Physiology and Biochemistry

Some Effects of Water Potential on Growth, Turgor, and Respiration of *Phytophthora cryptogea* and *Fusarium moniliforme*

D. M. Woods and J. M. Duniway

Department of Plant Pathology, University of California, Davis 95616.
Present address of first author: California Department of Food and Agriculture, 3288 Meadowview Road, Sacramento 95832.
Research supported in part by USDA Grant 80-CRCR-1-0426
Accepted for publication 12 May 1986 (submitted for electronic processing).

ABSTRACT

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.

Mycelial growth, turgor, and respiration of *Phytophthora cryptogea* and *Fusarium moniliforme* were evaluated in media adjusted to various constant water potentials (Ψ) by adding solutes. Growth by *P. cryptogea* in standing liquid cultures was reduced by 50% at −9 to −31 bars Ψ, whereas a 50% reduction in the growth of *F. moniliforme* occurred at −26 to −200 bars, the exact values depending on the medium and solutes used to vary Ψ. Growth measured as fresh weight was generally decreased more by decreases in Ψ than was growth in dry weight. Whereas *P. cryptogea* grew at lower Ψ values in a complex liquid medium of high nutritional content than in a defined medium, *F. moniliforme* gave the opposite results. Use of agar rather than liquid medium extended the Ψ range over which *P. cryptogea* grew. Mycelial turgor in *P. cryptogea*, as estimated with thermocouple psychrometers, gradually increased from 12 to 25 bars as medium Ψ was decreased from −5 to −24 bars, even though the same decreases in Ψ reduced growth. *F. moniliforme* maintained turgor pressures that averaged 15 bars over the entire Ψ range of −5 to −200 bars that allowed measurable growth. Respiration rates were significantly higher for mycelial mats grown at low Ψ values, and respiration rates of *P. cryptogea* increased proportionately more than did those of *F. moniliforme* as Ψ decreased. The results suggest that the metabolic costs of growth at low Ψ values influence growth rate more than does turgor pressure and are more limiting for *P. cryptogea* than for *F. moniliforme*.

Water potential (Ψ) is recognized as an important parameter in the ecology and growth of plant-pathogenic fungi (5,9–11). In fungal plant pathogens, the effects of Ψ on growth determine, to some extent, the conditions under which pathogenesis in a host plant is possible, and the effects of substrate Ψ on mycelial growth by many fungi have been researched. Recent reviews of the literature (5,9,12), however, show that most of the individual reports of research on the water relations of fungi employed a single osmoticum in one medium to evaluate the effects of Ψ on one measure of mycelial growth. Unfortunately, the influence of Ψ on the growth of mycelium may vary with the nature of the medium and osmoticum used and with the manner in which growth is measured (1,4,9,11,12,24,25).

Although the mechanisms by which low Ψ values reduce cell growth have been researched extensively in higher plants, algae, bacteria, and yeasts (3,12,15), much less is known about such mechanisms in mycelial fungi (3,9,11,12,16–19). A variety of experiments have shown that a positive turgor exists in hyphal tips, and there is considerable evidence that interactions between cell turgor and wall plasticity govern, to a large extent, the rate and morphology of mycelial growth (2,11,12,19,21,26). A few studies have also shown that the hyphae of several fungi are able to maintain positive turgors at low Ψ values (2,16–19). In addition, Wilson and Griffin (27) found that the rate of respiration per unit of growth by

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mycelial fungi increased greatly on media of low Ψ.

The present study evaluates the effects of Ψ on the growth, respiration, and maintenance of turgor by the mycelia of two plant-pathogenic fungi that differ greatly in their tolerance of water stress. Growth is measured on both liquid and agar media amended to low Ψ values with a variety of solutes. The two fungi studied are *Phytophthora cryptogea* Pethyb. & Laff., an Oomycete with aquatic affinities, and *Fusarium moniliforme* (Sheldon) Snyd. & Hans., a relatively xerophytic Hyphomycete in the Fungi Imperfecti (5,9,12). A preliminary report of this research has been given (29).

