Magneto: The effect of high magnetic field on the growth of *Neurospora crassa*[1,2]

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OBJECTIVE

To determine whether the fungus *Neurospora crassa* is sensitive to extremely high magnetic fields while irradiated with flavin-activating blue light.

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INTRODUCTION

The biological and/or biochemical response of organisms exposed to magnetic fields has long been a source of curiosity and speculation within the scientific community. In fact, a cursory search of the scientific literature on the subject reveals that experimental results are conflicting. In fungi, Sadauskas *et al*. (1987) observed morphological changes in the conidia of *Aspergillus puniceus,* as well as changes in pigmentation in colonies of *Aspergillus niger*, after growth in static and alternating (10 Hz frequency) magnetic fields. As well, there are those who maintain that the circadian rhythms inherent to all organisms are sensitive to slight changes in the earth's magnetic field and thus applied magnetic fields (Brown *et al*. 1969, 1978). However, several other reports have found no effects of magnetic fields (Bitz and Sargent, 1974, Beischer, 1972). Therefore, we decided to study the effects, if any, on *Neurospora crassa* cultures grown in static magnetic fields of considerable magnitude.

MATERIALS AND METHODS

Strains and Medium. Stock cultures of wild-type *N. crassa* (strain 74-OR23-1VA, FGSC 2489) were used in all experiments. Vogel's minimal medium plus 1.5% (w/v) sucrose and 2.0% (w/v) agar (VM) (Vogel, 1956) (25 ml) was added to race tubes 340 mm long and 15 mm wide. The major ions in VM (in mM) are: 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). Using sterile technique, the race tubes were inoculated with *N. crassa* conidia at one end and sealed at both ends using cotton. Cultures were grown in darkness at 28 °C for approximately 24 hours prior to experimentation.

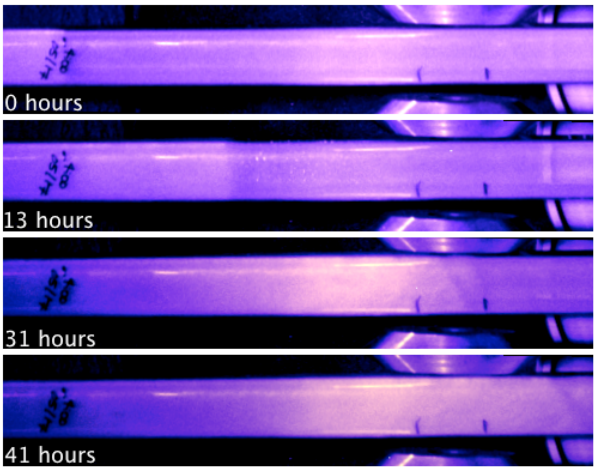

Figure 1. Experimental apparatus. Race tube containing the *N. crassa* colony under blue light. The edge of the colony can be seen marked on the left-hand side. The magnetic field is located between the two metal heads toward the right hand side. Colonies grew through the magnetic field so that growth before (0 and 13 hours), during (31 hours), and after (41 hours) magnetic field exposure could be monitored.

Growth Measurements. After a brief period of incubation, race tubes were positioned in a magnetic field of approximately 0.96 Tesla (9600 Gauss) strength, so that the colony edge would grow through the high strength magnetic field, continuously imaged using a time-lapse camera (Figure 1). The magnetic field was generated with a Varian Associates electromagnet, Model V3713, powered by a Aehr Test Systems 120 V DC power supply. The magnetic field strength was measured with an AlphaLab Inc. DC Gaussmeter, Model

GM-1-ST. The temperature of the magnet was measured with a Mastercraft Temperature Gun (an infrared temperature sensor). Cultures were illuminated with blue light (456 ± 16 nm) (a 40 LED cluster lamp [S1040-120A, Ledtronics] positioned about 0.8 m away from the race tube). The initial colony edge was marked, as well as the position of the magnetic field. Movement of the growth front was imaged with a Lumenera 2.0 scientific USB camera. The camera recorded photos of the colony every 20 minutes for a period ranging from 48 to 72 hours. Ambient temperature and relative humidity was monitored for the same time period using an Extech Instruments, Temperature/Humidity USB Datalogger, model RHT10. To ensure uniform temperature near the magnet, a portable fan was used. The experiments were replicated 7 times.

RESULTS

Overall linear extension, and the growth rate during each 20-minute interval were measured from the images using Image J. Data were analyzed using Excel (Microsoft). Linear regressions were used to determine the overall growth rate —growth rates per 20-minute interval ranged between 0 to 7 mm per hour. Figure 2 shows data for linear extension and growth rate for three experiments.

