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The Relation between Turgor and Tip Growth in *Saprolegnia ferax*: Turgor Is Necessary, but Not Sufficient to Explain Apical Extension Rates

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KAMINSKYJ, S. G. W., GARRILL, A., AND HEATH, I. B. 1992. The relation between turgor and tip growth in *Saprolegnia ferax:* Turgor is necessary, but not sufficient to explain apical extension rates. *Experimental Mycology* 16, 64–75. Linear growth rate of *Saprolegnia* was reduced in direct proportion to increased osmotic pressure (Π) of the medium, when sorbitol or PEG-400 was used as osmotica. However, increasing medium Π reduced hyphal turgor only to a minimum positive level, which was maintained while extension rates continued to decline. TPA, a K^+-channel agonist effective on *Saprolegnia* protoplasts, also caused dose-dependent linear growth rate reductions but did not substantially affect turgor. When turgor was compared with linear growth rate in the osmoticum experiments, there was a positive correlation only for hyphae growing faster than 12 μm/min; below this, there was a twofold range in extension rate despite essentially constant turgor. As well, TPA-treatments produced a twofold reduction in hyphal extension rate without substantially affecting turgor. Turgor should be consistent within a coenocyte, and is steady under constant growth conditions. However, under such conditions, we found average variations of fivefold in extension rate between hyphae, and twofold for hyphae over time. These results suggest that turgor is not the prime determinant of tip extension rate, and they are consistent with cytoskeletal regulation of that rate. Linear growth rates of *Saprolegnia* colonies were similar on basal medium containing 1% (w/v) glucose, sorbitol, or PEG and only slightly faster than without added carbohydrate. Increasing medium Π with glucose also reduced hyphal extension rate. © 1992 Academic Press, Inc.

INDEX DESCRIPTORS: tip growth; turgor pressure; oömycetes; fungi.

Tip growth models typically assume a linear relationship between turgor pressure and hyphal extension rate (Koch, 1982; Saunders and Trinci, 1979; Wessels, 1988). Assuming that turgor is inversely related to osmotic pressure (Π)[1] of the medium, studies of the relationship between medium Π and colony growth (measured by dry weight or by colony diameter) generally support these models, i.e., growth rates are usually depressed with increased medium Π. However, turgor may or may not be influenced by medium Π. The details of these relationships are variable and are summarized in Table 1. A further complication is that linear growth may (Griffin, 1978) or may not (Trinci, 1969) be simply related to increase in colony dry weight. To date, direct comparisons of turgor with tip extension rate are few and variable, suggesting either a positive or no correlation (Table 1). There has been no work on the relation between turgor and linear growth rates in oömycetes which, being coenocytic, constitute a simpler model system. In this report we show a complex relationship between turgor pressure and hyphal tip extension rate in the oömycete, *Saprolegnia ferax*: reduced linear growth rates may or may not be correlated with lower turgor.

MATERIALS AND METHODS

Cultures and Media

Saprolegnia ferax (Gruith.) Thuret

[1] Abbreviations used: DIC, differential interference contrast; OM, organic medium; Π, osmotic pressure; PEG, polyethylene glycol 400; TPA, tetrapentyl ammonium chloride.

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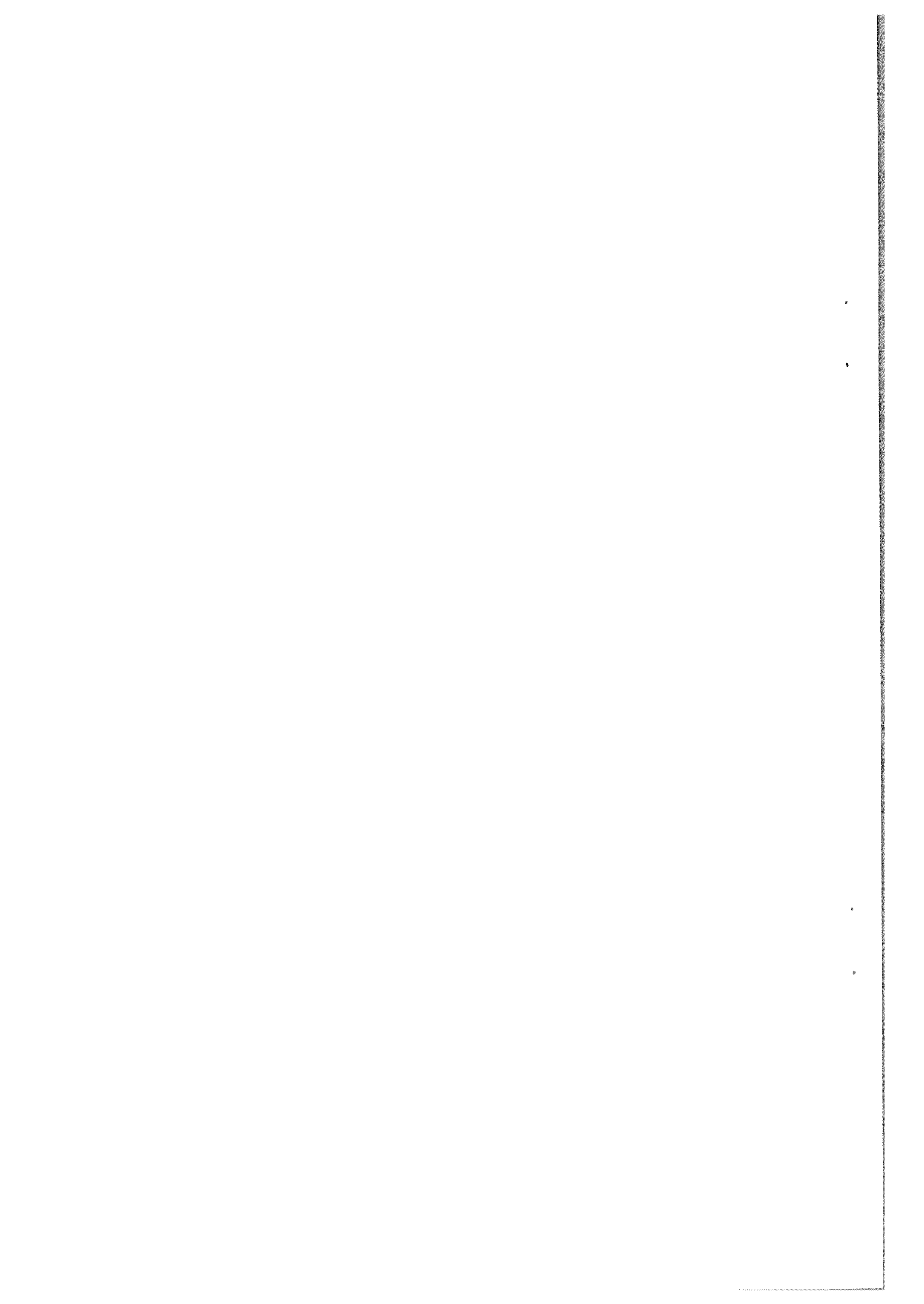