MATERIALS AND METHODS

Isolates. The isolate of *P. cryptogea* used was of the A2 mating type and was originally isolated from safflower (8). The isolate of *F. moniliforme* was originally obtained from corn by D. Hall, Department of Plant Pathology, University of California, Davis. Stock cultures of *P. cryptogea* were maintained on cornmeal agar, and those of *F. moniliforme* were maintained on potato-dextrose agar. Inoculum for individual experiments was grown on 20 ml of lima bean broth (LBB) in 125-ml flasks without shaking for 7–10 days. Mycelial disks were cut with a 7-mm-diameter cork borer, randomly distributed in sterile distilled water, and used as inoculum for individual experiments.

Media and osmotica. The effects of Ψ on the growth of fungi were measured on two media. The first was the P-3 medium originally developed by Hohl (14) for use with *Phytophthora* spp. It contained 20 g of glucose, 1 g of L-asparagine, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $CaCl_2 \cdot 2H_2O$, and 1 mg of thiamine HCl per liter. In some experiments, P-3 medium was solidified with 1.5% Difco-Bacto agar (Difco Laboratories, Detroit, MI). The second medium was a LBB prepared by boiling 200 g of frozen lima beans in 500 ml of distilled water for 10 min, blending at high speed for 2 min, and centrifuging for 20 min at $8{,}000 \times g$. Finally, 10 g of glucose and 10 g of yeast extract (Difco) were added to the supernatant and the volume was adjusted to 1 L with distilled water. The osmotic or solute potential (ψ_s) of both media was adjusted by adding various solutes before bringing the medium to full volume. Thus, although ψ_s varied, the concentration of nutrients remained constant. The solutes used individually to adjust ψ_s were polyethylene glycols with average molecular weights of 300 and 6,000 (PEG 300 and PEG 6000, Sigma), an artificial mixture of the salts in sea water (Instant Ocean, Eastlake, OH 44094), and reagent-grade sucrose, mannitol, NaCl, KCl, $MgSO_4$, and Na_2SO_4. The amended P-3 broth was sterilized by filtration except when agar or polyethelene glycols were used; agar and polyethylene glycols were dissolved in water and autoclaved separately, then added to a concentrated, filter-sterilized P-3 broth. The LBB was always autoclaved.

The total water potentials (Ψ) of the growth media used were measured in an isopiestic thermocouple psychrometer (7) at the end of each experiment. The measured Ψ values of unamended media varied from one batch to another by as much as 1 bar (1 bar = 0.1 MPa) for P-3 broth and by as much as 5 bars for P-3 agar. The Ψ values of P-3 agar were always lower than those of P-3 broth with the same added solutes, and the Ψ of P-3 broth increased by only 1–3 bars during 21 days of mycelial growth. Fungi were inoculated into 20 ml of standing broth in 125-ml flasks or onto 30 ml of solidified agar medium in 9-cm-diameter petri plates. Petri plates were placed in plastic containers and cultures were incubated at room temperature (20–25 C).

Measurement of growth. To measure the fresh weight of mycelium grown in standing liquid broth, the extracellular solution was drained in a defined and consistent manner. This was done using Büchner funnels as tension plates (8) with a water column adjusted to give a constant 200-mbar tension on the porous glass plate. Mycelial mats were removed from the broth with forceps, placed on the tension plate, and covered with an inverted small petri plate to prevent evaporation. The adhering liquid was allowed to drain for 1 min before the mat was removed to measure its fresh weight. Preliminary experiments showed that the tension and time used for drainage gave repeatable fresh weights that did not differ significantly from those obtained with longer periods of drainage (28). Young mycelial mats of *F. moniliforme* did not stay together when lifted with forceps, and the drainage procedure was modified for them. A filter with 1.2-μm pores (Gelmann Instrument Co., Ann Arbor, MI) at a predetermined dry weight was placed on the funnel and wetted with 15 ml of water. Immediately after 1 min of drainage, the filter was reweighed and replaced on the funnel. The loose mycelium of *F. moniliforme* was then transferred to the filter, rinsed with 15 ml of water, and allowed to drain for 1 min before the mycelium and filter were weighed and dried. (All of the rinse water appeared to drain away within 45 sec.) Three or four replicate flasks of broth-grown mycelium were harvested for each solute at each ψ_s value and time of sampling. Dry weights were measured after the mats had been at 90 C for 2 days. The diameters of fungal colonies on agar medium were measured at three positions on each of three plates for every treatment.