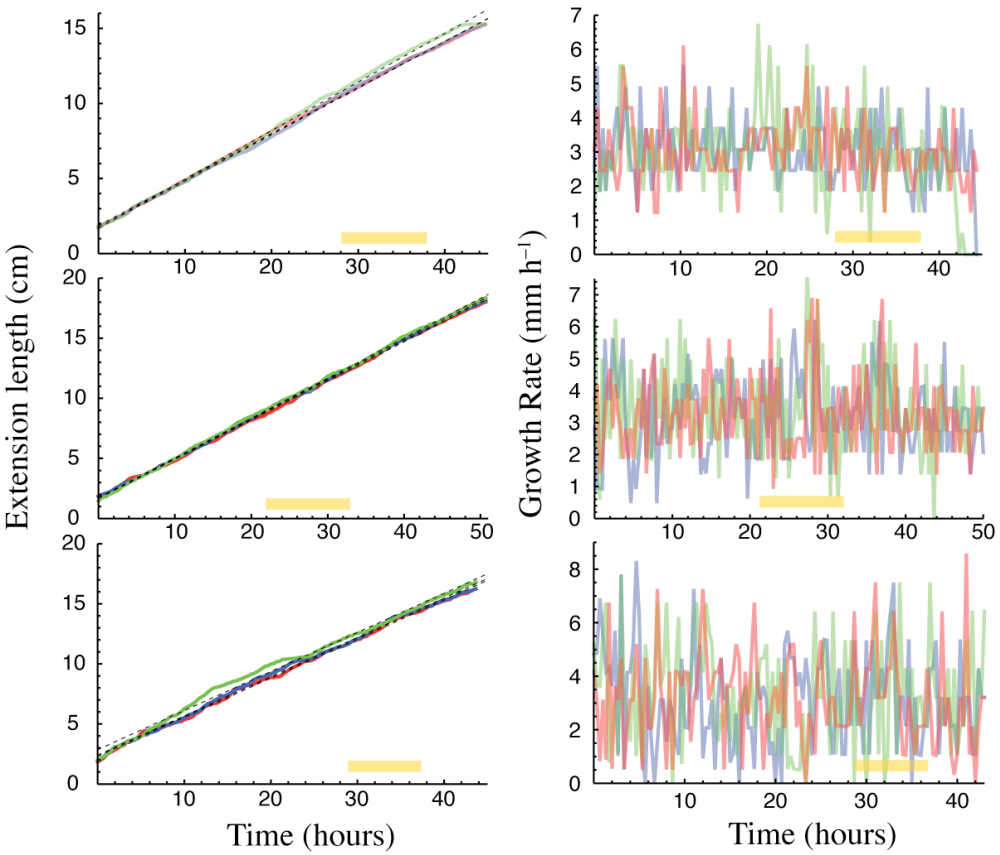

Figure 2. Linear extension of the colony edge and growth rates over time. (Left) The initial colony edge was marked on the exterior of the race tube. The extending colony edge was measured at the left edge (red), center (blue) and right edge (yellow) of the colony. Yellow bars indicate growth interval in the magnetic field. Linear regressions (black lines) were used to determine overall growth rate. (Right) Growth rate was measured for every 20-minute period. Results showed large variation between time periods, ranging from 0.0 mm/hr (no growth) to 7 mm/hr.

To determine if growth was rhythmic or pulsatile in any form, the growth rate data was analyzed using autocorrelation. A relationship between growth peaks at short lag intervals would be visible on a plot of autocorrelations as a sinusoidal function. No such

relationship was found, so variations in growth rate are solely random. An example of an autocorrelation plot is shown in Figure 3.

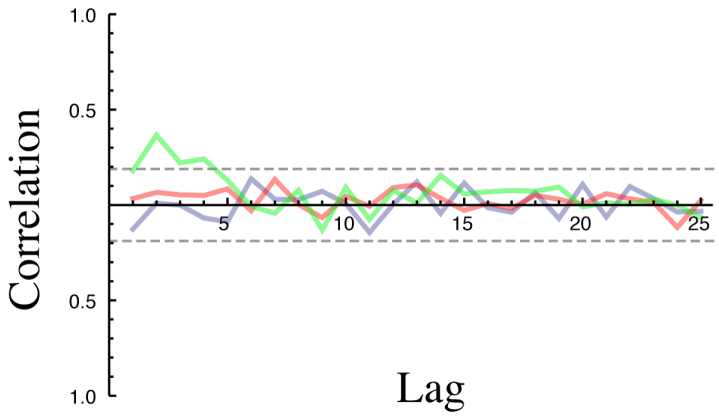

Figure 3. Autocorrelations for growth rates of *N. crassa.* Autocorrelation functions were preformed for growth rates at the left edge (blue), center (red), and right edge (green) of the colony edge. Confidence intervals (± 95%) are also shown (dotted lines above and below the autocorrelations). Significant periodicity would cause the autocorrelations to consistently be larger than the 95% confidence intervals, not observed.

