Auctus Electrica: Voltage field dependence of *Neurospora crassa* growth[1].

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OBJECTIVE

To determine the effect of external voltage fields on hyphal growth of the fungus *Neurospora crassa*.

[1]

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INTRODUCTION

Neurospora crassa is a mycelial fungus that is known to have polarized tip growth. The growth is associated with electrical currents and electrical fields that are generated near the growing hyphal tips (McGillvray and Gow, 1986; Gow, 1984). The exact mechanism of establishing and maintaining polarity of hyphal growth is unclear. Some studies suggest that growing hyphae tend to generate current from the hyphal tips to the more distal regions of the hyphae (Crombie, Gow and Gooday, 1989). Other studies found that current flow in growing hyphae is in the opposite direction —from distal hyphae to the growing tips (McGillvray and Gow, 1986). Experiments involving analysis of natural electric fields near hyphae showed high variation in electric field strength, which depends greatly upon the environment (Crombie, Gow and Gooday, 1989).

In order to gain a better understanding of the nature of polarized hyphal growth, the effects of external electric field on fungal growth have been investigated. In some cases, applying external electric fields does influence growth polarity of biological organisms Zygotes of the algae Fucus, Pelvetia and Ulva, the moss Funaria as well as spores of Equisetum all became polarized under the influence of external electrics field (reviewed in Crombie, Gow and Gooday, 1989).

Experiments exposing fungal cells (including Neurospora crassa) to external electric field supported the idea that the hyphae are affected by the external voltage field. *Neurospora crassa* hyphae showed initial growth towards the anode and eventual loss of anodal orientation as the strength of the field was increased (McGillvray and Gow, 1986). Cells of *Candidia albicans*, showed a different response to the external voltage field. The cells grew towards the cathode and away from anode. Unlike *Neurospora crassa*, cells of *Candidia albicans* did not align themselves perpendicular to the electrodes with an increase in the electric field strength (Crombie, Gow, Gooday, 1989).

In my research, I investigated the effects of electric fields (varying voltages and electrode orientations) on the growth of *Neurospora crassa*.

MATERIALS AND METHODS

Strain. A wildtype strain of *Nerurospora crassa* (74-OR23-IVA, FGSC #2489) was used for all experiments.

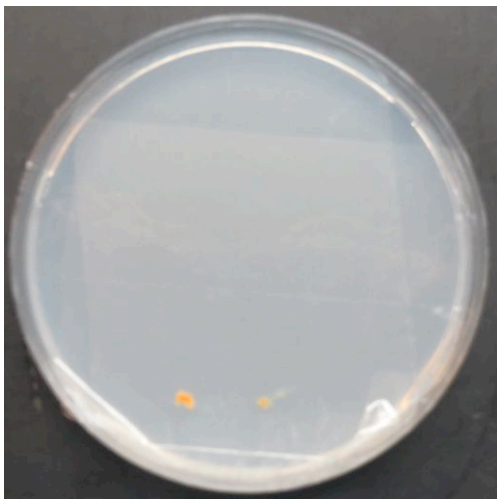

Figure 1: Petrie dish with agar gel, dialysis tubing and hyphea.

Growth conditions. Conidia were inoculated on Petrie dishes (diameter = 90.0 mm) containing Vogel's medium plus 2% agar. The following ions are present in Vogel's medium (mM): K^+ (36.7), P_i (36.7), Na^+ (25.5), NH_4^+ (25), NO_3^- (25), citrate (8.5), Cl^- (1.36), Mg^{2+} (0.81), SO_4^{2-} (0.81), and Ca^{2+} (0.68); pH is 5.8 (Lew, 2007). The conidia were sandwiched between two layers of dialysis tubing with molecular weight cut off of 14000 on top of agar (Figure 1). This was to create a 2-dimensional mycelial colony, and avoid shifting of surface hyphae during experiments. The Petrie dishes were incubated at 28° C in the dark.

Experimental conditions. Prior to the experiment, a buffer solution (22 ml) was added to Petrie dishes. Three solutions of different conductivity were used. All buffer solutions contained sucrose (6.83 gm/150ml [133 mM]), MES (0.293gm/150ml [9.1 mM]) at a pH of 5.8 (adjusted with KOH). Each solution also contained various amounts of potassium, which are listed in Table 1.

Table 1: Potassium concentrations for solutions of various conductivities.

Solution	mM
Low conductivity	4.0 K^+
Medium conductivity	20.0 KCl
High conductivity	100.0 KCl

Note: In low conductivity solution the potassium was due to pH adjustment with KOH. In medium/high conductivity solutions, KCl at the final concentrations is specified.

Experimental set up. A power supply (BK Precision Model 1685B) was connected to two platinum wires, used as electrodes. The electrodes were placed in a Petrie dish via special apparatus, specifically designed for these experiments (Figure 2). Depending on the experiment, the electrodes were positioned either parallel or perpendicular to the direction of hyphal growth. A microscope (Zeiss Stemi 2000-CS) was placed directly above the dish with the electrodes. In order to obtain a better quality image on the microscope a special lighting system was used (Figure 3). A piece of reflective material (paper) was placed inside the lighting mount. The paper reflector redirected the incident light coming from a lamp into the opening on the top surface of the lighting mount. This caused the light to be transmitted through the colony edge, resulting in a higher quality image.

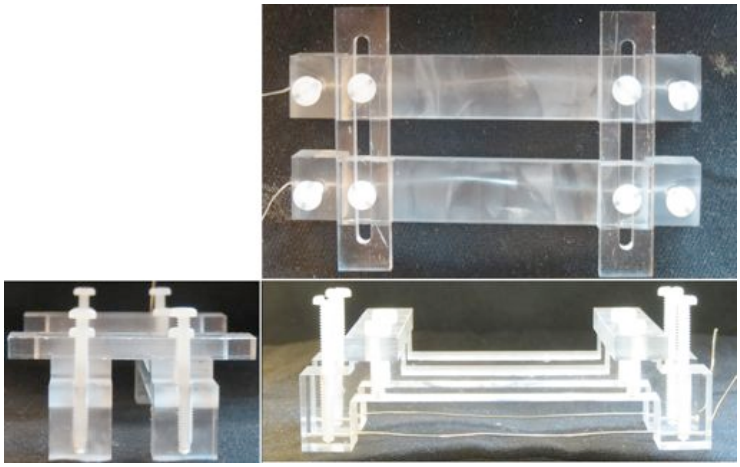

Figure 2. Apparatus used to hold platinum electrodes for positioning inside the Petrie dish.

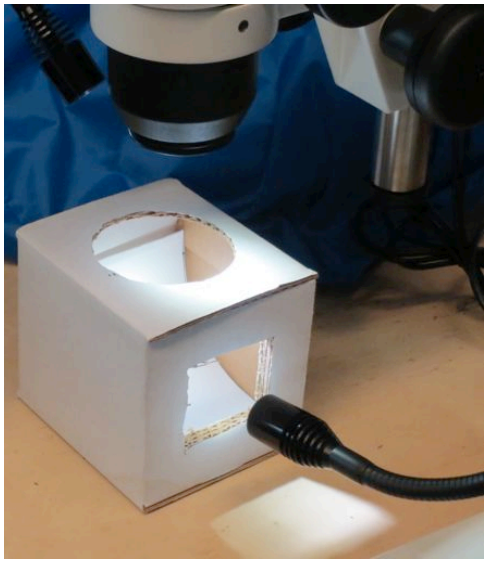

Figure 3: Lighting mount used to illuminate the Petrie dish.

Collecting Data. Once the Petrie dish with buffer solution and electrodes connected to power supply was placed on the lighting mount, the experimental set up was complete and data could be collected. The chosen zoom magnification for the microscope was 2.5. Images of hyphae at the colony edge were acquired every 60 or 120 seconds, depending on the experiment. In order to see whether the growth under the influence of electric field differed from regular growth, prior to turning on the field, the hyphal growth was recorded for 10-20 minutes. Once the field was turned on the hyphea continued to be recorded until it: a) stopped growing or b) grew out of the field of view.