Measurements of turgor and respiration. The turgor or positive pressure component (ψ_p) of cell Ψ was estimated in mycelium that was grown for 21 days on P-3 broth amended to various ψ_s values with NaCl or KCl. Mycelial mats were drained by the methods described, and four to eight were grouped to make a sample of mycelium large enough for use in one psychrometer (7). Each group of drained mats was quickly placed in one corner of a small plastic bag and sealed tightly with a minimum volume of air. The small bags of mycelium were immediately dropped into liquid nitrogen, then stored at −20 C, and mycelial mats were allowed to thaw completely while still enclosed in the plastic bags. Baggie brand plastic bags (Colgate-Palmolive Co., Jeffersonville, IN) were able to withstand freezing and thawing without breaking. The ψ_s values of the thawed mycelia were measured in the psychrometers. Examination of the frozen and thawed mycelium of both *P. cryptogea* and *F. moniliforme* revealed loss of ψ_p and a complete disruption of hyphal membranes. On the assumption that the Ψ values of the living mycelium and its medium were equal (2,13,19), ψ_p was calculated as the difference between the medium Ψ and the mycelial ψ_s.

Respiration measurements were made on mycelial mats growing on P-3 broth amended with KCl, NaCl, or sucrose. Measurements were made every other day for 21 days on three or four flasks per treatment; data from a representative experiment are presented. Flasks were immersed in a water bath at 25 C with their tops sealed tightly to tubing, and compressed air was forced through the flask at 7.8 $cm^3 \cdot s^{-1}$. A pump on a separate and short loop of tubing constantly mixed the air in the flask. Respiration rates were calculated from the difference in CO_2 concentration between the incoming and outgoing air measured with an infrared gas analyzer. Within 2–4 min, the gas composition within the system came to equilibrium and measured respiration rates became constant. After each respiration measurement, the contents of the flask were harvested to determine the fresh and dry weights of mycelium by the methods described.

RESULTS

Growth. Growth of *P. cryptogea*, as measured by colony diameter on agar for 7 days or as dry weight in P-3 broth for 21 days, was reduced by low Ψ values, but there was no measurable lag period and growth rates remained nearly constant over the periods of time and range of Ψ values employed. Growth of *F. moniliforme* on agar had a lag phase of 1 day on media at $\Psi \geq -78$ bars that increased to 3 days when the agar medium was at $\Psi \leq -98$ bars. After the lag phase, growth in colony diameter by *F. moniliforme* on P-3 agar was linear with time for up to 6 days. A lag phase was not apparent when *F. moniliforme* was grown on P-3 broth at $\Psi \geq -30$ bars, but the lag phase was as long as 3–5 days at Ψ values of −90 and −110 bars. On P-3 broth at Ψ values between −5 and −30 bars, maximum growth rates of *F. moniliforme* were observed in 3- to 8-day-old cultures, and although rates were somewhat reduced in older cultures, growth continued for at least 21 days; at $\Psi \leq -90$ bars, maximum growth rates were maintained

for 19–21 days. Both fungi grew more rapidly on LBB than on P-3 broth, and after 7 days, mycelial mats from LBB weighed about twice as much as those from P-3 broth.

After the initial experiments described above, the effects of Ψ on fungal growth on P-3 agar were measured after 6 days of incubation. The choice of an incubation period for additional experiments on P-3 broth represented a compromise between a desire to obtain mycelium during a period of maximum growth rate and the need to obtain mycelial mats that were large enough for psychrometric measurements of water relations parameters. At low Ψ values, the latter consideration necessitated the choice of 21 days of incubation on P-3 broth. Because of their more rapid growth, LBB cultures were incubated only 7 days.