(ATCC 36051) was grown at room temperature on an organic medium (Heath and Greenwood, 1968) designated OM, containing 1% (w/v) glucose, 1% (w/v) sorbitol (Sigma) or 1% (w/v) polyethylene glycol 400 (PEG: Baker Chemicals). For colony extension rate measurements, media of identical composition were solidified with 1.5% (w/v) Difco Bacto agar.

OM amended with glucose, sorbitol, or PEG (the latter two also used as plasmolyzing solutions) was prepared from stock solutions. Tetrapentyl ammonium chloride (TPA: Aldrich Chemicals) was prepared as a 10 m*M* stock solution in sterile distilled water and added to OM to the appropriate concentrations.

Observation Systems

Colonies were examined with a dissecting microscope and substage illumination. Hyphal diameter was measured using a 100× N.A. 1.32 objective, and differential interference contrast (DIC) optics. Plasmolysis was evaluated using a 100× N.A. 1.25 objective with phase-contrast optics. Hyphal extension rate measurements used a 10× N.A. 0.32, objective and phase-contrast optics. Trypan blue uptake was evaluated using a 10× 0.25 NA objective and DIC optics. Images were recorded with 35-mm film or on videotape.

Linear Growth Rates

Colony extension rates were measured on solid media inoculated with 1 × 1 × 3-mm pieces cut from the margin of a colony growing rapidly on OM. After 46 h, radial growth from the edge of the inoculum was measured to the nearest 0.5 mm at two places on replicate plates, using 6× magnification.

Extension rates for individual hyphae were determined from small colonies growing in liquid OM in 25-μl microscope chambers (Heath, 1988), continuously perfused with fresh medium. Rates were determined by recording groups of hyphae at intervals, with the illumination turned off between records. All hyphae within the field of view for the entire observation period were measured from the video monitor to the nearest 1 mm (4.4 μm).

Diameter

Hyphal diameter was measured from videotapes to the nearest millimeter on the monitor (0.44 μm). Measurements were made at 50 and 75 μm from the tip and the values were averaged. Diameters of plasmolyzed hyphae were measured between walls.

Osmometry

The osmolality of OM amended with sorbitol or PEG was measured using a Wescor Model 5100C vapor pressure osmometer (Wescor, Logan, UT). Osmolality values were used to construct standard curves for sorbitol and PEG (which are not ideal solutes) solutions, after subtracting the osmotic contribution of OM which was common to all media. Quadratic regression curves were fitted using Cricket Graph 1.3.1 (Cricket Software, Malvern PA); the residual variations were ≤ 0.001. These equations were used to convert molar osmoticum concentrations into osmolalities, and then Π of these solutions was derived according to the relationship $\Pi = RTc$, where c is osmolality in moles $\times$ kg^{-1}, and RT is 2.446 kg $\times$ MPa $\times$ mol^{-1} at 21°C (Money, 1989).

Turgor Estimation—Incipient Plasmolysis

Colonies were grown undisturbed for at least 16 h in liquid media; sectors were cut and left to recover in the growth medium for at least 15 min. Colonies in media of $\Pi > 0.75$ MPa needed a longer recovery (at

least 30 min) for consistent results. Sectors were mounted in their growth medium in microscope chambers (Heath, 1988) which had a volume of approximately 25 μl, and challenged by flow-through of at least 400 μl of media with higher Π. Incremental change in Π of plasmolyzing solutions was at least 0.25 MPa; each solution was tested on a fresh sector.

Hyphal turgor was estimated by determining the minimum medium concentration which could induce moderate (type 2, see below) plasmolysis after 2 min. Results were scored qualitatively as (1) some shrinkage, but plasmolysis in $<50\%$ of the hyphae, (2) all hyphae shrunken and plasmolysis in 50% of the hyphae, beginning by 2 min, (3) rapid and violent shrinkage accompanied by plasmolysis in $\geqslant 50\%$ of the hyphae.

The diameter of *Saprolegnia* hyphae changed with Π of the growth medium, and all hyphae shrank significantly in plasmolyzing solutions with respect to their growth diameters (see Results). Without correction, this shrinkage would lead to overestimating the absolute value of hyphal osmotic pressure because a shrunken protoplast is more concentrated than that in a fully turgid hypha (assuming that no solute is lost). However, since the product of internal hydrostatic pressure and cell volume is approximately constant (Nobel, 1983) hyphal turgor pressures were adjusted by multiplying with the factor (r^2 after plasmolysis/r^2 before plasmolysis), which is proportional to the change in volume assuming that the change in length is relatively small; this correction has been employed elsewhere (Money, 1990). Volume-correction factors were calculated for each growth medium formulation.

Turgor Estimation—Osmometry

Colonies were grown undisturbed in liquid media for at least 16 h (longer in media with $\Pi > 0.75$ MPa) until they were about 1 cm in diameter, harvested by vacuum filtration on a 10-μm Nitex mesh filter supported by the sintered glass plate of a Büchner funnel, and then drained of medium using 240 kPa suction (monitored by an in-line vacuum gauge) for 5 s after the bulk of the medium had been removed. The mycelium was lifted from the filter surface, transferred to a microfuge tube (which was then sealed), and frozen with liquid nitrogen. Both the mycelium and a growth medium reference sample were stored at −20°C until further processing and/or osmometry.

