Time series analysis demonstrates the absence of pulsatile hyphal growth

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Hyphal tip growth has been previously reported as pulsatile, defined as regularly alternating fast and slow rates of extension. The growth of pollen tubes, and hyphae of Neurospora crassa and Saprolegnia ferax were analysed using high spatial and temporal resolution. By using long (100–500 s) records of growth rate, sampled every second, it was possible to apply rigorous statistical analysis of the time series. As previously demonstrated, pollen tubes can show pulsatile growth, detectable with this system. In contrast, hyphal growth rates do not show any evidence of pulsatile growth; instead, growth rates appear to fluctuate randomly. It is concluded that pulsatile growth is not a common feature of hyphal tip growth.

Establishing the presence of pulsatile growth in hyphal organisms would create a new and valuable tool for analysing the regulation of tip growth in general.

In the fungi, there is an early report of pulsatile growth of mature Phycomyces sporangiophores (Castle, 1940), but mature sporangiophore cells extend by intercalary growth, not directly relevant to tip growth. More recently, pulsed growth was reported for diverse fungi and oomycetes (López-Franco et al., 1994), described as fluctuating “continuously with alternating periods of fast and slow growth at more or less regular intervals”. The published datasets were limited to short time durations and not analysed statistically. Since then there have been few reports of hyphal growth analysis. Money (1997) presented a very limited set of data for Achlya, showing fluctuating, but certainly not periodic, growth and subsequently showed fluctuating force exerted by hyphal tips of the same species (Johns et al., 1999). More recently, Jackson (2001) showed that potentially undetected growth oscillations in the vertical plane could create artefactual growth pulses. In spite of the limited data supporting pulsatile hyphal tip growth, it has been accepted as a universal feature of hyphae, indicated by the conclusion to a recent commentary: “I suspect that pulsatile growth will be recognized as a universal feature of polarized cellular development” (Money, 2001). Such a conclusion should be viewed with caution since even pollen tubes show extensive initial growth with no evidence for pulsation (Messerli & Robinson, 1997; Messerli et al., 1999; Pierson et al., 1996).

Pulsatile phenomena are common in biological processes. In many cases, for example circadian rhythms and pulsations in blood flow, the periodic fluctuations are clearly discernible. However, if the periodic fluctuations are small in amplitude, it becomes necessary to use analytical tools to reveal their presence. Because of the potential importance...

INTRODUCTION

Tip growth is a complex and highly regulated process wherein cell surface extensibility is primarily restricted to an apical dome that moves forward, leaving behind a tubular cell. This type of growth is the hallmark of the fungal kingdom, and also produces pollen tubes and root hairs in angiosperms, diverse cells in lower plants and algae and hyphae of oomycetes. The key feature of tip growth is the regulation of apical extensibility, because the mechanisms that achieve this determine both growth rate and cell morphology and, in the case of fungi and oomycetes, colony morphology. There is no comprehensive model for the regulation of tip extensibility, but it is clear that the cytoskeleton, localization of exocytosis, regulation of cell-wall properties and turgor regulation must all be involved. Coordination and regulation of these processes is critical to tip growth and one facet of such regulation is the distribution of cytoplasmic Ca$^{2+}$. A tip-high gradient of these ions seems to be a universal and obligatory component of tip growth.

There is no a priori reason why tip growth should not be a continuous and uniform process, fluctuating randomly around a mean growth rate. However, at least in older pollen tubes, there is compelling evidence for pulsatile tip growth, defined as regularly alternating fast and slow rates of extension. Initial observations of regular periodic fluctuations in rate of tip extension are matched by similar (typically phase-shifted) periodicity in cytoplasmic calcium concentrations and fluxes of Ca$^{2+}$ and other ions (Holdaway-Clarke et al., 1997; Messerli et al., 1999, 2000; Messerli & Robinson, 1997; Pierson et al., 1996). These matching observations obtained by very different techniques provide strong support for the reality of pulsatile tip growth, a process that has been very valuable in understanding regulation of pollen tube growth (Feijó et al., 2001).
of pulsatile growth in hypha as a means to explore the mechanisms regulating tip growth in greater detail, we have undertaken an extensive time series analysis of growth data for two hyphal species using a system that is capable of revealing pulsatile growth in pollen tubes, and find no evidence for periodic fluctuations. Instead, growth fluctuates randomly. Although it is not possible to exclude the possibility that hyphae may show pulsatile growth, our observations show clearly that pulsatile growth is not a universal attribute of hyphal growth.