The effects on growth of various Ψ values obtained by adding NaCl or KCl to three media are shown in Figure 1. Although the magnitudes of the growth reductions varied with the osmoticum and medium, the growth of *P. cryptogea* was uniformly reduced more by decreasing Ψ values than was the growth of *F. moniliforme*. The growth of *P. cryptogea* was affected somewhat less by decreases in Ψ of LBB than of P-3 broth (Fig. 1A,C), whereas growth by *F. moniliforme*, at least in relative terms, was reduced less by decreases in Ψ of P-3 broth than LBB (Fig. 1B,D). Both fungi grew more at low Ψ values on broth media amended with KCl than with NaCl, but the differences between the effects of the two solutes on growth were small for *P. cryptogea* on P-3 broth (Fig. 1C) and for both fungi on P-3 agar (Fig. 1E,F). The addition of KCl to give Ψ values between −10 and −70 bars in LBB and between −10 and −120 bars in P-3 broth actually stimulated growth of *F. moniliforme* measured as dry weight, but growth measured as fresh weight was not stimulated (Fig. 1B,D). In fact, with the single exception of *P. cryptogea* on P-3 broth (Fig. 1C), growth on broth media measured as fresh weight was reduced more by moderate decreases in Ψ than was growth in dry weight. *P. cryptogea* grew relatively more on P-3 agar at low Ψ values than on P-3 broth (Fig. 1C,E); use of agar, however, did not increase the growth of *F. moniliforme* at low Ψ values (Fig. 1D,F).