Membrane disruption after freeze–thaw permeabilization was evaluated using light microscopy and trypan blue exclusion (Gahan, 1984). Colonies were stained in 0.1% aqueous trypan blue for 30 s, washed in OM for 5 min, and examined for staining of the cytoplasm. There was cytoplasmic staining in $<5\%$ of the living hyphae, whereas $>95\%$ stained after freeze–thaw permeabilization. While this result suggested that membranes had indeed been disrupted, the Π of fluid which seeped from permeabilized mycelium was lower than that which was pressed out, suggesting that the hyphal walls were retaining osmotically active components. Therefore, to ensure valid and reproducible measurements, thawed mycelia were disrupted at 0°C using two 4 s, 35 W treatments with the microprobe of a Sonifier Cell Disruptor, Model W185 (Heat Systems-Ultrasonics, Plainview, NY). This treatment produced 10- to 50-μm-long hyphal fragments, plus cytoplasmic debris. Debris were pelleted by centrifuging for 90 s in an Eppendorf Model 5412 centrifuge (Brinkmann Instruments, Rexdale, Ontario) and then the sample was kept at 0°C until the lysate Π was measured as described above.

Statistical Analysis

Statistical analyses were performed according to the procedures of Zar (1984) aided by the statistical software, StatView

SE + Graphics version 1.02 (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

As with other fungi (see Table 1), the linear growth rate of *Saprolegnia* colonies was depressed as medium Π increased, using sorbitol or PEG as osmotica (Fig. 1). Adding glucose to standard OM also suppressed extension rates (data not shown) to levels intermediate between the osmotica when compared as millimolar levels. While

TABLE 1
Summary of Relationships Between Medium Π and Fungal Growth Characteristics

Species	Medium Π vs linear growth	Medium Π vs colony weight	Medium Π vs turgor	Turgor vs linear growth	Ref.
Oömycetes					
Saprolegnia	3	5	4	2	This report; Harrison and Jones (1971)
Aphanomyces	—	—	1	—	Hoch and Mitchell (1973)
Phytophthora	3,4	3,4	1	—	Luard (1982); Luard and Griffin (1981); Sommers *et al.* (1970); Sterne *et al.* (1976); Wilson (1973); Woods and Duniway (1986)
Ascomycetous Fungi					
Aspergillus	2,3,4,5	3	6	6	Adebayo *et al.* (1971); Beever and Laracy (1986); Luard and Griffin (1981); Pitt and Hocking (1977); Smith and Hill (1982); Stevens *et al.* (1983)
Chrysosporium	5	—	5	—	Luard and Griffin (1981); Pitt and Hocking (1977)
Coprophilous fungi[a]	4	—	—	—	Kuthubutheen and Webster (1986)
Eurotium	4,5	—	2	—	Andrews and Pitt (1987); Luard and Griffin (1981); Pitt and Hocking (1977)
Fusarium	3,4,5	3	1,6	—	Luard and Griffin (1981); Cook *et al.* (1972); Kuthubutheen and Webster (1986); Magan and Lynch (1986); Wilson (1973); Woods and Duniway (1986)
Gibberella	3	3	—	—	Wearing (1976)
Neocosmospora	—	3	3	—	Kelly and Budd (1990)
Paecilomyces	3	3	—	—	Inch and Trinci (1987)
Penicillium	4,5	—	1,5	—	Hocking and Pitt (1979); Luard and Griffin (1981); Magan and Lynch (1986)
Soil ascomycetes[b]	3,4	—	—	—	Magan & Lynch (1986)
Xerophiles[c]	5	—	—	—	Andrews and Pitt (1987)
Basidiomycetous Fungi					
Clavulina	3	—	—	—	Wilson (1973)
Phallus	3	—	3	1	Eamus and Jennings (1986)
Phellinus	6	—	1	—	Luard and Griffin (1981)
Serpula	3	—	1	1,6	Eamus and Jennings (1986); Thompson *et al.* (1985)
Soil basidiomycetes[d]	3,4,6	—	—	—	Wilson and Griffin (1979)
Wood rotting fungi[e]	4	—	—	—	Boddy (1983)
Xeromyces	5	—	1	—	Luard and Griffin (1981); Pitt and Hocking (1977)
Zygomycetes					
Mucor	4,5	—	1	6	Adebayo *et al.* (1971)
Pilobolus	4	—	—	—	Kuthubutheen and Webster (1986)

Note: Numbers in the table refer to the type of correlation between factors over the range studied: 1, positive, linear; 2, positive, nonlinear; 3, negative, linear; 4, negative, nonlinear; 5, positive at lower Π and negative at higher Π; 6, none.

[a] *Doratomyces, Isaria, Onychophora, Podospora, Saccobolus, Sporormiella, Stibella.*

[b] *Acremonium, Chaetomium, Gliocladium, Penicillium, Trichodema.*

[c] *Basipetospora, Geomyces, Polypaecillium.*

[d] *Agaricus, Boletus, Bovista, Clavulina, Clitocybe, Geastrum, Lactarius, Polystictus, Rhizopogon.*

[e] *Bjerkandera, Coriolus, Exidia, Hyphoderma, Peniphora, Phanerochaete, Phlebia, Schizopara, Stemum, Vuilleminia.*

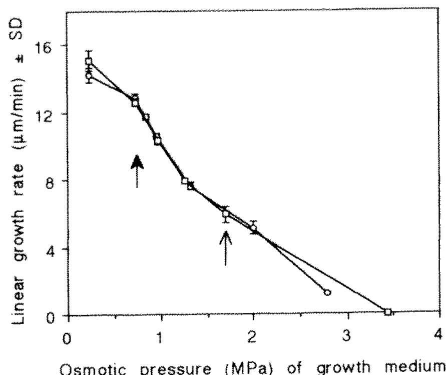

FIG. 1. The relation between medium Π and extension rate in *Saprolegnia ferax*. Osmotica were sorbitol (circles) and PEG (squares). Growth media with Π greater than indicated by the solid arrow were not associated with substantial turgor variation (see Fig. 3). Colonies growing on solid media with Π greater than the open arrow had abnormal morphology and would not grow in comparable liquid media.

it is likely that changing medium Π will affect turgor, this is by no means certain: positive, negative or no correlation between these factors has been reported for various species (Table 1). Thus, we needed to directly measure the turgor of colonies grown in media with different Π. Because turgor is difficult to measure accurately (Jennings, 1990; Nobel, 1983), we chose to use both incipient plasmolysis and hyphal lysate osmometry.

