as viscous as 1.5 M sucrose solution, which has a viscosity of $7\mathrm{gs}^{-1}\mathrm{m}^{-1}$ (Bancal & Soltani, 2002). Here we use a value of $\eta = 2\mathrm{gs}^{-1}\mathrm{m}^{-1}$.

The velocity of fluid flow and the diameter of the tubular vessels within the cords may vary considerably throughout the fungi, but even the most extreme plausible values yield a Reynolds number several orders of magnitude smaller than one (Lew, 2005). This tells us that smooth, laminar flow is occurring (Nobel, 1991; Lew, 2005).

**Pressure gradients and speed of flow**

Suppose that a cord has a cross-sectional area $a$ and carries a current $f$. The mean speed of flow within the cord as a whole will be $f/a$. However, only a fraction $\lambda$ of the cross-sectional area of each cord will be occupied by the interior of the vessels that carry mass flows. Thus the mean speed within the vessels will be $f/\lambda a$. If we want to use our model to obtain estimates for the speeds of mass flow we need to choose an appropriate value for $\lambda$. Similarly, if we want estimates of the pressure gradients we need to choose a sensible value for the parameter $\sigma$. Here we assume that $\lambda = 0.5$, and that the tubes carrying mass flows all have an internal radius $r = 6\mu\mathrm{m}$. In this case

$$v = \frac{f}{\lambda a}, \tag{7}$$

where $v$ is the mean velocity of mass flow, $f$ is the current through the cord and $\lambda a$ is the cross-sectional area through which the current passes. The Hagen-Poiseuille equation tells us that the pressure gradient $\frac{\mathrm{d}P}{\mathrm{d}x}$ must satisfy the equation

$$\frac{\mathrm{d}P}{\mathrm{d}x} \equiv \frac{\Delta P}{l} = f\frac{8\eta}{n\pi r^4} = f\frac{\pi r^2}{\lambda a}\frac{8\eta}{\pi r^4} = v\frac{8\eta}{r^2}, \tag{8}$$

where $\eta$ is the dynamic viscosity of the fluid, $n$ is the number of tubes within the cord and $r$ is the radius of each tube.

**Deriving pressure gradients from the time lapse model**

To estimate the pressure gradients needed to drive the flows predicted by the time lapse model, we need estimates for the conductances of cords. Here we assume that half the cross-sectional area of each cord was occupied by transport vessels of internal radius of $6\mu$m, and we also assume that the viscosity of the moving fluids was $2\text{gs}^{-1}\text{m}^{-1}$ (Eamus, 1985; Howard, 1981; Lew, 2005). By Equation (8), these values give us the relationship

$$\frac{\mathrm{d}P}{\mathrm{d}x} \approx 4v \times 10^{-5},$$

where $v$ is measured in $\mu\text{m s}^{-1}$, and $\frac{\mathrm{d}P}{\mathrm{d}x}$ is measured in bar $\text{cm}^{-1}$. Our estimate of conductance per unit area tells us that maintaining a velocity of $1\mu\text{m s}^{-1}$ only requires a pressure gradient of around $4 \times 10^{-5}$ bar $\text{cm}^{-1}$. This is very small compared to the hydrostatic pressure of hyphae, which is about $4 - 5$ bar (Amir *et al*., 1995b; Lew *et al*., 2004a; Lew, 2005; Money, 1997). Pressure gradients of this scale could plausibly be maintained over tens or even hundreds of meters.

In *Neurospora crassa* the cytoplasm moves forward with the growing tips at a rate of $0.2 - 0.5\mu\text{m s}^{-1}$. Mass flows in the hyphae behind the tips typically reach $5\mu\text{m s}^{-1}$, and currents as fast as $60\mu\text{m s}^{-1}$ have also been directly observed (Lew, 2005). In *P. velutina* the highest reported velocities are around $900\mu\text{m s}^{-1}$ (Wells *et al*., 1995b), though obtaining accurate estimates of velocity is a major challenge. Our estimate for the conductance of cords implies that maintaining a velocity as large as $900\mu\text{m s}^{-1}$ requires a pressure gradient of around $0.04$ bar $\text{cm}^{-1}$. This is a significant pressure gradient compared to the hydrostatic pressure of hyphae, and pressure gradients of this scale could only be sustained over a few tens of centimeters.

**Deriving wall shear stresses from the time lapse model**

Fluid flows induce wall shear stresses on the vessels within cords. A good estimate for the wall shear stress $\tau$ can be obtained using the formula

$$\tau = \frac{4\eta v}{r}, \tag{9}$$

where $\eta$ is the dynamic viscosity of the fluid, $v$ is the mean velocity of fluid flow and $r$ is the radius of the vessels within cords (Sherman, 1981). Using the previously indicated values for $\eta$ and $r$ tells us that

$$\tau \approx v \times 10^{-3},$$

where the mean velocity $v$ is measured in $\mu$m s$^{-1}$ and the wall shear stress $\tau$ is measured in pascals or Nm$^{-2}$. By way of comparison, the wall shear stresses in mammalian arterial systems are in the range $0.2 - 2$Nm$^{-2}$ (Kamiya *et al*., 1984; Rodbard, 1975).