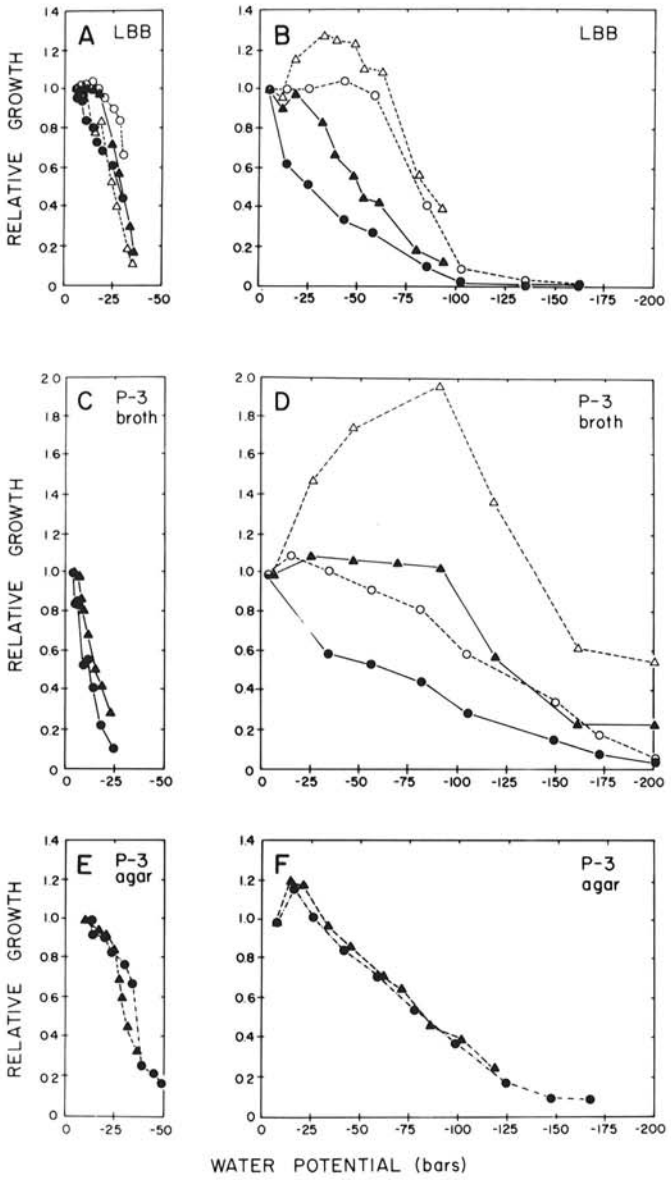

Fig. 1. Growth of *Phytophthora cryptogea* and *Fusarium moniliforme* on media amended to various water potentials (Ψ) by adding NaCl (circles) or KCl (triangles): **A,** *P. cryptogea* on lima bean broth (LBB), **B,** *F. moniliforme* on LBB, **C,** *P. cryptogea* on P-3 broth, **D,** *F. moniliforme* on P-3 broth, **E,** *P. cryptogea* on P-3 agar, and **F,** *F. moniliforme* on P-3 agar. Mean fresh weights (solid lines) and dry weights (dashed lines) of mycelia on broth media and mean colony diameters on agar are expressed relative to those obtained on the same medium without NaCl or KCl. Although they are not shown, the relative dry weights of *P. cryptogea* on P-3 broth were the same as the relative fresh weights shown in C.

The influence of a variety of solutes on the growth of *P. cryptogea* was measured in experiments similar to the one shown in Figure 1. The results for all solutes are summarized in Table 1, which gives the Ψ values at which the final amount of growth was reduced to one-half of that obtained in the same medium without an osmoticum added. With the exception of the addition of sucrose to LBB, the shapes of the curves relating the final growth to medium Ψ resembled those shown in Figure 1 (28). Additions of sucrose that depressed the Ψ of LBB by 1–12 bars increased final growth of both fungi by 1.6–2.6 orders of magnitude over that in unamended LBB. The effects of solutes other than sucrose on *P. cryptogea* resembled the effects of NaCl and KCl (Fig. 1) in that a greater tolerance to low Ψ was observed in LBB and P-3 agar than in P-3 broth, and with few exceptions, decreases in Ψ had greater effects on fresh weight than on dry weight. *P. cryptogea* grown in P-3 broth was relatively tolerant of low Ψ when the major solute was sucrose or mannitol, of intermediate tolerance with a mixture of the salts in sea water or pure $MgSO_4$, NaCl, or KCl, and rather intolerant with Na_2SO_4 and PEG 300 (Table 1). Medium containing PEG 6000 was extremely viscous and did not drain freely from the mycelium on tension plates. Therefore, the Ψ values at which PEG 6000 was observed to reduce growth of *P. cryptogea* by one-half may be artificially low. The use of LBB rather than P-3 broth greatly reduced the differences between solutes in their effects on the growth of *P. cryptogea*, i.e., the effect of Ψ was more uniform among the solutes used (Table 1). The solubility of many

TABLE 1. Water potentials (bars) at which adding osmotica to various media reduced growth of *Phytophthora cryptogea* and *Fusarium moniliforme* to one-half of that on media without added osmotica[a]

Fungus and osmoticum	P-3 broth[b]		P-3 agar[c]	LBB[b]	
	Dry weight	Fresh weight	Colony diam.	Dry weight	Fresh weight
P. cryptogea					
Sucrose	−25	−11	−32	−27	−26
Mannitol	−24	−19	···	−25	−26
PEG 300	−9	−9	···	−20	−17
PEG 6000	−29	−21	···	···	···
Sea salt	−17	−16	···	···	···
$MgSO_4$	−17	−16	−24	−31	−28
Na_2SO_4	−10	−9	···	−25	−23
NaCl[d]	−13	−12	−36	−31	−25
KCl[d]	−13	−11	−31	−29	−25
F. moniliforme					
NaCl[d]	−112	−65	−84	−81	−26
KCl[d]	−200	−127	−84	−85	−50

[a] Values extrapolated using straight lines to connect the individual data points for each solute (e.g., Fig. 1).
[b] Growth measured as fresh and dry weight after incubation for 21 days on P-3 broth and 7 days on LBB (lima bean broth).
[c] Growth measured as colony diameter after incubation for 6 days.
[d] Data extrapolated from Figure 1.