Incipient plasmolysis uses hypertonic treatments, and because organisms can adapt to changes in external Π (Jennings and Burke, 1990), and the related genus *Aphanomyces* has been reported to equilibrate rapidly (Hoch and Mitchell, 1973), we first needed to determine how *Saprolegnia* adapted to sudden changes in Π. We chose a medium with a moderate increase in Π, OM plus 0.5 MPa due to added sorbitol, which was near-isotonic but not plasmolyzing (Heath, unpublished observations), and then determined turgor by incipient plasmolysis. If *Saprolegnia* were unable to adapt to osmotic stress, turgor would be reduced to zero by this treatment and remain there. In contrast, perfect adaptation would be indicated by consistent turgor despite changes to medium Π; incomplete adaptation is characterized by an equilibrium turgor value which is different from that before challenge (Jennings and Burke, 1990). Turgor was reduced by the near-isotonic medium, and hyphal diameter was reduced as well ($P < 0.05$, ANOVA; data not shown). After 5 min, turgor had partially recovered (Fig. 2), and after 10 min turgor was similar to that of colonies grown in the higher Π medium for many hours. However, the equilibrium turgor was lower than for OM-grown hyphae, suggesting incomplete adaptation (see also Fig. 3). Therefore, *Saprolegnia* can make rapid and substantial accommodation to near-isotonic increases in external Π, and so incipient plasmolysis turgor estimations were evaluated at 2 min, before substantial accommodation was likely. Because some accommodation may already have occurred by 2 min, these estimations may be higher than the true value. Similarly, the fact that these estimations used 50% plasmolysis as an indicator of incipient plasmolysis (in isotonic medium no plasmolysis should occur), this

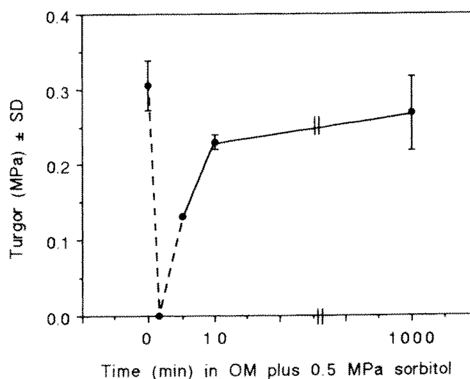

FIG. 2. Accommodation by *Saprolegnia ferax* hyphae to nonplasmolyzing hypertonic stress, with turgor estimated by incipient plasmolysis with sorbitol. Control colonies (0 min) were mounted in liquid OM; 5- and 10-min treatments were similarly mounted, subjected to hypertonic stress with near-isotonic solution (OM plus 0.5 MPa sorbitol) at 0 min, followed by turgor estimation. The 1000-min treatment corresponded to the turgor in colonies grown in the test solution for 16½ h. The dashed line refers to the theoretical change in turgor with time, the solid line to experimental data.

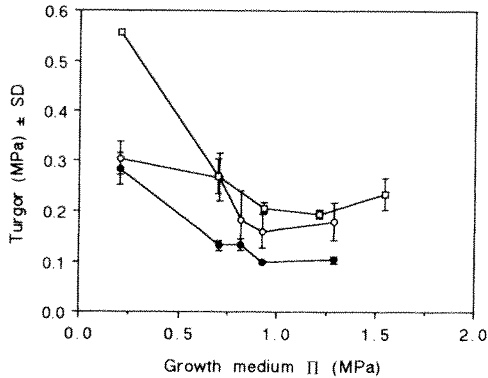

FIG. 3. The effect of growth medium Π on hyphal turgor in *Saprolegnia ferax*. Colonies were grown in OM containing sorbitol (circles) or PEG (squares) as osmotica. For the incipient plasmolysis estimates (open symbols), turgor was evaluated with the same osmoticum as the growth medium. Turgor values estimated by osmometry are indicated by closed circles.

will add to the tendency to overestimate the actual turgor using this technique.

Using osmometry to measure turgor, the osmolality of hyphal lysate is compared to that of the growth medium; the difference between these two values (the lysate being higher) is equivalent to turgor. However, this technique is affected by growth medium retained in the walls and between hyphae: media may drain incompletely, thus reducing the measured osmolality of the lysate. Beever and Laracy (1986) suggest that extracellular fluid, even after aggressive extraction, may still amount to as much as 26% of fresh weight. Mycelium is sometimes washed after harvest but, depending on the washing solution, washing may cause hypotonic stress leading to adaptation and lower lysate osmolality. We compared lysate osmolality of colonies which were not washed after harvesting with those which were washed with OM (0.28 MPa) immediately after the growth medium was removed. Although washing and draining required less than 1 min, lysate osmolality of washed colonies grown in the lowest level of sorbitol was reduced from 0.97 to 0.56 MPa, well below that of the growth medium, which was 0.81 MPa. Consequently, we did not wash our mycelia after harvest and therefore our osmometry measurements will be below the true turgor pressure. However, by using both incipient plasmolysis (too high) and osmometry (too low), we should have bracketed the real turgor pressure. Our recorded values are consistent with this suggestion (Figs. 3 and 4).