As well as growth, the morphology of the *N. crassa* cultures was documented to determine whether there were any significant deviations from a normal growth pattern. Observations on growth before and after the colony had entered the magnetic field revealed no noteworthy differences. Humidity and temperature were tracked over the same time period to see if oscillations in temperature or relative humidity would need to be taken into consideration. Temperature was found to remain constant, however the relative humidity showed a large variance. Representative data are shown in Figure 4.

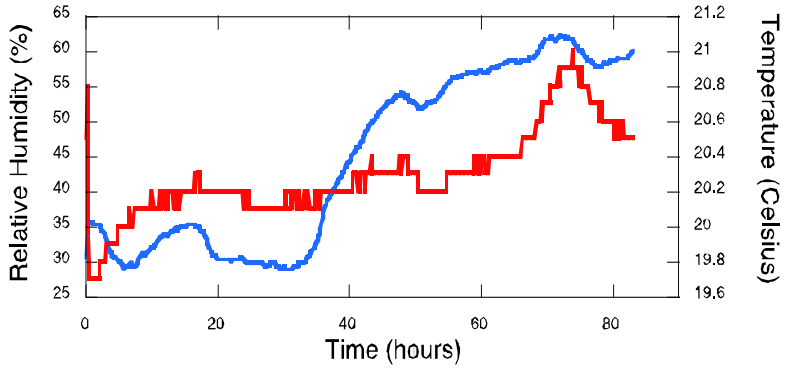

Figure 4. Changes in temperature and relative humidity over time. The Extech Datalogger took Readings every 10 minutes for the duration of the experiment. Data graphed using Excel. Relative humidity (blue) increased over time, whereas temperature (red) remained relatively stable.

DISCUSSION

Relationships have been found between exposure to blue light and magnetoreception in several bird species (Wiltschko and Wiltschko, 1999). Furthermore, *Neurospora* is known to have two blue-light receptor proteins, WC1 and WC2, which play a key role in regulating processes of growth and differentiation (Linden *et al*. 1997). For this reason, we grew our colonies under constant blue light exposure, in the hopes of maximizing any magnetic field effects. Since light entrains the circadian rhythm (and induces the

formation of conidia in *Neurospora*) (Davis, 2000), this guaranteed that any perceived changes in morphology could not be due to the intrinsic circadian growth rhythm of the fungus.

A previous study showed that magnetic field strengths of 6.36 and 32.25 Gauss had no effect on the growth of *N. crassa* (Bitz and Sargent, 1974). In our estimation, these are relatively weak magnetic fields, which could have contributed to the lack of any significant effect. We found that even at magnetic field strengths ranging from 9400 to 9600 Gauss (approximately 1 Tesla), no changes in the growth or discernible morphology of the colonies was detectable. From a biological point of view, this is both surprising and interesting. The electrophysiological properties of fungi are dependent on the flow of ions across the cell membrane, primarily in the form of H^+. Moving electrical charge sets up a current, which produces its own magnetic field. We expected that these internal fields could interact with an external magnetic field, possibly producing changes in growth pattern or orientation. One possible reason for our inability to detect an effect is that these internal fields are too small to have large-scale effects.

The synthesis of carotenoid pigments in conidia is increased by exposure to blue light (Davis, 2000). For this reason, bright orange pigmentation was seen in all colonies in contrast to the normally whitish color seen in cultures grown in the dark. Another factor we had initially noticed was a large increase in the number of aerial hyphae after the colonies had been placed in the magnetic field. We monitored the ambient temperature to determine if this was a byproduct of growth conditions, but temperature was relatively stable overall. However, relative humidity over the experimental period did change dramatically (Figure 4). High humidity encourages the formation of aerial hyphae, while low humidity causes shorter aerial hyphae (Siegel *et al*. 1968).

Figure 5. Pigmentation and aerial growth of hyphae. The colony was permitted to grow for a period of 24 hours under blue LED light of approximately 456 nm, in a magnetic field of 0.96 T. Strong orange pigmentation can be seen on the right hand side, where inoculation had taken place. Formation of aerial hyphae can be seen in the majority of the race tube, compared to relatively little aerial hyphae near the inoculation site.

CONCLUSION

Our results showed no evident effects on form and growth of *Neurospora crassa* when subjected to a high strength constant magnetic field. The effect of low frequency oscillating magnetic fields on fungal growth would be an interesting avenue for further research.

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