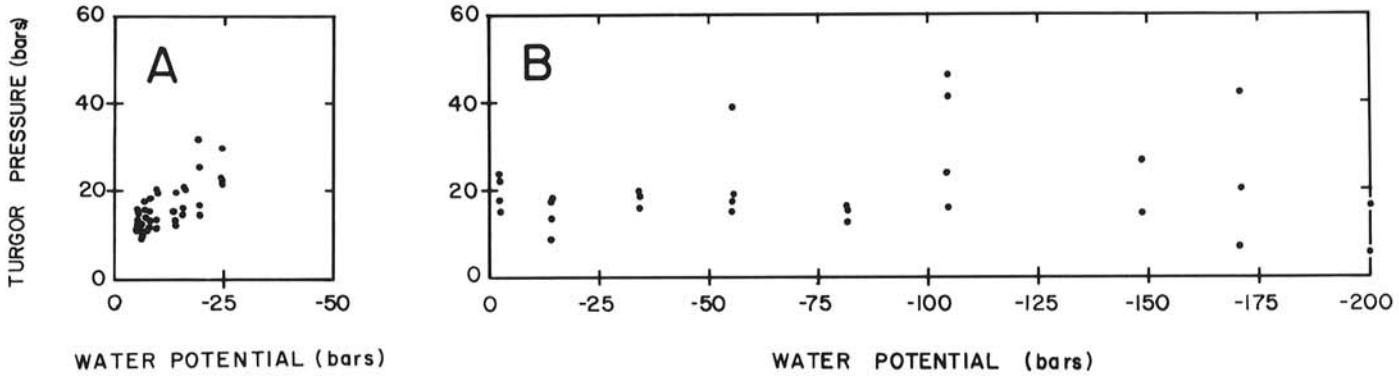

Fig. 2. Calculated turgor pressures (ψ_p) of mycelia of **A,** *Phytophthora cryptogea* and **B,** *Fusarium moniliforme* grown 21 days on P-3 broth medium amended to various water potentials (Ψ) by adding NaCl. Turgor (ψ_p) was calculated as the difference between the measured ψ_s of frozen and thawed mycelial mats and the measured Ψ of the growth medium; all replicate values obtained are shown individually.

TABLE 2. Respiration and growth rates of *Phytophtora cryptogea* and *Fusarium moniliforme* grown on P-3 broth amended to various water potentials with NaCl

Fungus	Water potential (bars)	Mean growth rate[a] ($mg \cdot day^{-1}$)	Mean respiration rate[b] (nmol $CO_2 \cdot g^{-1} \cdot s^{-1}$)
P. cryptogea	−5.0	5.8	67
	−8.5	6.7	180
	−12.0	1.9	294
	−23.0	0.9	457
F. moniliforme	−5.0	3.7	325
	−13.0	5.6	400
	−30.0	3.5	364
	−90.0	1.6	559

[a] Growth rate measured as mean increase in oven-dry weight of mycelium from three or four flasks from day 3 through day 7 or 8.
[b] Mean rate of CO_2 efflux per unit dry weight of mycelium measured from three or four replicate cultures on day 5.

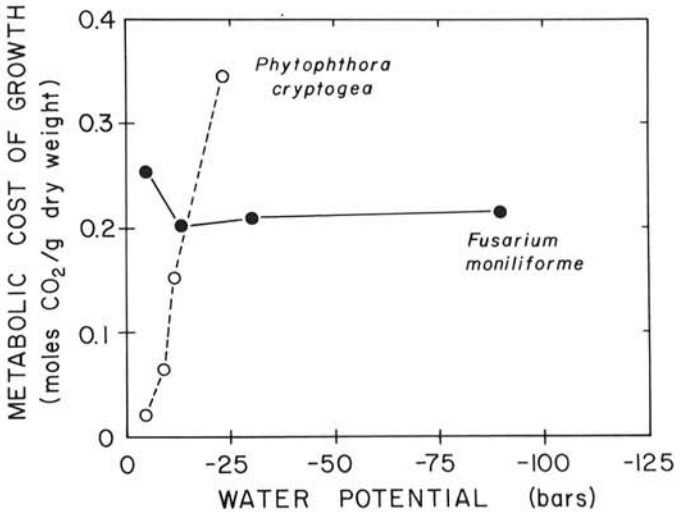

Fig. 3. Metabolic cost of growth for mycelium of *Phytophthora cryptogea* and *Fusarium moniliforme* on P-3 broth amended with NaCl to various water potentials (Ψ). The metabolic cost of growth was calculated as the rate of CO_2 production divided by the rate of growth. Respiration was measured as the rate of CO_2 efflux (nmol $CO_2 \cdot s^{-1}$) on 5-day-old cultures, and growth rate ($mg \cdot day^{-1}$) was measured as the increase in mycelial dry weight between days 3 and 7 or 8. (Mean values are given in Table 2.)