Data analysis. Once the recording of hyphal growth was complete, the images were converted into an image stack using the program ImageJ (Rasband, 2014). To track the growth rate of the hyphae, the positions of the hyphal tips on each slice of the image stack were marked and the coordinates recorded. The coordinates of positions of hyphal tips at different points in time were transferred to Microsoft Excel.

The coordinate values were normalized by subtracting the values of coordinates of the tip on the first image

$$x_{n(normalized)} = x_n - x_0 \quad \text{(eqn 1)}$$

$$y_{n(normalized)} = y_n - y_0 \quad \text{(eqn 2)}$$

This was done in order to obtain relative growth response of the hyphea from the start of the experiment. The normalized values were then converted from pixels to microns by multiplying them by a conversion factor of 280 pixels / 1000 microns (for the ×2.5 zoom). Finally the growth rate was obtained by calculating average rate of change of position over time

$$V_{xn} = (x_n - x_{n-1})/\Delta t \quad \text{(eqn 3)}$$

$$V_{yn} = (y_n - y_{n-1})/\Delta t \quad \text{(eqn 4)}$$

In some cases the apparent position of the hyphae relative to the microscope lens changed due to refraction of light, rather than hyphal growth. To account for change of position of hyphea due to refraction of light, an object on the image that is expected to be nearly stationary (such as an air bubble) is selected. The position of the object is tracked and recorded. The coordinates of the object at different points in time are normalized using equations 1 and 2 and subtracted from the normalized positions of hyphal tip. These calculations were performed for some plates, where a significant light refraction was present. For each experiment the measurements and calculations were performed for 3 to 4 hyphae.

Graphic representation of data. Experimental results were represented by both a positional graph (figure 4 panel A) and a vector field graph (figure 4 panels B and C). The positional graph was simply obtained by plotting the normalized x position *versus* normalized y position of the hyphae in the experiments where hyphae grew perpendicular to the field and vice versa (normalized y *versus* normalized x) when hyphae were aligned parallel to the electric field. The vector field graph shows the velocity vectors of each hypha over the time interval of the experiment. The x-axis represents the time, negative values represent time before the field was applied. The y-axis represents the y-component of the growth rate in plates with hyphae growing along electric field and the x-component of the growth rate in plates where hyphae grow perpendicular to the electric field. The reason for such a distinction is that electrodes happen to run along the x-axis and hyphae that grow parallel to the field grow perpendicular to the electrodes-along the y-axis. The opposite is true for hyphae growing perpendicular to the electric field. Vector arrows point in the direction of growth. The graph was plotted such that each arrow is represented by the average of three velocity vectors, centered at the middle time point. This was done to improve the clarity of the graph and decrease the overlap between arrow vectors. The majority of vector field graphs were normalized in order to improve visualization. Normalization was done by setting the initial direction of growth to be along the y-axis. In figure 4B, both normalized (left panel) and regular (right panel) vector field graphs were shown in order to clearly illustrate the behaviors of the hyphae. Figure 4B shows the vector fields for individual hyphea, while figure 4C shows the vector field averaged over the four measured hyphae.

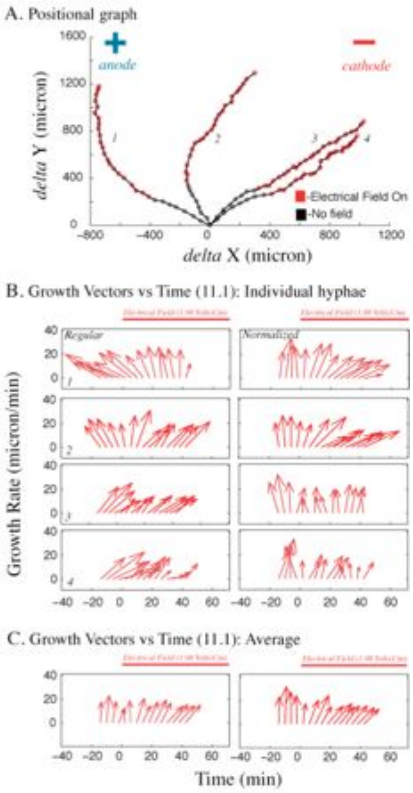

Figure 4: Experiment 11_1 graphs

Polarization calculations:
Polarization is a change in the direction of growth since the moment electric field was applied. It is found by averaging the angle of hyphal growth over the time period where no field was present and subtracting it from the average angle of growth over selected time period, which occurs after the field was applied. The angle of growth is calculated by taking the arctangent of two components of growth rate (eq 5 and 6)

$$\theta_{perpendicular} = \arctan(Vy_n / Vx_n) \qquad \text{(eqn 5)}$$

$$\theta_{parallel} = \arctan(Vx_n / Vy_n) \qquad \text{(eqn 6)}$$

The choice of the equation depends on type of the experiment being analyzed. Equation 5 is used for trials where electric field is perpendicular to the direction of growth and

initial, normalized growth is along the x-axis while deflection occurs along the y-axis. Equation 6 —with reversed axes— was used for experiments where the electric field was along the direction of hyphal growth. In both equations an angle of 0° represents the growth along the initial path. In equation 5, a positive angle represents deflection towards cathode, while a negative angle represents deflection towards anode. In equation 6 both positive and negative angles represent deflection away from the electrode, the hyphae were growing towards initially (cathode or anode, depending on the experiment). For all experiments, the calculated angles were then averaged (eqn 7) over selected time periods.

$$\theta_{av} = \frac{\sum_{i=1}^{n} \theta_i}{n} \qquad \text{(eqn 7)}$$

As stated above, the first time period is simply the interval when no field was applied. The second time period, over which the angles are averaged is depends on the experiments being averaged. The interval always starts at the second vector after the field was applied. The hyphal response to the voltage field is not immediate and that is why the first arrow is omitted from the average. The interval ends at the point where hyphae slowed their growth rate. Since hyphal growth does not stop at the same time for all plates, the average growth rate will decrease over time, even though the growth rate for individual hypha might not be changing. The sample size that contributes to the value of growth angle also decreases. As a result the values at longer time intervals are less reliable. So the endpoint was selected in order to keep the results fairly accurate, while not excluding too many vectors. The polarization angle is calculated by equation 8.

$$\theta_{pol} = \theta_{avf} - \theta_{avi} \qquad \text{(eqn 8)}$$

Where θ_{avf} is the average angle over the second interval, while θ_{avi} is the angle averaged over the first interval.

RESULTS

The effects of various voltages and electric currents. Fields with magnitude ranging from 1.98 to 20 Volts/cm were applied to the hyphae. For all trials where electric fields were significantly stronger than 1.98 Volts/cm, hyphal growth stopped within 900 seconds after being exposed to the field so no directional response could be observed. The value of 1.98 Volts/cm was found to be optimal for continued growth and the majority of the experiments were performed with a field of this magnitude. The power supply was operated in a constant voltage mode, meaning the current adjusted accordingly to the set voltage. The value of optimal current was 0.05 mA. These values of electric field strength and current were optimal for all three solutions of low to high conductivity used in the experiments.

Experiments involving various solutions: All of the experiments discussed, were performed with electric field being oriented perpendicular to the direction of growth and at optimal values of voltage and current.

Low conductivity solution. Cells grown in a low conductivity solution did not show a strong response to the electric field. The averaged growth vectors (n = 5 plates) (figure 5), suggests that no significant change of direction of growth occurred. The polarization angle was -11.7 degrees. There was a significant decrease of the average growth rate over time. This was partly due to some hyphae stopping growth altogether and partly due to a decrease of the growth rate of individual hyphea.