Increasing growth medium Π with either osmoticum was correlated with a reduction in turgor pressure to a minimum but positive level (Fig. 3), regardless of technique-

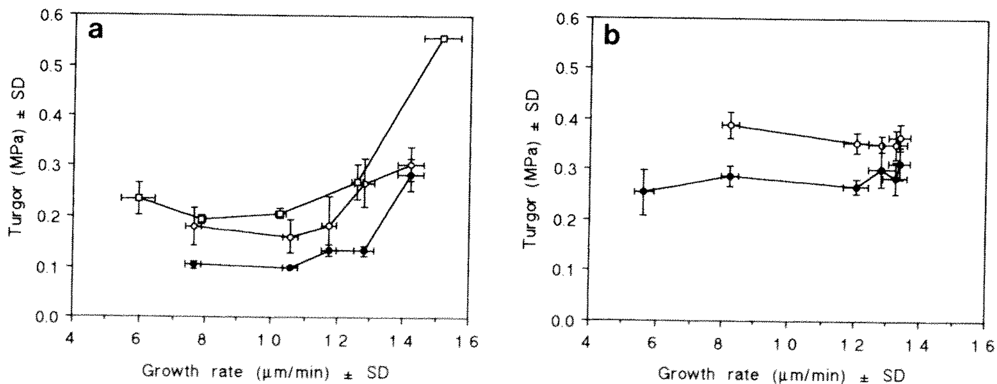

FIG. 4. The relation between turgor and extension rate in *Saprolegnia*. (a) Osmoticum-induced extension rate inhibition: sorbitol, with turgor estimated by incipient plasmolysis (open circles) or osmometry (closed circles), PEG, with turgor estimated by incipient plasmolysis (open squares). (b) TPA-induced extension rate inhibition. Turgor estimated by incipient plasmolysis (open circles) or osmometry (closed circles).

dependent variations in the absolute level. This minimum turgor was maintained, presumably by synthesis or uptake of osmotically active molecules, despite increases in medium Π from 0.75 to 1.6 MPa. All of these colonies looked qualitatively normal. We were unable to measure turgor for colonies in media above about 1.6 MPa, although extension rate reduction continued in media with $\Pi > 1.75$ MPa (Fig. 1). In high Π media, colony and hyphal morphology were no longer normal: colonies would grow only on the agar surface, and hyphae were very highly branched; we did not investigate these further.

As an alternate strategy to changing medium Π in order to reduce turgor, we inhibited the activity of K^+ channels using the specific inhibitor TPA (Garrill *et al.*, 1992). K^+ is an important osmoregulatory ion in fungi and plants (Clipson and Jennings, 1990; Salisbury and Ross, 1985; Winter and Kirst, 1991) and so inhibiting K^+ uptake might reduce turgor. However, TPA produced essentially no change in turgor (Fig. 4b), regardless of which turgor-estimation technique was used. Nevertheless, there was a near-linear, TPA-concentration-dependent reduction in linear growth rate up to 250 μ*M* TPA, at which dose colonies were still growing at almost half the control rate.

Turgor and linear growth rates are both affected by medium Π, but the relationship is complex; this is most apparent when turgor is plotted as a function of rate (Fig. 4a). With osmotica, increased turgor correlated with increased linear extension rate only above 12 μm/min. Below this level, there was a twofold range in linear growth rates (6–12 μm/min) despite essentially constant turgor. Similarly, TPA-induced twofold changes in extension rate, independent of turgor pressure (Fig. 4b).

A complementary approach to studying the relationship between turgor and extension rate by attempting to manipulate turgor, is to describe the natural variability in linear growth rates between hyphae in a colony, and for individual hyphae over time. All hyphae growing close together in coenocytes are likely to be part of a single pressure compartment, and thus should have similar turgor and therefore similar extension rate if the two factors were directly correlated.

We measured linear growth of hyphae in microscope chambers. Typical data for one chamber showed that all hyphae grew substantially between observations; however, absolute amount varied between tips. In order to examine the differences in more detail, we expressed these same data as growth rates (Fig. 5). Rates for individual hyphae varied widely and erratically over succeeding 5- to 11-min periods. However, this was not due to a general decline in hyphal health, since the average growth rate for the group did not change significantly with time (0–5 min, 8.4 ± 1.1; 5–15 min, 8.6 ± 2.5; 15–26 min, 7.2 ± 1.8; and 26–36 min, 8.3 ± 1.9 μm/min, respectively) ($P > 0.05$, ANOVA). Overall, we measured linear growth rates for 156 hyphae in 11 chambers; for 4 chambers we made two to four successive rate determinations, which we treated as separate for calculation of between-tip variation. The growth rate variation (fastest/slowest) between all growing tips in a chamber at any one time was 4.8 ±

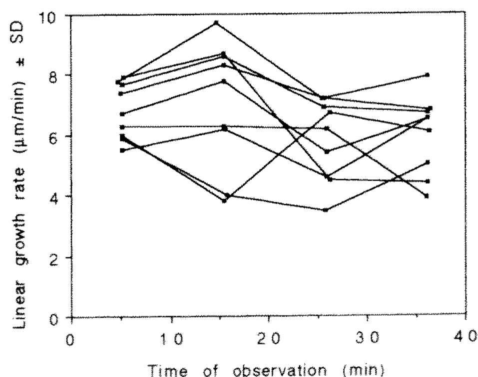

FIG. 5. Linear growth rates of nine neighboring hyphae of *Saprolegnia* in a microscope slide chamber, from a colony mounted 5 min before the first observation (time 0). Rates are the length extended (μm) since the previous observation, divided by the time since the previous observation (min).

3.8 (n = 18, range 1.5–14.0). For hyphae where successive rates were determined the variation for any one tip was 1.9 ± 1.4 (n = 42, range 1.0–8.9).

During the course of our observations on turgor, we saw changes in hyphal diameter (Fig. 6), which is another manifestation of alterations to tip plasticity. There were significant medium Π-dependent increases in diameter for growing hyphae ($P < 0.05$, ANOVA), but no relationship between turgor and diameter. Growing hyphae were always significantly wider than the same population of tips after plasmolysis ($P < 0.05$, ANOVA), and the diameter of plasmolyzed hyphae also increased in a medium-Π-dependent manner. In contrast to our results with osmotica, but consistent with our results for turgor, TPA did not affect diameter: plasmolyzed hyphae were always significantly narrower than growing ones and both values were similar to the corresponding values for OM without osmoticum.