METHODS

Organisms, cultures and microscopy. Saprolegnia ferax (ATCC 36051) and Neurospora crassa (wild-type strain 987A, Fungal Genetics Stock Culture Collection) were grown on dialysis membrane overlying a solid medium (OM containing 1.5% agar (Heath & Greenwood, 1970)). Small portions of dialysis membrane bearing hyphae from the growing edge of a colony were removed, mounted in liquid OM on a slide and covered with a coverslip. Hyphal tip growth resumed after a recovery period of 10–30 min. Cultures were grown and observed at room temperature, ~22°C.

Pollen grains of Turnera ulmifolia (courtesy J. S. Shore, York University, Toronto, Canada) and Lilium longiflorum (courtesy C. Hasenkampf, University of Toronto at Scarborough, Canada) were collected from anthers on the day of experimentation. T. ulmifolia grains were placed on a slide in a pollen germination medium (30% sucrose, 0.5 mM H3BO3, 10 mM CaCl2, pH 4.2; S. J. Molnar & J. S. Shore, personal communication), a coverslip was added and the pollen was left to germinate in a humid environment at ~22°C for 30–60 min. L. longiflorum grains were similarly mounted and germinated in modified Dickinson’s medium (Messnerl et al., 2000) at 25°C for 60–90 min. Following germination preparations were returned to ~22°C.

Growing tips were observed with a Reichert Polyrav microscope using green light, Nomarski-DIC optics, a ×100 NA 1.32 objective, a ×2 intermediate lens and alternative intermediate lens systems in the camera tube for the different final magnifications. Images were captured using a Princeton Instruments MicroMax camera and WinView software at a rate of 1 frame s⁻¹, controlled by external synchronization with a square pulse from a BK Precision (model 3010) function generator (Maxtec International). The system gave final magnifications of ×5933 or ×8500 on the screen, a single pixel representing 0.050 or 0.035 μm, respectively. The lower magnification images facilitated longer observation series, while the higher magnification images offered greater measurement precision.

Growth measurements were made on the screen using a cursor positioned by eye at the leading edge of the cells. The image of the tip is complex, the real cellular origin of each component (e.g. plasma membrane, inner side of cell wall, cell wall–medium interface) is not easily identified, but this does not affect the data if a consistent feature of the image is chosen. The position of the cursor was measured in pixels using ImageJ (http://rsb.info.nih.gov/ij/) and growth rates determined by changes in pixel positions versus time. Pixel sizes were calibrated from images of a stage micrometer.

Data analysis

Interpolation. Because the microscope stage had to be moved, typically twice during a measurement sequence, the measurements contained gaps of 2–5 s. Interpolation was used to fill the data gaps, by inserting the mean of the growth rate prior to and after the stage was moved. In the case of a gap of 4 s, the preceding growth rate was inserted into the first two gap positions and the succeeding rate in the last two positions. For a gap of 5 s, the middle point was the mean of the growth rates before and after the gap.

Error analysis. Growth rates were measured by two individuals and twice by the same individual. The differences in measurements were tabulated and fit to a normal distribution of the form:

\[
\text{Percent} = \frac{e^{-\frac{(x - \mu)^2}{2s^2}}}{s\sqrt{2\pi}}
\]

where \(x\) is the observed value, \(\mu\) the population mean and \(s\) the standard deviation.

Test for randomness. In the statistical software package SYSTAT (Wilkinson, 1990), datasets were analysed sequentially as follows. (1) The distribution of growth rates was examined graphically to assure that it was near normal. (2) The datasets were tested using linear regression to confirm that the signals were statistically stationary; that is, the growth rate was not changing, neither increasing nor decreasing, over the period of the measurements. The slope, the Pearson correlation coefficient (\(r^2\)) and a t-test of the significance of the correlation between growth rate and time were examined. In all cases, the slope was less than ±0.033 (range −0.012 to ±0.033 μm min⁻¹); 88% of measurements were less than ±0.01, the largest \(r^2\) was 0.13 and t-tests yielded two-tail probabilities that were non-significant for 20/25 experiments. Therefore, most data were statistically stationary.