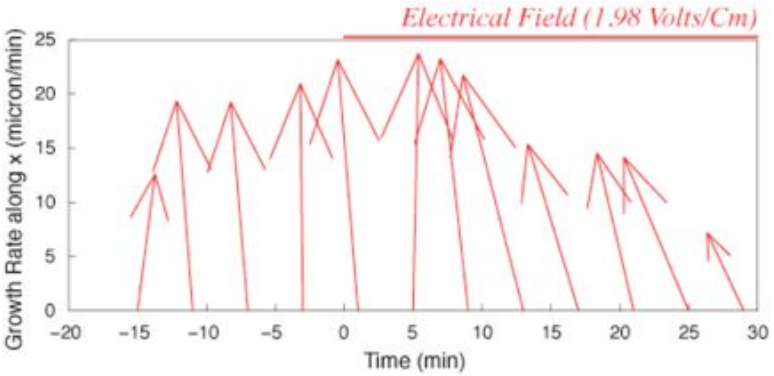

Figure 5. Average growth rate for low conductivity plates

Medium conductivity solution. Cells grown in the medium conductivity solution appeared to have the healthiest response to the applied field. That is why majority of experiments were performed with a medium conductivity solution. Growth vectors were averaged over 10 plates (figure 6). Contrary to expectations, there was no significant change in growth direction. The polarization angle was 2.9 degrees.

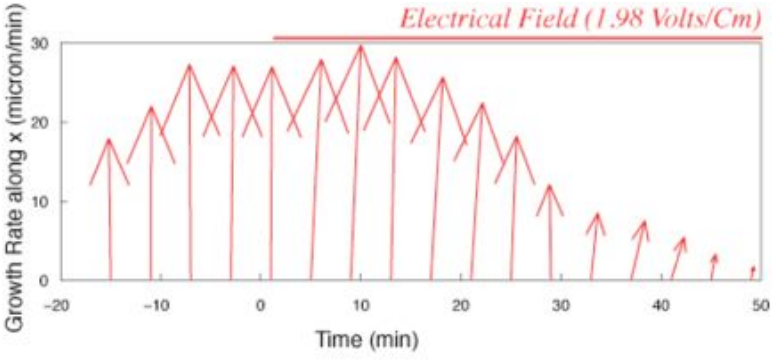

Figure 6. Average growth rate for medium conductivity plates

Half of the plates stopped growing after 37 minutes of being exposed to the field. Just as

in experiments with low conductivity solutions, there was a significant decrease in growth rate was noted as the time progressed. It was partly due to some of the hyphae stopping growth completely and partly due to the actual decrease of the growth rate of individual hyphae.

High conductivity solution. Cells grown in high conductivity solution showed a response that was similar to the ones in low/medium conductivity experiments (figure 7). The polarization angle was 6.7 degrees. Therefore no significant deflection occurred. Half of the plates stopped growing after 13 minutes of being exposed to the field. There was a significant decrease in growth rate, due to both a decrease in growth rate of individual hypha and complete stop of growth of certain hypha over time. On average hyphea in high conductivity solution had the lowest growth rate and the quickest stoppage of growth.

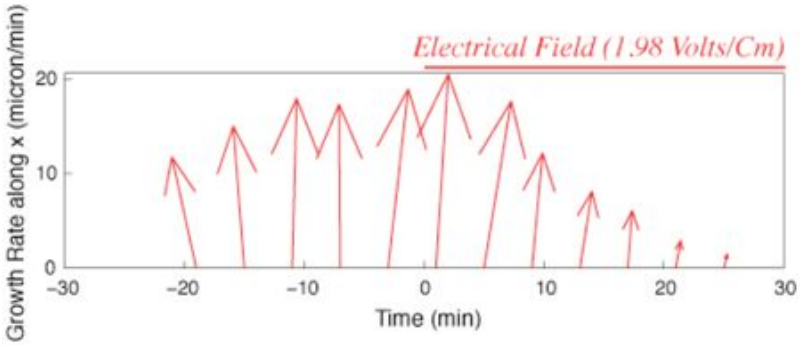

Figure 7. Average growth rate for high conductivity plates

Experiments involving growth parallel to the electric field:
All experiments involving hyphae oriented parallel to the electric field were done in themedium conductivity solution, as cells tended to grow for longer periods of time, thus allowing more observations to be collected. Two types of experiments were performed: hyphae growing towards the cathode and hyphae growing towards the anode.

Hyphae growing towards the cathode. Hyphal cells growing towards cathode showed a fairly complex response to the voltage field (figure 8). There was a strong, but brief decrease in growth rate (minutes 20-32), followed by a partial recovery of growth rate. Eventually the growth rate started decreasing again. The decrease in the average growth rate is associated with both a slowed down rate of growth of individual hypha and completely aborted growth of some

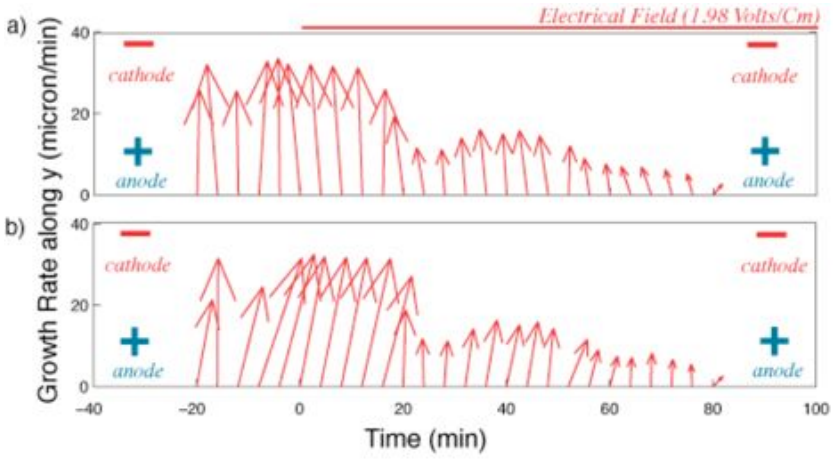

Figure 8. Normalized (a) and regular (b) averaged over 10 plates vector field for hyphea initially oriented towards cathode.

hypha. Note that in the parallel field experiments, hyphae grew for significantly longer period of time compared to experiments, where the growth was perpendicular to the electric field. Half of the plates stopped growing by 56th minute. In three out of ten plates observed, hyphae did not stop growing by 80th minute (experiments had to be ended because hyphal tips grew out of the field of view. The polarization angle for the experiments was -1.4°, meaning a slight deflection towards cathode occurred.

Hyphea growing towards the anode. Hyphal cells that were initially facing anode, responded similarly to the cells growing perpendicular to the field (figure 9). The growth vector showed steady growth, followed by a gradual decrease. Unlike hyphae growing towards the cathode, growth did not recover. Just as in previous experiments, there was no significant shift in growth direction. The polarization angle was -2.2° (a slight deflection away from anode). Growth in half of the plates stopped at 24 minutes.

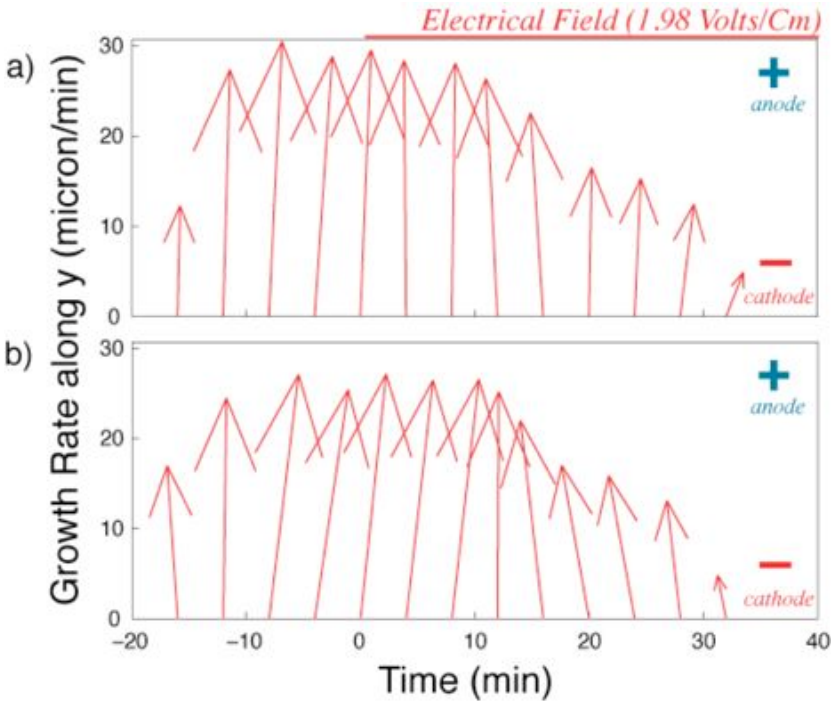

Figure 9. Normalized (a) and regular (b) averaged over 8 plates vector field for hyphea initially oriented towards cathode.