It is widely accepted that a local adaptive response to wall shear stress is a key mechanism that enables the optimisation of mammalian vascular systems (Kamiya *et al*., 1984; Rodbard, 1975; Sherman, 1981). By analogy it is certainly plausible that hyphae could detect and respond to velocities of the order $100 - 1000\mu$m s$^{-1}$, as we estimate that such currents would induce wall shear stresses of the order $0.1 - 1$Nm$^{-2}$. It is less likely that fungi can detect the difference between much slower moving currents, as the corresponding changes in wall shear stress would be very small.

# 4 Further Results

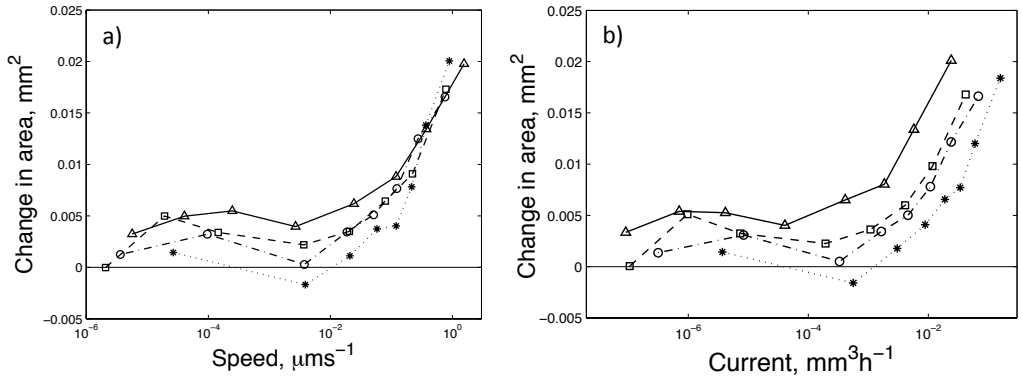



Figure 2: **Correlation between the change in cross-sectional area and the predicted flow for edges of similar thickness.** The data from all experiments and all time steps were partitioned into four bins according to the thickness of the cords. The lines were generated by data from cords with cross-sectional area less than $0.02$mm$^2$ ($\triangle$), cross-sectional area between $0.02$mm$^2$ and $0.04$mm$^2$ ($\square$), cross-sectional area between $0.04$mm$^2$ and $0.06$mm$^2$ ($\circ$) and cross-sectional area greater than $0.06$mm$^2$ ($*$). Each of these bins was then subdivided into ten subsets of equal size, according to the calculated speed (a) or current (b). Each marker indicates the mean speed and mean change in cross-sectional area for one of these subsets.

**a)** Regardless of cross-sectional area, there was a similar relationship between the speed of flow in a cord and the change in cross-sectional area. Note that fewer large cords have a very low speed (less than $10^{-2}$, say).

**b)** Larger cords tended to carry more current, but regardless of cross-sectional area, there was a similar correlation between predicted current and the measured change in area.

# 5 Growth, Mass Flows and Nutrient Translocation

The currents calculated by the time-lapse model represent a minimal total flux, found by calculating the unique set of currents that account for the observed changes in cord volume, while minimising the work required to overcome viscous drag. A different distribution of currents could be established by additional transport mechanisms, though the conservation of volume is a constraint on all possible patterns of fluid flow. In particular, osmotic gradients between adjacent fungal vessels could produce flows that differ from the calculated minimum. Indeed, if transport were solely driven by apical mass flow, it would be difficult to account for simultaneous bi-directional movement and concurrent basal transport (Fricker *et al*., 2007; Lindahl *et al*., 2001; Olsson & Gray, 1998; Tlalka *et al*., 2003; Tlalka *et al*., 2008). However, any currents beyond those predicted by our model necessarily require the fungi to do additional work.

Our central claim is that nutrients loaded into the mass-flow transport pathway will move towards the hyphal tips at a velocity that is partly determined by the volume of 'downstream' growth, and the network architecture. However, to actually reach the tips, the nutrients need to move at a greater speed than the column of water that advances in tandem with the tips (see Fig. 1a). This will be achieved in part automatically at a slow rate by diffusion, but could be increased substantially by local evaporation at the tips, the movement of vesicles by motor proteins, or by other means of active transport.

Finally, we note that as growth, mass-flow and nutrient transport are coupled, there may be an interesting interaction between nutrient availability, control of branching and nutrient transport. It is well known that the rate of hyphal branching increases when tips encounter resource rich environments (Gow and Gadd, 1995). Turgor pressure and the build up of vesicles have both been implicated, but whatever the mechanism behind this response, differential branching rates may constitute a unique kind of foraging strategy. In resource-poor regions tips rarely branch, and consequently tip growth in resource-poor environments induces relatively low flux densities in the trailing hyphae. In resource-rich environments, where branching rates are high, flux densities will also be high. We speculate that regions of the mycelium that do not receive a sufficient supply of resources will regress, and that the resulting networks constitute an efficient response to the given resource environment.