Time series analysis relies upon two related analytical techniques to identify non-random periodic fluctuations in the parameter being measured over time: autocorrelation and Fourier (frequency) analysis (MacDonald, 1962). A practical introduction to these time series analysis techniques is presented by the National Institute of Standards and Technology (NIST/SEMATECH, 2003). Autocorrelation was performed to determine whether changes in growth rate over time were either random, exhibiting no self correlation over time, or periodic, in which case there would be significant autocorrelation. The autocorrelation analysis tests for correlations between data values in the datasets based on an increasing lag interval (\(k\)) between the data according to:

\[
r_k = \frac{\sum_{i=1}^{n-k} (Y_i - \bar{Y})(Y_{i+k} - \bar{Y})}{\sum_{i=1}^{n} (Y_i - \bar{Y})^2}
\]

where \(Y_i\) is the value at the \(i\)-point in the data series, \(Y_{i+k}\) is the value at the \(i\)-th plus \(k\) lag point in the data series and \(\bar{Y}\) is the mean for all data points.

If data exhibit any periodicity, this appears as significant correlation coefficients at a \(k\) value that will depend upon the peak to peak interval of the periodicity. Confidence intervals of 0.05 were calculated using the formula:

\[
\pm \frac{1.96}{\sqrt{n - 1 - k}}
\]

where \(p\) is the length of the time series and \(k\) is the lag interval (Legendre & Legendre, 1983). Note that as the length of the time series increases, the confidence intervals for the autocorrelation become smaller because the sample size for a given lag interval increases. That is, a statistically significant presence of pulsatile growth is easier to reveal using experiments with a larger number of data measured over a longer duration.

Comparison with randomly fluctuating growth rates. A time series of a randomly fluctuating growth rate was generated to compare
random fluctuations with experimental measurements of hyphal growth rates. A normally distributed random variate (mean ± SD of 0 ± 1) was generated in SYSTAT and transformed such that the mean ± SD was 16.2 ± 4.3 μm min⁻¹, similar to the mean hyphal growth rate for N. crassa. Autocorrelation was performed as described above.

### RESULTS

#### Measurement accuracy

Examination of tip growth for possible subtle fluctuations in growth rates demands accurate determination of the extension of the cell surface at the set time intervals. This was determined by experienced observers. As seen in Fig. 1, this pollen tube was 1.65 mm long. Another tube that was only 1.52 mm long did not show pulsatile growth, suggesting that pulsatile growth begins at about 1.6 mm, but we were unable to determine the length of other non-pulsatile tubes because they typically grow in tight circles. Most interestingly, the pulsatile tube was periodic when first examined, then reverted to non-pulsatile growth during observation, showing reversibility of pulsations.

**Fig. 1.** Error analysis of growth rate measurements. A single video sequence from one hypha was measured by two individuals (circles) or twice by the same individual (squares). One set of measurements was subtracted from the other. The growth rates were the same for 32–34% of the measurements. A further 41–45% differed by one pixel. The differences between the two sets of measurements were fit to normal distributions, yielding a mean difference (± SD) of 0.000 ± 1.39 (circles) or 0.000 ± 1.261 (squares).

#### Pollen tubes

Because pollen tubes have an extensive literature documenting pulsatile growth by complementary methods, we analysed the pollen tubes of two different species (Table 1). It is important to note that the mean rates of growth for the pollen tubes covered the range shown by the subsequently observed hyphae, thus presenting model cells of similar size with similar growth increments. *L. longiflorum* has been extensively analysed previously (see Introduction) and shown to exhibit fluctuating, but non-pulsatile, growth in short tubes and pulsations in longer tubes. Consistent with these observations, we observed three tubes with non-periodic growth and one with very clear periodicity of about 59 s (Fig. 2). Evidently, our methodology is well able to detect pulsatile growth. Similarly, in the previously unreported *T. ulmifolia*, we were able to detect pulses in part of one dataset (Fig. 2), but in no other tubes examined. This pollen tube was 1.65 mm long. Another tube that was only 1.52 mm long did not show pulsatile growth, suggesting that pulsatile growth begins at about 1.6 mm, but we were unable to determine the length of other non-pulsatile tubes because they typically grow in tight circles. Most interestingly, the pulsatile tube was periodic when first examined, then reverted to non-pulsatile growth during observation, showing reversibility of pulsations.