DISCUSSION

Experiments involving various voltages showed a correlation between applied voltage and the cessation of hyphal growth. In all such experiments hyphae stopped growing at some point. Higher voltages lead to an earlier stoppage of growth. At the optimal voltage of 3.95 V (1.98 V/cm), on average, the hyphae grew for longest time intervals.

Experiments involving solutions of various conductivity and electric field oriented perpendicular to the direction of growth did not match our expectations. Unlike results discussed in earlier reports (McGillvray and Gow, 1986), there was no significant polarization; that is, change in growth direction at all of the solution conductivities tested. The highest average polarization angle was 11.7° towards the anode in the low conductivity solution. Hyphae grown in medium and high conductivity solution did show a slight deflection towards the cathode. Based on these results it is unclear whether the applied electric field had any effect on the direction of growth.

In previous experiments where effects of external field on fungal cells were investigated, voltage fields of 0 to 58 V/cm were generated and the hyphae grew under the influence of the field for several hours (Crombie, Gow and Gooday, 1990; McGillvray and Gow, 1986). For unclear reasons, the experimental set up, described in materials and methods of this report does not allow prolonged exposure to electric fields, especially if they are of the high magnitude-cessation of growth occurs before any deflection could be observed.

One of the potential causes of stoppage of hyphal growth is the insufficient amount of spacing between cells and electrodes. In our experimental set up, hyphea was placed between two electrodes, approximately 2cm apart. Meanwhile in earlier experiments, where deflection was observed a small DNA electrophoresis cell was used (Bio-Rad; mini sub cell) (Crombie, Gow and Gooday, 1990; McGillvray and Gow, 1986). The width of gel tray in such cell is 7 cm therefore the distance between electrodes is close to that value (www.bio-rad.com).

It's possible that the medium of growth is the cause of cessation, however in terms of the solutions used, the set up in our experiment is quite similar to the set up used in earlier experiments. McGillvray and Gow (1986) grew cells of *Neurospora crassa* on agar gel with Vogel's medium. Low conductivity medium and a 20 mM potassium-phosphate buffer, with a pH of 6-5 were used in the experiment. These substances are nearly identical to the ones described in the materials section of this report. The only extra component that was present in this experiment is dialysis tubing. It is very unlikely to cause the cessation of growth and earlier trials that were performed without confirm it. Hyphea still stopped growing in the experiments where dialysis tubing was absent.

The major difference in the hyphal behavior of the cells, grown at different conductivities was in the duration of growth. Half of the cells placed in low and high conductivity solutions grew for 17 and 13 minutes, respectively, after being exposed to the field.

Meanwhile half of the cells from plates with medium conductivity solution continued to grow, stopping after 37 minutes under the influence of field.

Experiments with hyphae aligned towards the anode showed unusual results. Growth rate vectors of hyphae that initially grew towards the anode were surprisingly similar to growth rate vectors of cells that grew perpendicular to the field. The averages from both types of experiments fit the pattern of nearly constant growth rate followed by steady decline, without any significant deflections. These observations may suggest that hyphae do not respond to the external electric field, since rotation of the field by 90° did not lead to any noticeable changes in the growth pattern of the cells. The only difference in behavior of the cells growing towards the anode versus cells growing perpendicular to the voltage field was the duration of growth. On average, the growth of cells towards the anode stopped 13 minutes earlier than the growth of cells perpendicular to the electrodes (24 versus 37 minutes).

However, hyphae that grew towards cathode demonstrated a response that is significantly different from the response shown in any other experiments. The growth vectors showed a phase of steady growth rate followed by sharp decline, partial recovery and finally steady decrease of growth rate. Cells also grew for a significantly longer period of time than in any other experiment.

An interesting pattern can be observed from experiments where direction of growth is along the electric field. Hyphea that grows towards cathode has significantly longer growth duration than hyphea growing along the electrodes, which has a growth duration longer then of cells growing towards the anode (in medium conductivity solution). These observations suggest that cessation of growth may relate to the distance or relative velocity of the cells towards the anode.

Experimental results did not show a significant polarization of hyphea under the influence of external field. It was observed that electric field can induce cessation of growth, the origins of which are unclear. Some of the observations taken indicated that cessation of growth is depends upon relative position of the cells to the electrodes.

Future experiments can use the set up with greater separation between electrodes in order to increase the duration of hyphal growth. If a problem of growth cessation will be solved, then experiments involving non-constant field/current and reversal of field will the next step in investigating effects of external field on hyphal growth.

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Crombie T., Gow N. A. R., Gooday G. W. (1990) Influence of applied electrical fields on yeast and hyphal growth of *Candidia albicans*. J Gen Microbiol. 136:311-317.

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Gow N. A. R. (1984) Transhyphal Electrical Currents in Fungi. J Gen Microbiol. 130:3313-3318.

APPENDIX A-CREATING PLOTS IN MATLAB

Vector Field:

(1) quiver(Time,ans,Vfx,Vfy,scale) or (2) quiver(Time,ans,Vfy,Vfx,scale)

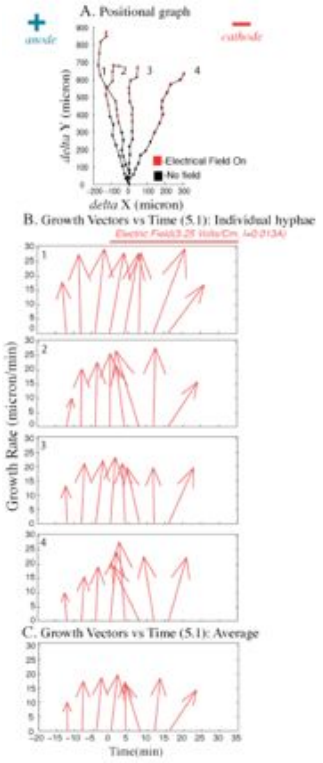
Time=x-coordinate of the vector tail. Represented by time interval over which growth occurs.
Vfx=x-component in (1)/y-component in (2) of the vector. Represented by growth rate in x direction
Vfy= y-component in (1)/x-component in (2) of the vector. Represented by growth rate in y direction
ans= x-coordinate of the vector tail. Represented by zero matrix equal in size to Time, Vfx, Vfy
scale=1x1 zero matrix. Makes vectors unscaled.

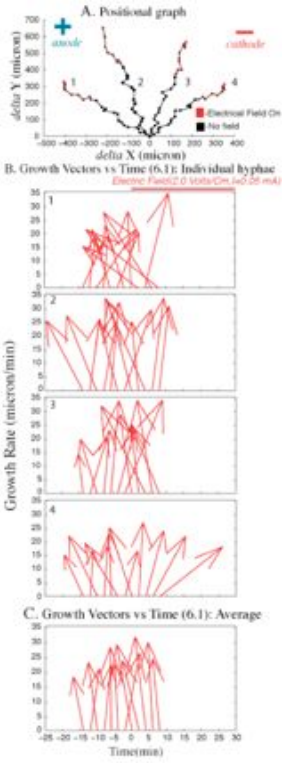
Scatter Plot:

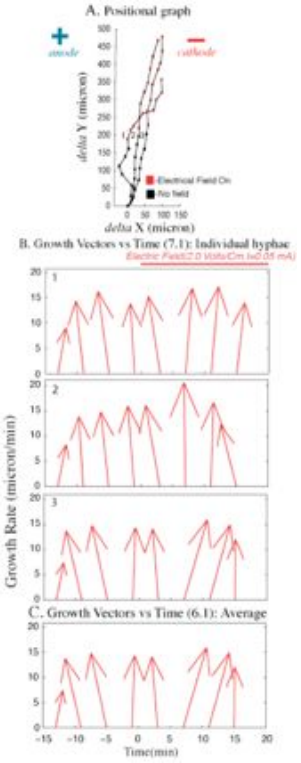
(1)	or	(2)
scatter(y1,x1,S,C) hold on plot(y1,x1) scatter(y2,x2,S2,C2) plot(y2,x2) scatter(y3,x3,S3,C3) plot(y3,x3) scatter(y4,x4,S4,C4) plot(y4,x4)		scatter(x1,y1,S,C) hold on plot(x1,y1) scatter(x2,y2,S2,C2) plot(x2,y2) scatter(x2,y2,S3,C3) plot(x3,y3) scatter(x4,y4,S4,C4) plot(y4,x4)