Although not affecting the interpretation of our experiments, since the osmotica caused both linear growth rate and turgor reductions, we were surprised to discover that *Saprolegnia* colonies grew at similar linear extension rates, with only a slight increase with added carbohydrate: a) basal OM (no added carbohydrate), 13.0 ± 0.4 μm/min; b) glucose, 13.9 ± 0.2 μm/min; c) PEG, 14.1 ± 0.1 μm/min; d) sorbitol, 14.7 ± 0.1 μm/min (a ≠ b, c, d; b = c = d. ANOVA $P < 0.05$, plus Tukey test).

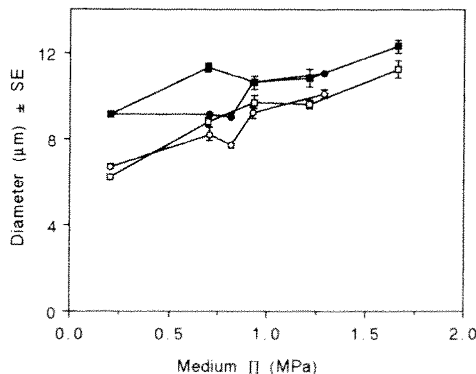

FIG. 6. Relation between medium Π and hyphal diameter before (closed symbols) and after (open symbols) plasmolysis. Π was adjusted with sorbitol (circles) or PEG (squares) as osmotica.

DISCUSSION

Increased growth medium Π was directly correlated with linear growth rate reduction, in general agreement with the results summarized in Table 1. The suggestion of Woods and Duniway (1986) and Sterne *et al.* (1976), that decreased growth rates at higher medium Π is due to higher metabolic costs of maintaining positive turgor, seems a likely explanation of this effect. Jennings and Burke (1990) propose that "energy-spilling" reactions account for a portion of metabolism at high medium Π.

Our best estimate of turgor for *Saprolegnia* hyphae growing in OM is 0.3 MPa, which we determined using both osmometry and incipient plasmolysis using sorbitol. The high turgor value determined in OM-grown colonies by incipient plasmolysis with PEG may be peculiar to that solute; variations of this type have been reported by Money (1990).

If turgor pressure and extension rate were interdependent, then treatments which affected one characteristic should have a corresponding effect on the other. For extension rates above about 12 μm/min, this expectation appears to be met. However, even in this range, an approximate 2-fold increase in turgor gives only about a 1.2-fold increase in linear growth rate. Below 12 μm/min, a 2-fold range in rate occurs at essentially constant turgor. These generalizations hold irrespective of the technique-dependent variations in the absolute level of turgor. Consistent with the lack of turgor-colony extension rate correlation under most experimental conditions, there is high natural variation in extension rate between hyphae in a colony or for individual tips over short time periods. Turgor is likely to be constant within a cell (Nobel, 1983) or coenocyte since membranes

are unable to withstand pressure differentials greater than 60 mbar (0.006 MPa) (Duniway, 1979). Turgor is also likely to be consistent for short periods of time under constant growth conditions. These suggestions are supported by direct pressure probe measurements in *Achlya* (Money, 1990) which show turgor variations of less than 50% between hyphae and only 6% for individual hyphae over 39 min, both under standard growth conditions. However, the variations in turgor reported by Money (1990) are much smaller than those we saw for hyphal extension rate, which averaged 5- and 2-fold, respectively.

Our results differ from reports on terrestrial saprobes showing a linear relationship or no relationship between turgor and extension rate, and also from reports on soil-borne plant pathogenic oömycetes, which show a positive correlation between medium Π and turgor (Table 1). These differences may be related to habitat, since *Saprolegnia* is an aquatic saprobe. However, and most curiously, our results contrast with those of Money (1991) who suggests that *Achlya* is unable to regulate turgor. As yet we have no explanation for this discrepancy.

Our results are comparable to elongation growth of internodal cells of the algae, *Chara* (Zhu and Boyer, 1991) and *Nitella* (Green *et al.*, 1985), which is relatively independent of turgor: artificially applied short-term changes in turgor are correlated with elongation rate changes, but within broad limits the cell compensates in about 15 min to re-establish the initial extension rate; there also appears to be a threshold turgor required for extension. The turgor-salinity response of *Chara canescens*, a brackish water species (Winter and Kirst, 1991), is strikingly similar to our data. As well as studies on algae, additional turgor applied to wheat and barley shoots produces only transient increases in extension rates, and NaCl-induced reductions in extension rate cannot be overcome by applying hydrostatic pressure (Termaat *et al.*, 1985). Since these systems are not tip-growing the mechanics of extension are not strictly analogous.

Factors which may influence extension rates in *Saprolegnia* include cytoskeletal support for the tip where the wall is weak (Jackson and Heath, 1990) and cell wall extensibility (plasticity and rate of crosslinking). Jackson and Heath (1990) have shown that the length of the putative supporting actin cap is positively correlated with linear growth rate, when naturally varying rates were calculated from hyphal extension after Calcofluor staining. However, if their data are compared for rates above and below 12 μm/min, marking the transition between turgor-extension rate dependence-independence, the relationships differ. Below 12 μm/min the extension rate is strongly correlated with the length of the actin cap ($r = 0.742$), but above 12 μm/min it is not ($r = -0.013$). This suggests the intriguing possibility that cytoskeletal factors may regulate extension rates below 12 μm/min, in lower turgor situations, while turgor dominates at high linear growth rates.

While the rate of tip extension is the most obvious parameter likely to be affected by turgor, hyphal diameter could also be increased by high turgor. However, consistent with the general lack of turgor-extension rate correlation, there was a similar lack of correlation between turgor and hyphal diameter. The diameter of hyphae growing on high Π media was significantly greater than on OM without osmotica (where turgor was lower) and, additionally, significant changes in diameter occurred on media with $\Pi > 0.75$ MPa, where turgor was constant. Our results agree with pressure probe-diameter data for *Achlya* (Money, 1990), showing that these factors are not related. This suggests that hyphal diameter is not the passive result of turgor acting on the deformable wall of the hyphal tip, consistent with the idea that the actin

cytoskeleton supports the extending apex (Jackson and Heath, 1990) and that the diameter of *Saprolegnia* hyphae is regulated by the cytoskeleton (Kaminskyj *et al.*, 1992).