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of cells</th>
<th>Series length (s)</th>
<th>Pixel size (μm)</th>
<th>Mean rates (μm min⁻¹)</th>
</tr>
</thead>
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<tr>
<td>Pollen</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>L. longiflorum</em></td>
<td>4</td>
<td>300</td>
<td>0.035</td>
<td>5–12</td>
</tr>
<tr>
<td><em>T. ulmifolia</em></td>
<td>14</td>
<td>66–300</td>
<td>0.035</td>
<td>7–39</td>
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<tr>
<td>Hyphae</td>
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<tr>
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<td>0.035</td>
<td>16–17</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>520</td>
<td>0.05</td>
<td>13–22</td>
</tr>
<tr>
<td><em>S. ferax</em></td>
<td>3</td>
<td>129–147</td>
<td>0.035</td>
<td>15–19</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>520</td>
<td>0.05</td>
<td>12–18</td>
</tr>
</tbody>
</table>

**Table 1.** Summary of cells analysed
The mean growth rate of both pulsatile and non-pulsatile phases was unchanged at about 13 μm min⁻¹. The periodicity, when present, was about 30 s and was clear by simple observation of the growth rate traces and by analysis with autocorrelation (Fig. 2) and Fourier transformation (Fig. 5).

Because the only objective of the analysis of the pollen tubes was to validate the ability of our observational system to demonstrate periodic pulsatile growth, we did not pursue the analysis of these cells any further.

**Hyphae**

Hyphae of both *N. crassa* and *S. ferox* (Table 1) showed fluctuating growth rates, but in no case was there any evidence of pulsatile growth (representative examples are

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**Fig. 2.** Growth rates and autocorrelations for *Turnera* sp. (a) and *L. longiflorum* (b). Growth rates for six tubes were measured to a resolution of 0.035 μm per pixel (left panels) and corresponding autocorrelations of the same tubes calculated with ±95% confidence intervals (smooth lines above and below the autocorrelations) (right panels). Various patterns of autocorrelation are apparent for pollen growth rates over time. Autocorrelations at short lag intervals are due to the fact that nearby data points do exhibit some inter-relation. Pulsatile fluctuations are clearly visible in the top panels of both (a) and (b). The growth rates in the top panels of (a) and (b) were used for Fourier analysis (Fig. 5).
shown in Figs 3, 4 and 5). These observations are independent of higher or lower magnification datasets and variations in mean growth rates (range 12·6–21·6 μm min$^{-1}$ for N. crassa and 11·7–19·0 μm min$^{-1}$ for S. ferax, similar ranges to those of the pollen tubes). Noticeably, the mean rates typically remained constant throughout the datasets, suggesting no adverse physiological factors developed during observation. The growth rates were also comparable to those shown by colonies on agar plates. Not only was there no evidence of pulsatile growth by simple observation of the growth rate traces, both autocorrelation and Fourier transform analysis showed only white noise behaviour, supported by a direct comparison to a computer-generated randomly fluctuating growth rate.

**Fig. 3.** Growth rates and autocorrelations for *N. crassa*. Growth rates (left panels) for three hyphae measured at a resolution of 0·05 μm per pixel (a) and at 0·035 μm per pixel (b) and corresponding autocorrelations (right panels) of the same hyphae with ±95% confidence intervals (smooth lines above and below the autocorrelations). Note that there is no indication of autocorrelation, with the exception of the noisy growth in the second hypha down, which shows some autocorrelation with a period of about 3 s. This could be an artefact and was never observed in higher resolution measurements of growth rate. The growth rate of the top panel in (a) was used for Fourier analysis (Fig. 5).
Comparison with randomly fluctuating growth rates

The experimental measurements of hyphae of both species examined are very similar to a randomly fluctuating growth rate generated using a normally distributed random variate (Fig. 6). The autocorrelation is also very similar to experimental measurements, suggesting that hyphal growth normally exhibits random fluctuations.

DISCUSSION

Technical considerations

To demonstrate pulsatile growth it is essential to have adequate spatial and temporal resolution. Previous reports (Introduction) have defined the range of period lengths and amplitudes (trough to peak) found in tip-growing systems as being about 4–60 s and 1·6–15 μm min⁻¹, respectively. More specifically, the reported values for N. crassa and S. ferax, the hyphae examined in the present work, are periods of 4·7 and 5·1 s and growth rates of 5·6 and 2·2 μm min⁻¹, respectively (López-Franco et al., 1994). We examined these two organisms as both are used routinely in our laboratories. By using time series analysis of measurements of longer duration than previously reported (Lopez-Franco et al., 1994), and using the standard techniques of autocorrelation and Fourier analysis, we expected to present a more convincing case for pulsatile growth, but

![Fig. 4. Growth rates and autocorrelations for S. ferax. Data are presented as described in the legend to Fig. 3. Note that there is no indication of autocorrelation. The growth rate of the top panel in (a) was used for Fourier analysis (Fig. 5).](image-url)
Fourier-transformed. The linearly compensated data were Hanning-filtered and then truncated to 256 measurements and linear trends removed. The squared real and imaginary coefficients of the Fourier transform.