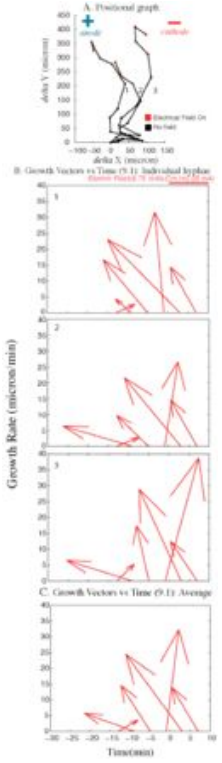
Plots 4 scatter plots, one for each hypha, overlapped by a regular line plot on the same graph.
x1-4=y-coordinate in (1) x-coordinate in (2) of hyphal tip position
y1-4=x-coordinate in (1) y-coordinate in (2) of hyphal tip position
S1-4=Size of the markers on scatter plot, set to constant value of 30. Matrix is equal in size to x1-4 and y1-4.
C1-4=Color of markers on scatter plot

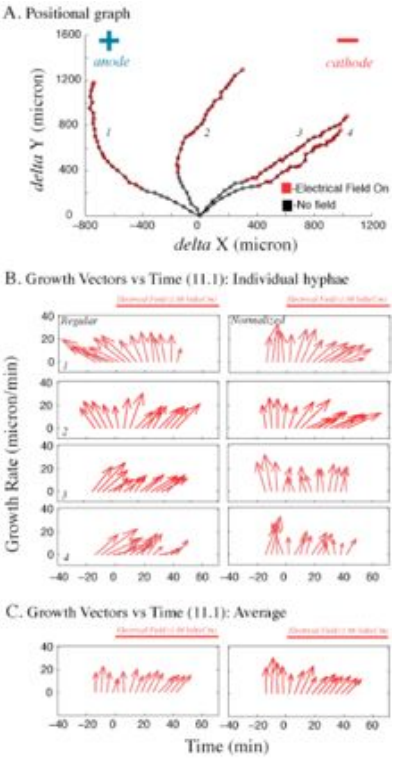
APPENDIX B-GRAPHS OF INDIVIDUAL EXPERIMENTS

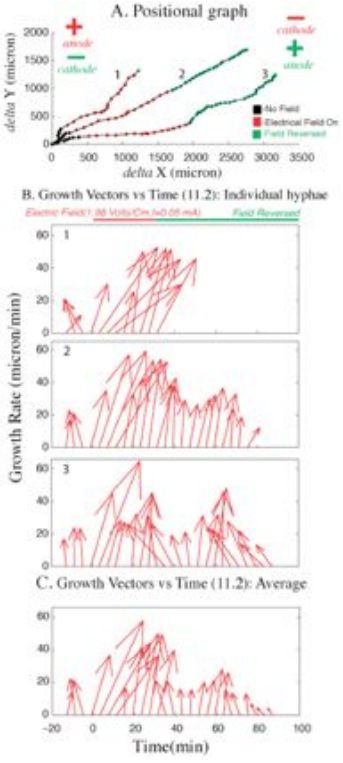


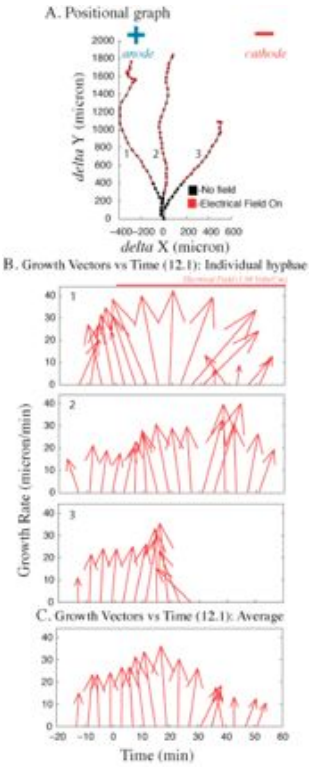


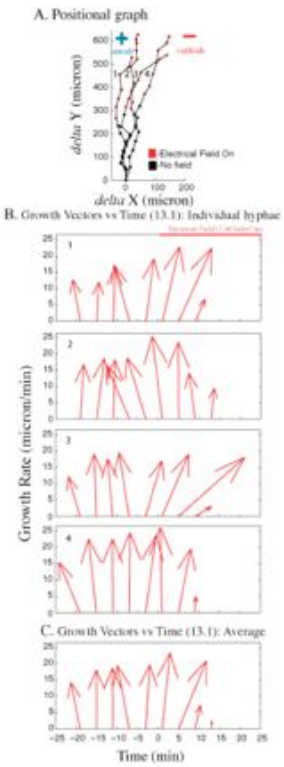


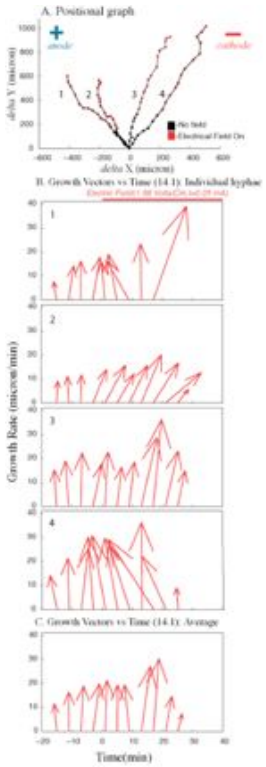


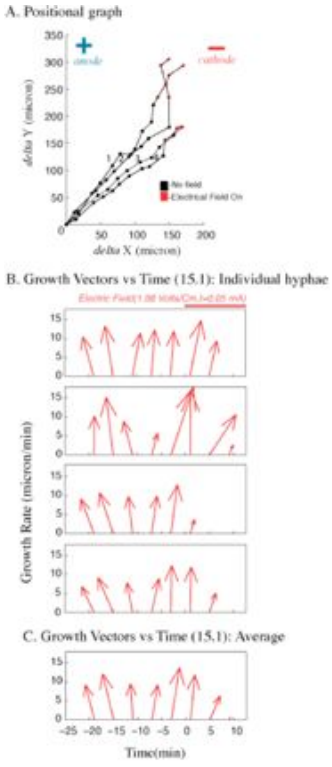


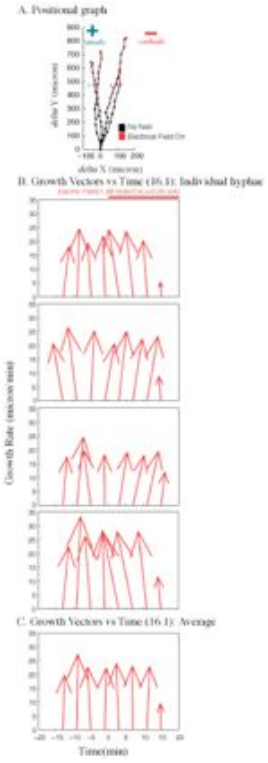


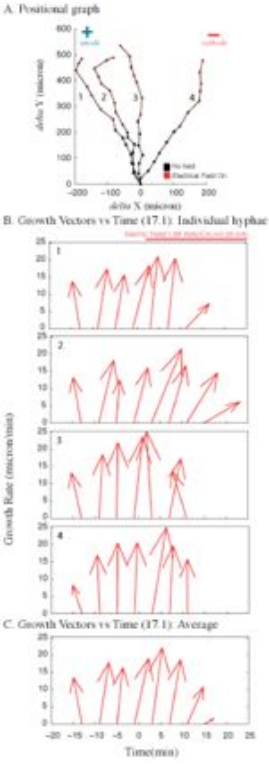

A. Positional graph
anode
cathode
delta Y (micron)
delta X (micron)
No field
Electrical Field On
B. Growth Vectors vs Time (12.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (12.1): Average
Time (min)