TPA inhibits K^+ channels in *Saprolegnia* protoplasts (Garrill *et al.*, 1992); however, it does not affect turgor in hyphae despite dose-dependent linear growth inhibition. Therefore, although ions such as K^+ are important in fungal and plant osmoregulation, as discussed earlier, their role in oömycete water relations is uncertain. Luard (1982) showed that the K^+ content of *Phytophthora* actually decreased as medium Π was increased using sucrose, although intracellular K^+ did increase if KCl was used as the osmoticum. However, proline levels in *Phytophthora* were well correlated with increases or decreases in growth medium Π, whether adjusted by sucrose or KCl. One possibility, if indeed K^+ were important in *Saprolegnia* osmoregulation, is that fluxes of other ions may compensate in the event of K^+-channel inhibition (Flowers and Läuchli, 1983; Lew, 1991). As well, Winter and Kirst (1991) show that Na^+, K^+, and Cl^- are all important for osmoregulation in *Chara*. Alternatively, K^+ may be metabolically important for reasons independent of turgor regulation (Flowers and Läuchli, 1983), consistent with TPA-induced inhibition.

Our finding that linear growth rates of *Saprolegnia* colonies were similar with only a slight increase due to added carbohydrate, regardless of type, is most unexpected and shows that this organism can use proteinaceous carbon sources in addition to carbohydrates.

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REFERENCES

ADEBAYO, A. A., HARRIS, R. F., AND GARDNER, W. R. 1971. Turgor pressure of fungal mycelia. *Trans. Br. Mycol. Soc.* **57**: 145–151.

ANDREWS, S., AND PITT, J. I. 1987. Further studies on the water relations of xerophilic fungi, including some halophiles. *J. Gen. Microbiol.* **134**: 669–677.

BEEVER, R. E., AND LARACY, E. P. 1986. Osmotic adjustment in the filamentous fungus *Aspergillus nidulans*. *J. Bacteriol.* **168**: 1358–1365.

BODDY, L. 1983. Effect of temperature and water potential on growth rate of wood-rotting basidiomycetes. *Trans. Br. Mycol. Soc.* **80**: 141–149.

CLIPSON, N. J. W., AND JENNINGS, D. H. 1990. The role of potassium and sodium in the generation of the osmotic potential of the marine fungus *Dendryphiella salina*. *Mycol. Res.* **94**: 1017–1022.

COOK, R. J., PAPENDICK, R. I., AND GRIFFIN, D. M. 1972. Growth of two root rot fungi as affected by osmotic and matric water potentials. *Soil Sci. Soc. Am., Proc.* **36**: 78–82. (cited in Griffin [1978])

DUNIWAY, J. M. 1979. Water relations of water molds. *Ann. Rev. Phytopathol.* **17**: 431–460.

EAMUS, D., AND JENNINGS, D. H. 1986. Turgor and fungal growth: Studies on water relations of mycelia of *Serpula lacrimans* and *Phallus impudicus*. *Trans. Br. Mycol. Soc.* **86**: 527–535.

FLOWERS, T. J., AND LÄUCHLI, A. 1983. Sodium versus potassium: Substitution and compartmentation. In *Inorganic Plant Nutrition. Encyclopedia of Plant Physiology, New Series, Vol. 15B* (A. Läuchli and R. L. Bieleski, Eds.), pp. 651–681. Springer-Verlag, Berlin.

GAHAN, P. B. 1984. *Plant Histochemistry and Cytochemistry, An Introduction*. Academic Press, New York.

GARRILL, A., LEW, R., AND HEATH, I. B. 1992. Stretch-activated Ca^{2+} and Ca^{2+}-activated K^+ channels in the hyphal tip plasma membrane of the oomycete *Saprolegnia ferax*. *J. Cell Sci.*, in press.

GREEN, P. B., ERIKSON, R. O., AND BUGGY, J. 1985. Metabolic and physical control of cell elongation rate. *In vivo* studies in *Nitella*. *Plant Physiol.* **47**: 423–430.

GRIFFIN, D. M. 1978. Effect of soil moisture on survival and spread of pathogens. In *Water Deficits and Plant Growth. Vol. V. Water and Plant Disease*. T. T. Kozlowski Ed.), pp. 175–197. Academic Press, New York/London.

HARRISON, J. L., AND JONES, E. B. G. 1971. Salinity tolerance of *Saprolegnia parasitica* Coker. *Mycopathol. Mycol. Appl.* **43**: 297–307.

HEATH, I. B. 1988. Evidence against a direct role for cortical actin arrays in saltatory organelle motility in hyphae of the fungus *Saprolegnia ferax*. *J. Cell Sci.* **91**: 41–47.

HEATH, I. B., AND GREENWOOD, A. D. 1968. Electron microscopic observations of dividing somatic

nuclei in *Saprolegnia*. *J. Gen. Microbiol.* **53**: 287–289.

HOCH, H. C., AND MITCHELL, J. E. 1973. The effects of water potentials on *Aphanomyces euteiches* during zoosporogenesis. *Can. J. Bot.* **51**: 413–420.

HOCKING, A. D., AND PITT, J. I. 1979. Water relations of some *Penicillium* species at 25°C. *Trans. Br. Mycol. Soc.* **73**: 141–145.

INCH, J. M. M., AND TRINCI, A. P. J. 1987. Effects of water activity on growth and sporulation of *Paecilomyces farinosus* in liquid and solid media. *J. Gen. Microbiol.* **133**: 247–252.

JACKSON, S. L., AND HEATH, I. B. 1990. Evidence that actin reinforces the extensible hyphal apex of the oomycete *Saprolegnia ferax*. *Protoplasma* **157**: 144–153.