There is no indication of any periodic growth in previously reported pulsatile growth. Furthermore, our best spatial resolution of about 1 pixel s<sup>-1</sup> in our data are more than adequate to reveal variations in growth rates for the two hyphal organisms are completely random, explicitly shown in Fig. 6.

Fig. 5. Fourier analysis of tip growth rate. Data for N. crassa (a), S. ferax (b), Turnera sp. (c) and L. longiflorum (d) were truncated to 256 measurements and linear trends removed. The linearly compensated data were Hanning-filtered and then Fourier-transformed. The y axis shows power, the sum of the squared real and imaginary coefficients of the Fourier transform. There is no indication of any periodic growth in N. crassa and S. ferax, but Turnera sp. shows a clear periodicity of about 30 s and L. longiflorum shows a similar periodicity of about 55 s. The power spectra for the two hyphal organisms is essentially flat, independent of frequency. This result is commonly described as white noise. In concert with the absence of autocorrelation, the results indicate that the fluctuations in growth rates for the two hyphal organisms are completely random, explicitly shown in Fig. 6.

arrived at the opposite conclusion. Both the temporal resolution (1 s) and length of time series ( ~ 25–100 period lengths) in our data are more than adequate to reveal previously reported pulsatile growth. Furthermore, our best spatial resolution of about 1 pixel s<sup>-1</sup> equals 1·8 μm min<sup>-1</sup>, less than the reported amplitudes and the same as the pixel size used previously (López-Franco et al., 1994). Alternatively, with a mean growth rate of 15 μm min<sup>-1</sup>, a typical rate (Table 1), the growth increment is 7 pixels s<sup>-1</sup>, well above the repeatability of 1 pixel determined for the location of the tip at each measurement point. It may be argued that the 27 % of measurements that differed by more than a single pixel in repeat observations of a single data series (Fig. 1) could be responsible for underestimating peak deviations from the mean, but such an argument would require the additional assumptions that this error is always in the requisite direction and systematically in synchrony with any hypothetical periodicity in the data – highly unlikely assumptions. We conclude that our observational system is at least comparable to that of previous reports, and more than adequate to reveal variations in growth rates of magnitudes previously reported. This conclusion is supported by the clear observation of pulsatile growth of some pollen tubes.

Absence of pulsatile growth

We cannot rule out the existence of pulsatile growth with lower amplitudes or periodicity than previously reported, below the sensitivity of our system, but demonstration of such becomes problematic due to spatial resolution; 0·035 μm is already well below the theoretical limit of resolution of the microscope and is unlikely to be bettered due to the constraints of the wavelength of light. Thus, we conclude that pulsatile growth of the magnitude previously reported for hyphae of the same species as we have examined is not a common feature of hyphal tip growth.

The absence of pulsatile growth is not unexpected. Pollen tubes are the best-studied tip-growing cells in this respect. Tubes growing soon after germination (<30 min, up to about 300 μm at reported growth rates (Messerli & Robinson, 1997) or <700 μm (Pierson et al., 1996) show fluctuating, but not pulsatile growth rates. Similarly, the present observations of pollen tube growth reveal fluctuating but non-pulsatile growth in shorter tubes, up to at least 1·5 mm long. In all these studies, the mean growth rates during the pulsatile and non-pulsatile phases were essentially the same, thus pulsatile growth does not correlate with variation in growth rate. Furthermore, while limited to a single fortuitous tube, our observation of reversion from pulsatile to non-pulsatile growth shows that the engagement of pulsatile growth is not irreversible, and again the mean rate did not change during the transition.

The only other tip-growing cell type studied in sufficient detail to evaluate pulsatile growth is the plant root hair. Approximately the first third of root hair growth may be non-pulsatile and slower than the later pulsatile phase (Wymer et al., 1997), yet in another species, growth was reportedly non-pulsatile throughout hair elongation (Shaw et al., 2000). Thus, again pulsatile growth is apparently neither obligatory nor common in this tip-growing cell type.

While we do not imply that the well-established pulsatile growth reported in pollen tubes and perhaps root hairs is not both real and potentially very significant in understanding the mechanisms of tip growth, it is important to
emphasize that in all critically examined tip-growing cells, such growth is not obligatory. Thus, it would be a mistake to try to base explanations of the process on models requiring pulsatility. Perhaps the most interesting question involves the factors regulating switching between the two modes of growth, but, as indicated above, obvious correlates such as mean growth rates and tube lengths do not seem to offer an explanation.