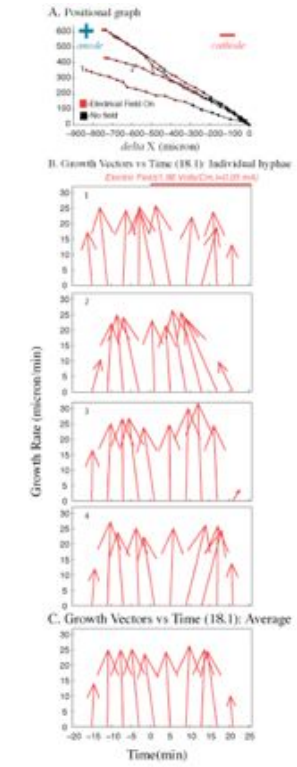

A. Positional graph
anode
cathode
delta Y (micron)
delta X (micron)
Electrical Field On
No field
B. Growth Vectors vs Time (13.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (13.1): Average
Time (min)

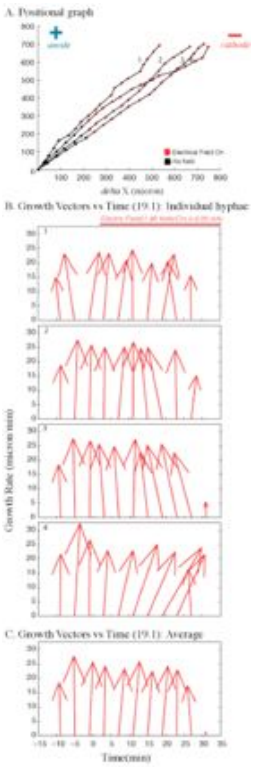

A. Positional graph
anode
cathode
delta Y (micron)
delta X (micron)
No field
Electrical Field On
B. Growth Vectors vs Time (14.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (14.1): Average
Time(min)

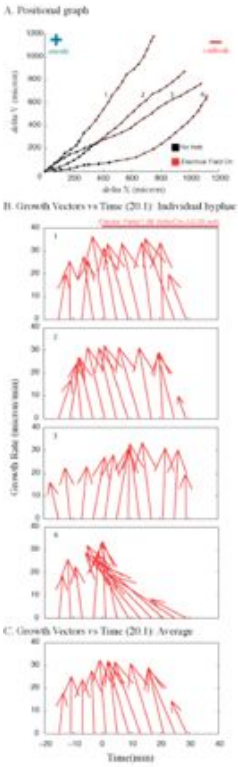

A. Positional graph
anode
cathode
delta Y (micron)
delta X (micron)
No field
Electrical Field On
B. Growth Vectors vs Time (15.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (15.1): Average
Time(min)

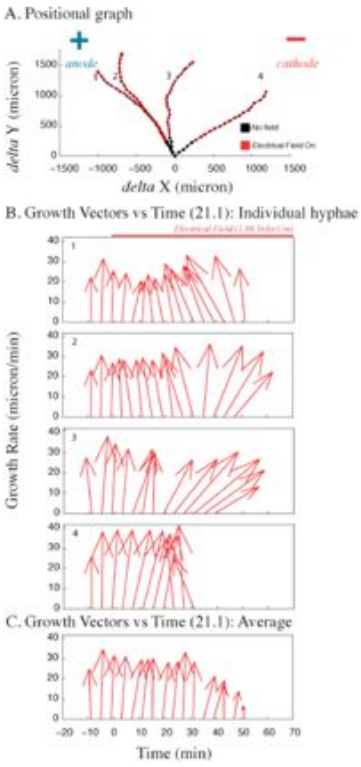

A. Positional graph
anode
cathode
delta Y (micron)
delta X (micron)
No field
Electrical Field On
B. Growth Vectors vs Time (16.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (16.1): Average
Time(min)

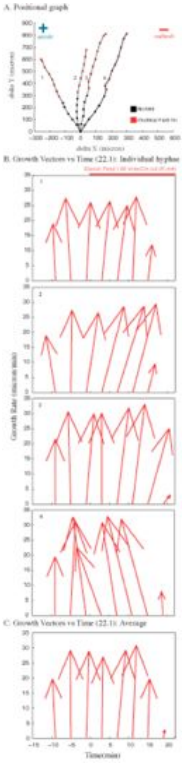

A. Positional graph
anode
cathode
delta Y (micron)
delta X (micron)
No field
Electrical Field On
B. Growth Vectors vs Time (17.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (17.1): Average
Time(min)

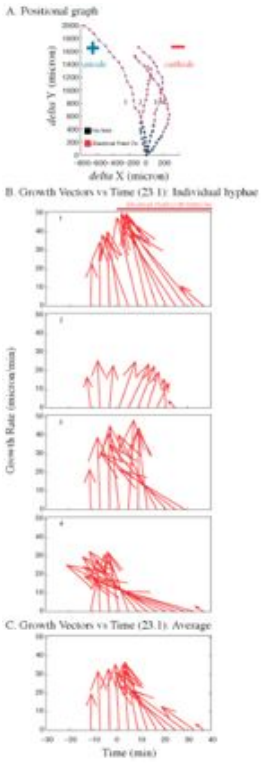

A. Positional graph
anode
cathode
delta X (micron)
B. Growth Vectors vs Time (18.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (18.1): Average
Time(min)

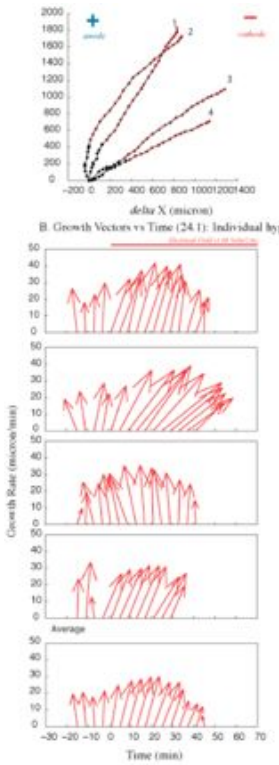

A. Positional graph
anode
cathode
delta X (micron)
B. Growth Vectors vs Time (19.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (19.1): Average
Time(min)

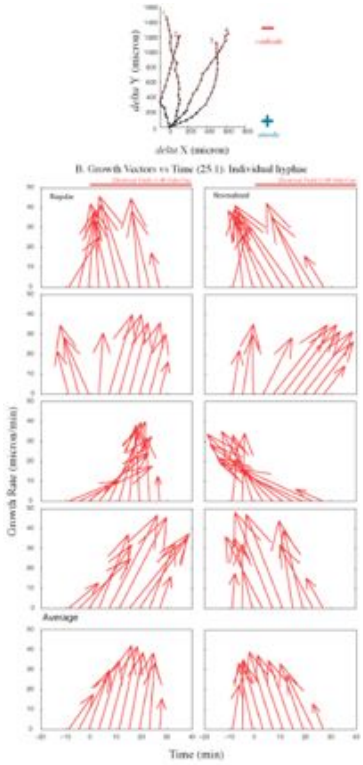

A. Positional graph
anode
cathode
delta X (micron)
B. Growth Vectors vs Time (20.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (20.1): Average
Time(min)

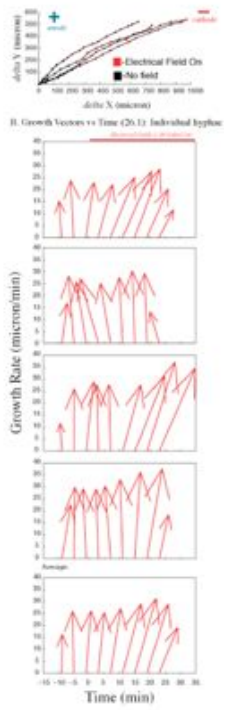

A. Positional graph
anode
cathode
delta Y (micron)
delta X (micron)
No field
Electrical Field On
B. Growth Vectors vs Time (21.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (21.1): Average
Time (min)