JENNINGS, D. H. 1990. Osmophiles. In *Microbiology of Extreme Environments* (C. Edwards Ed.), pp. 117–146. Open University Press, Stony Stratford, Milton Keyes.

JENNINGS, D. H., AND BURKE, R. M. 1990. Compatible solutes—Their mycological dimension and their role as physiological buffering agents. *New Phytol.* **116**: 277–283.

KAMINSKYJ, S. G. W., JACKSON, S. L., AND HEATH, I. B. 1992. Fixation induces differential polarized translocations of organelles in hyphae of *Saprolegnia ferax*. Submitted for publication.

KELLY, D. J. A., AND BUDD, K. 1990. Osmotic adjustment in the mycelial ascomycete *Neocosmospora vasinfecta* (E. F. Smith). *Expl. Mycol.* **14**: 136–144.

KOCH, A. L. 1982. The shape of the hyphal tips of fungi. *J. Gen. Microbiol.* **128**: 947–951.

KUTHUBUTHEEN, A. J., AND WEBSTER, J. 1986. Effects of water availability on germination, growth and sporulation of coprophilous fungi. *Trans. Br. Mycol. Soc.* **86**: 77–91.

LEW, R. R. 1991. Electrogenic transport properties of growing *Arabidopsis* root hairs: The plasma membrane proton pump and potassium channels. *Plant Physiol.* **97**: 1527–1534.

LUARD, E. J. 1982. Growth and accumulation of solutes by *Phytophthora cinnamomi* and other lower fungi in response to changes in external osmotic potential. *J. Gen. Microbiol.* **128**: 2583–2590.

LUARD, E. J., AND GRIFFIN, D. M. 1981. Effect of water potential on fungal growth and turgor. *Trans. Br. Mycol. Soc.* **76**: 33–40.

MAGAN, N., AND LYNCH, J. M. 1986. Water potential, growth and cellulolysis of fungi involved in decomposition of cereal residues. *J. Gen. Microbiol.* **132**: 1181–1187.

MONEY, N. P. 1989. Osmotic potential of aqueous polyethylene glycols. Relationship between molecular weight and vapor pressure deficit. *Plant Physiol.* **91**: 766–769.

MONEY, N. P. 1990. Measurement of hyphal turgor. *Expl. Mycol.* **14**: 416–425.

MONEY, N. P. 1991. Magnitude, role and regulation of hyphal turgor. (Abstr.) *Mycol. Soc. Amer. Newslett.* **42**: 28.

NOBEL, P. S. 1983. *Biophysical Plant Ecology and Physiology*. Freeman, San Francisco.

PITT, J. I., AND HOCKING, A. D. 1977. Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. *J. Gen. Microbiol.* **101**: 35–40.

SALISBURY, F., AND ROSS, C. 1985. *Plant Physiology*, 3rd ed. Wadsworth, Belmont CA.

SAUNDERS, P. T., AND TRINCI, A. P. J. 1979. Determination of tip shape in fungal hyphae. *J. Gen. Microbiol.* **110**: 469–473.

SMITH, S. L., AND HILL, S. T. 1982. Influence of temperature and water activity on germination and growth of *Aspergillus restrictus* and *A. versicolor*. *Trans. Br. Mycol. Soc.* **79**: 558–560.

SOMMERS, L. E., HARRIS, R. F., DALTON, F. N., AND GARDNER, W. R. 1970. Water potential relations of three root-infecting *Phytophthora* species. *Phytopathology* **60**: 923–934.

STERNE, R. E., ZENTMEYER, G. A., AND BINGHAM, F. T. 1976. The effect of osmotic potential and specific ions on growth of *Phytophthora cinnamomi*. *Phytopathology* **66**: 1398–1402.

STEVENS, L., DIX, N. J., AND THOMPSTONE, A. 1983. Effects of high water activity on growth and metabolism in *Aspergillus sejunctus*. *Trans. Br. Mycol. Soc.* **80**: 527–529.

TERMAAT, A., PASSIOURA, J. B., AND MUNNA, R. 1985. Shoot turgor does not limit shoot growth of NaCl-affected wheat and barley. *Plant Physiol.* **77**: 869–872.

THOMPSON, W., EAMUS, D., AND JENNINGS, D. H. 1985. Water flux through mycelium of *Serpula lacrimans*. *Trans. Br. Mycol. Soc.* **84**: 601–608.

TRINCI, A. P. J. 1969. A kinetic study of the growth of *Aspergillus nidulans* and other fungi. *J. Gen. Microbiol.* **57**: 11–24.

WEARING, A. H. 1976. *Preliminary Studies on the Saprophytic Behaviour of Fusarium roseum 'graminearum.'* Ph.D. Thesis, University of Sydney, Sydney, Australia. (cited in Griffin [1978])

WESSELS, J. G. H. 1988. A steady-state model for apical wall growth in fungi. *Acta Bot. Neerl.* **37**: 3–16.

WILSON, J. M. 1973. *The Effect of Water Potential on the Growth and Respiration of Various Soil Microorganisms*. M.Sc. Thesis. Australian National University, Canberra. (cited in Griffin [1978])

WILSON, J. M., AND GRIFFIN, D. M. 1979. The effect of water potential on the growth of some soil basidiomycetes. *Soil Biol. Biochem.* **11**: 211–212.

WINTER, U., AND KIRST, G. O. 1991. Partial turgor pressure regulation in *Chara canescens* and its implication for a generalized hypothesis of salinity response in charophytes. *Bot. Acta* **104**: 37–46.

WOODS, D. M., AND DUNIWAY, J. M. 1986. Some effects of water potential on growth, turgor, and respiration of *Phytophthora cryptogea* and *Fusarium moniliforme*. *Phytopathology* **76**: 1248–1254.

ZHU, G.-L., AND BOYER, J. S. 1991. Turgor and elongation in *Chara* cells. (Abstr.) *Plant Physiol.*, (*Suppl.*) **96**: 38.

ZAR, J. H. 1984. *Biostatistical Analysis*, 2nd ed. Prentice-Hall, Toronto.