Possible explanations of previous data

One possible artefactual source of periodic pulsatile growth patterns has been described by Jackson (2001). She argues that periodic oscillations of growth direction in the z plane could be sufficient to create an illusion of pulsatile growth in many species, yet remain undetected within the plane of focus of the microscope. This may be related to the well-described phenomenon of helical growth. In fact only planar sinusoidal growth in the z plane could generate such an effect. Horizontal helical growth would give constant elongation in the x-y plane because the tip would always be elongating at a constant angle to the long axis of the hypha. Only helical growth at some inclination to the horizontal could generate apparent pulses as envisaged by Jackson (2001). It is possible to model the apparent growth pulses from such an effect (Table 2). Determination of the radius of the helix is uncertain, but Jackson (2001) has argued that if it is less than about 0.5 μm it might be undetectable. As seen in Table 2, amplitudes and periods comparable to those previously reported for hyphae can be generated from inclined helical growth, but only at inclinations of about 10° to the horizontal. However, such an inclination would result in hyphae moving out of the optical plane of focus within about 6 μm, which would be easily detectable and is unlikely in datasets of experienced observers. Thus, while inclined, but not horizontal, helical growth could generate artefactual pulsatile growth, such seems unlikely.

As previously observed (López-Franco et al., 1994), many older reports of hyphal tip growth simply lacked sufficient temporal or spatial resolution to detect pulsatile growth. In this regard, the report by Lopez-Franco et al. (1994) served a very useful purpose, emphasizing that relatively fast

changes in hyphal growth rate can occur. However, to demonstrate unequivocal pulsatile growth requires objective criteria and sufficiently long datasets. To date, among recent studies of hyphal tip growth, only the current report presents such objective analysis and finds no evidence for pulsatility. Lopez-Franco et al. (1994) incubated hyphae in the slide chambers for 24–36 h before measuring growth rates, while we measured hyphal growth soon (30 min) after placing the hyphae in the chamber. However, when we plotted the presented data in Fig. 2 from López-Franco et al. (1994) using autocorrelation analysis, none of the presented species showed objective evidence for pulsatile growth based on the 95% confidence interval criterion. Only Gilbertella persicaria came close, with peaks just under the 95% value. With a longer series of measurements, it may have been possible to demonstrate statistically significant pulsatile growth in this species. Even subjective observation of the other traces, and those in Money (1997), can easily be interpreted as non-pulsatile, fluctuating to be sure, but randomly. Although we have demonstrated that two hyphal organisms have growth rates which fluctuate randomly, it may be that some fungal species exhibit pulsatile growth, possibly age-dependent. Until similarly

Table 2. Apparent pulsatile growth rates due to inclined helical growth

These values were calculated using the equation: \( V_x = a \cos \phi \) \( R \sin \phi \cos(t) \), where \( V_x \) is the apparent velocity in the x direction, \( a \) is the velocity in x which is independent of velocity parallel to the long axis of the hypha, i.e. the actual rate of hyphal tip expansion, \( R \) is the radius of the helix and \( \phi \) is the angle of inclination. Values used were: \( a = 15 \mu \text{m min}^{-1} \); \( \phi = 10^\circ = 1.5 \times 10^{-1} \).

<table>
<thead>
<tr>
<th>Radius (μm)</th>
<th>Amplitude (toward peak, μm min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period (s): 10  20   30   60</td>
</tr>
<tr>
<td>0.2</td>
<td>2.5  1.1  0.9  1.4</td>
</tr>
<tr>
<td>0.5</td>
<td>6.1  4.3  2.2  1.1</td>
</tr>
<tr>
<td>1</td>
<td>12.3* 8.6  4.3  2.2</td>
</tr>
<tr>
<td>2</td>
<td>24.5* 17.1* 8.7  4.4</td>
</tr>
</tbody>
</table>

*These values require unrealistic tip velocities > 40 μm min\(^{-1}\).
detailed analyses demonstrate pulsatile growth of hyphae, it may be wiser to accept fluctuations of growth rate, but not periodicity in those fluctuations. Thus, it may also be wiser to seek regulatory models that do not invoke pulsatility.

ACKNOWLEDGEMENTS

This work was supported by Discovery Grants from NSERC to R. R. L. and I. B. H. Michael Slattery very kindly pointed out the need for inclined helical growth to generate apparent pulsatility and prepared the modelling presented in Table 2.

REFERENCES