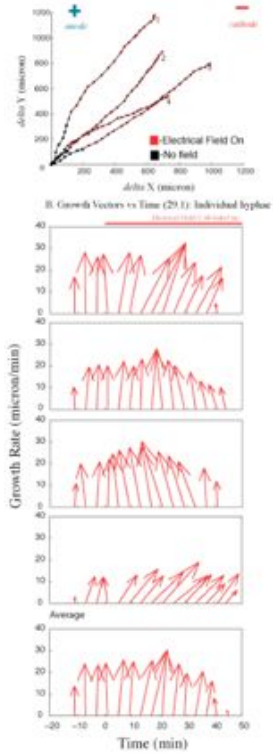

A. Positional graph
B. Growth Vectors vs Time (22.1): Individual hyphae
C. Growth Vectors vs Time (22.1): Average

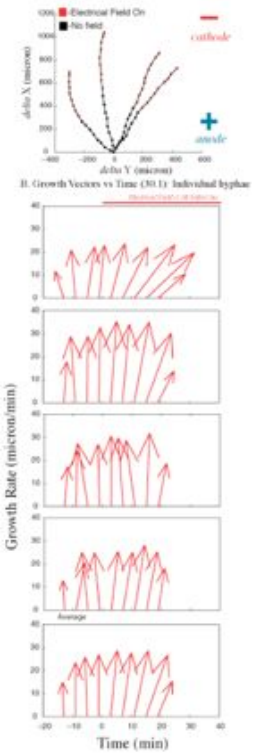

A. Positional graph
delta Y (micron)
anode
cathode
delta X (micron)
B. Growth Vectors vs Time (23.1): Individual hyphae
Growth Rate (micron/min)
C. Growth Vectors vs Time (23.1): Average
Time (min)

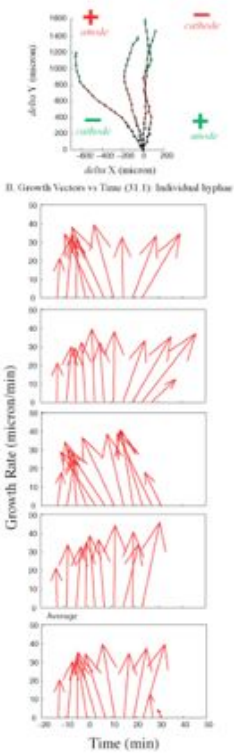

delta X (micron)
B. Growth Vectors vs Time (24.1): Individual hyphae
Growth Rate (micron/min)
Average
Time (min)

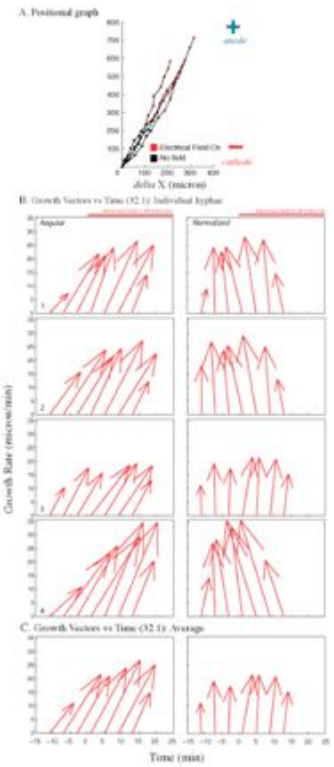

delta Y (micron)
delta X (micron)
B. Growth Vectors vs Time (25.1): Individual hyphae
Growth Rate (micron/min)
Average
Time (min)

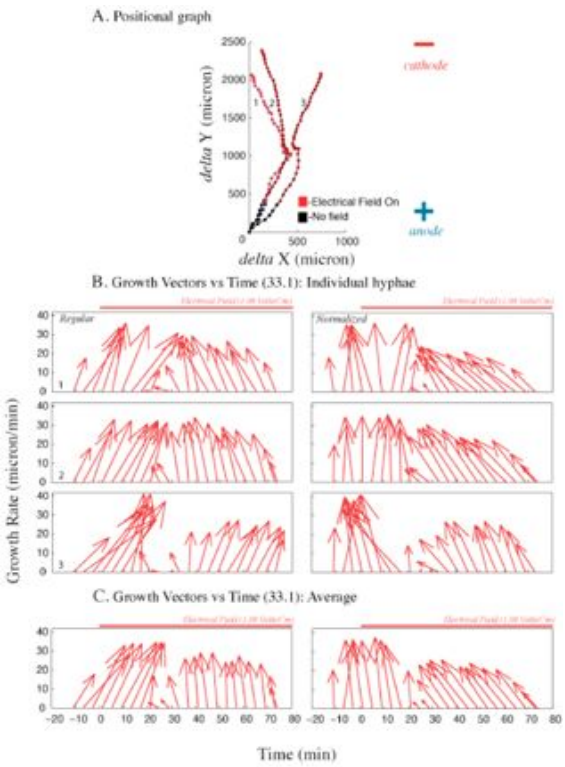

Electrical Field On
No field
delta X (micron)
B. Growth Vectors vs Time (26.1): Individual hyphae
Growth Rate (micron/min)
Time (min)

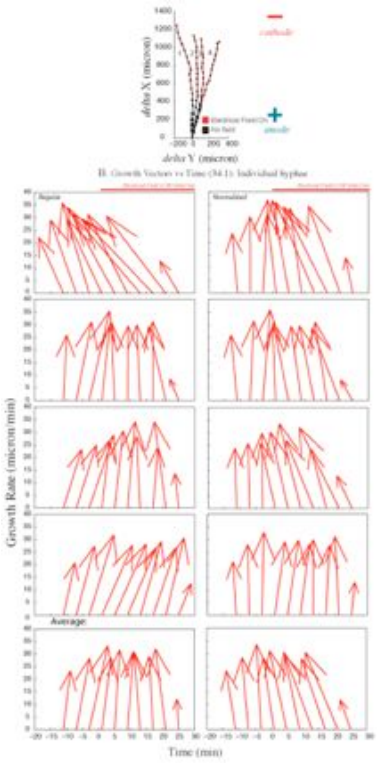

Electrical Field On
No field
delta X (micron)
B. Growth Vectors vs Time (29.1): Individual hyphae
Growth Rate (micron/min)
Average
Time (min)

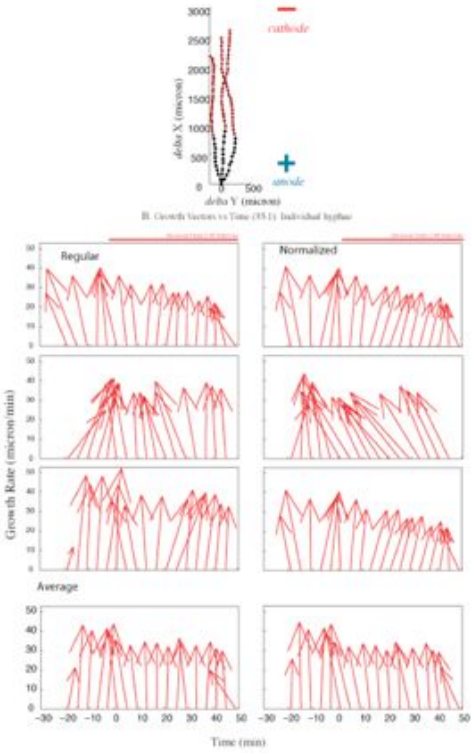

Electrical Field On
No field
cathode
anode
delta Y (micron)
B. Growth Vectors vs Time (30.1): Individual hyphae
Growth Rate (micron/min)
Average
Time (min)

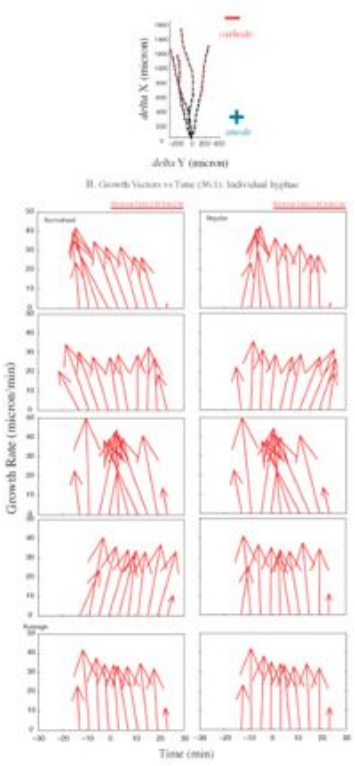

cathode
anode
delta Y (micron)
delta X (micron)
B. Growth Vectors vs Time (31.1): Individual hyphae
Growth Rate (micron/min)
Average
Time (min)

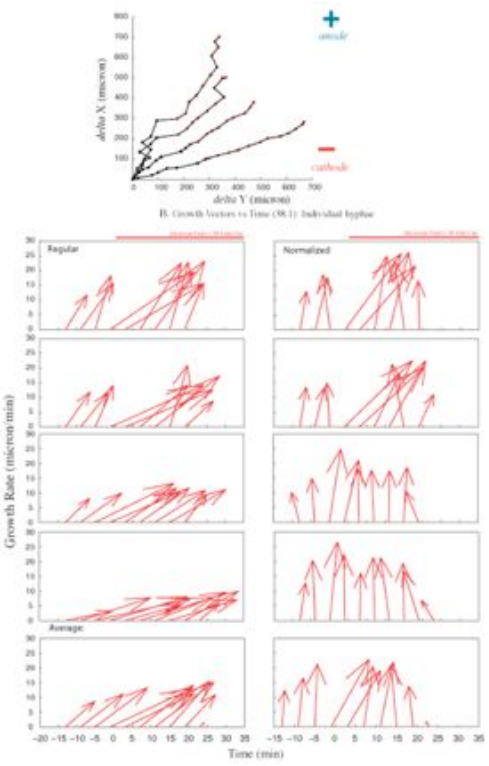

A. Positional graph
anode
Electrical Field On
No field
cathode
delta X (micron)
B. Growth Vectors vs Time (32.1): Individual hyphae
Regular
Normalized
C. Growth Vectors vs Time (32.1): Average
Growth Rate (micron/min)
Time (min)

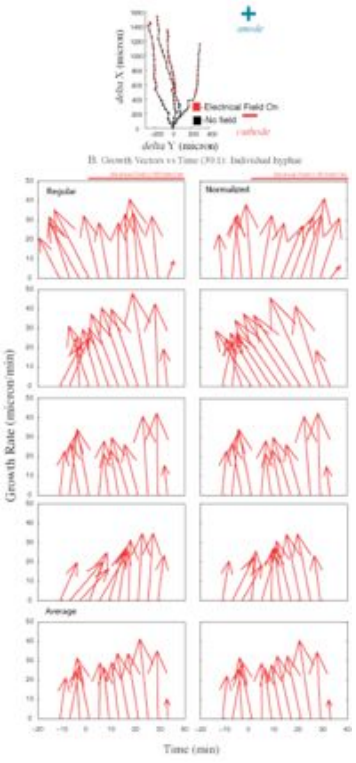

A. Positional graph
cathode
delta Y (micron)
Electrical Field On
No field
anode
delta X (micron)
B. Growth Vectors vs Time (33.1): Individual hyphae
Regular
Normalized
Growth Rate (micron/min)
C. Growth Vectors vs Time (33.1): Average
Time (min)

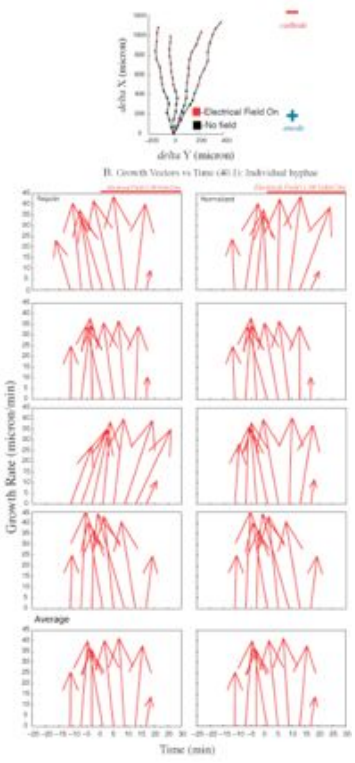

cathode
anode
delta Y (micron)
B. Growth Vectors vs Time (34.1): Individual hyphae
Regular
Normalized
Average
Growth Rate (micron/min)
Time (min)

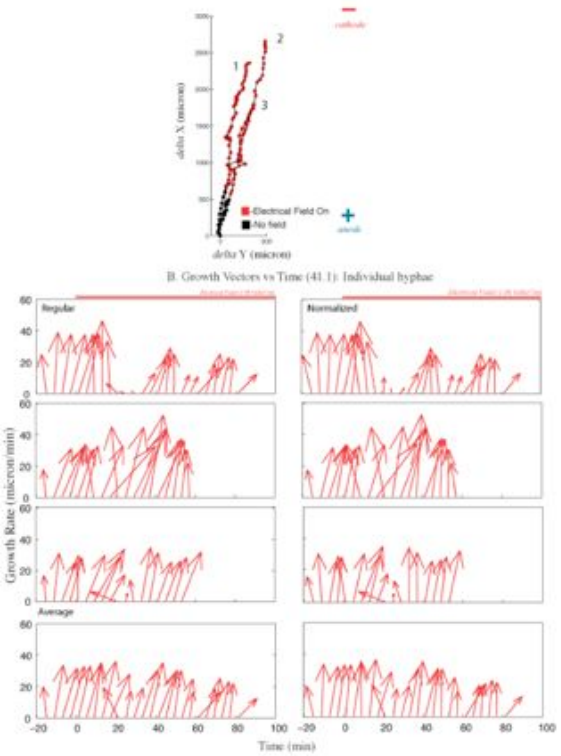

cathode
anode
delta Y (microns)
B. Growth Vectors vs Time (35.1): Individual hyphae
Regular
Normalized
Average
Growth Rate (micron/min)
Time (min)

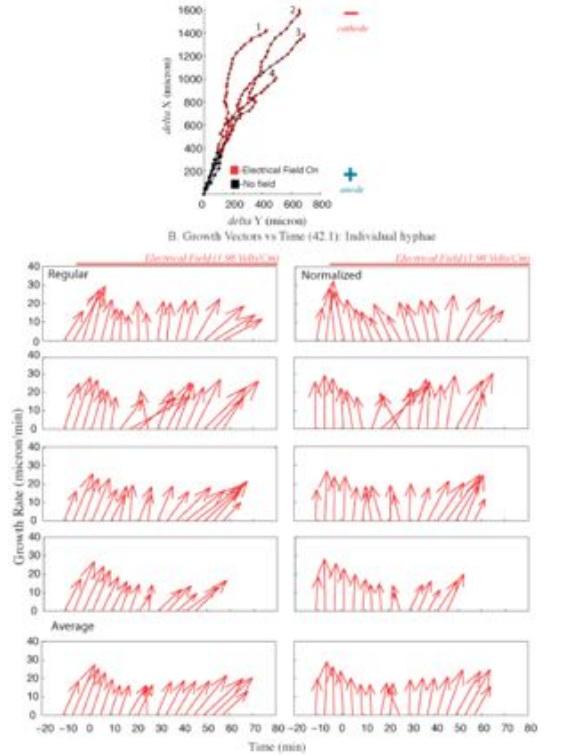

delta X (micron)
delta Y (micron)
Growth Rate (micron/min)
Time (min)


delta X (micron)
delta Y (micron)
Regular
Normalized
Average
Growth Rate (micron/min)
Time (min)


delta X (micron)
Electrical Field On
No field
delta Y (micron)
Regular
Normalized
Average
Growth Rate (micron/min)
Time (min)


delta X (micron)
Electrical Field On
No field
delta Y (micron)
Average
Growth Rate (micron/min)
Time (min)


1
2
3
delta X (micron)
Electrical Field On
No field
delta Y (micron)
B. Growth Vectors vs Time (41.1): Individual hyphae
Regular
Normalized
Average
Growth Rate (micron/min)
Time (min)


1
2
3
delta X (micron)
Electrical Field On
No field
delta Y (micron)
B. Growth Vectors vs Time (42.1): Individual hyphae
Regular
Normalized
Average
Growth Rate (micron/min)
Time (min)