of the solutes used was insufficient to obtain Ψ levels low enough to reduce the growth of *F. moniliforme* greatly, and only the effects of NaCl and KCl were tested. With the single exception of the fresh weight obtained on LBB containing NaCl, the Ψ values that reduced the growth of *F. moniliforme* by one-half were one or more orders of magnitude lower than for *P. cryptogea* on the same medium (Table 1, Fig. 1).

Turgor and respiration. To examine some of the physiological differences between *F. moniliforme* and *P. cryptogea* that may underlie their responses to decreasing Ψ, the effects of medium Ψ on turgor and respiration were measured. Although there is variation in the data, both fungi maintained significantly positive turgors throughout the Ψ range that permitted growth. For example, on P-3 broth amended with NaCl, *F. moniliforme* maintained a relatively constant turgor that averaged about 15 bars or more at Ψ values ranging from −5 bars down to −200 bars (Fig. 2B). Turgor in the mycelium of *P. cryptogea* increased from a minimum of 9–15 bars to an average maximum of about 25 bars as Ψ decreased from −5 to −24 bars (Fig. 2A). The effects of Ψ on growth of the mycelial mats used to measure turgor were nearly identical to those shown in Figure 1C,D. Although the data obtained with KCl are not shown, turgor values were similar when KCl was used instead of NaCl as the major osmoticum.

Respiration rates in 1- and 3-day-old cultures on P-3 broth were too small and variable to be measured with the methods used. Repeatable and consistent measurements of respiration, however, were made on 5- to 21-day-old cultures on P-3 broth and showed a general but slow decline for any given treatment with culture age. In 5-day-old cultures at the highest Ψ value used, the respiration rates of *F. moniliforme* were much higher than those of *P. cryptogea* (Table 2). The respiration rate of *P. cryptogea*, however, increased more than that of *F. moniliforme* with decreasing Ψ values in NaCl-amended P-3 broth (Table 2). The differences between the two fungi in respiratory responses to decreased Ψ were accentuated when the metabolic cost of growth was calculated by dividing the rate of CO_2 production by the corresponding rate of growth. As is shown in Figure 3, the metabolic cost of growth in *P. cryptogea* increased greatly with decreasing Ψ while that of *F. moniliforme* remained relatively unchanged. In P-3 broth amended with NaCl, both the respiration and growth rates showed the largest differences between Ψ values in 5- to 8-day-old cultures. Although the increase in respiration at $\Psi \leqslant -20$ bars was less than with NaCl, respiration in *P. cryptogea* did increase when KCl or sucrose was used to lower the Ψ of P-3 broth; although KCl was used only at $\Psi \geqslant -70$ bars, its effects on respiration in *F. moniliforme* were similar to those of NaCl. Transfers of 5-day-old mycelial mats of *P. cryptogea* from P-3 broth, amended with NaCl to $\Psi = -5, -12$, or -20 bars, into new batches of the same media containing 0–20 $g \cdot L^{-1}$ glucose indicated that the amount of glucose that limited growth was much less than the amount present in 5-day-old cultures, and such transfers never increased growth rate.

DISCUSSION

The effects of Ψ on growth of *P. cryptogea* reported here are generally similar to those reported previously for species of *Phytophthora* (9,18,19,24,25,27) and *Pythium* (9). Compared with *P. cryptogea*, *F. moniliforme*, like many Fungi Imperfecti and Ascomycetes, is a xerophyte; in fact, its lower Ψ limit for growth of about −200 bars (Fig. 1) is exceeded greatly by only a limited

number of other fungi (9–12,19). The data of Wilson and Griffin (27) describing the effects of Ψ on the growth of *F. moniliforme* on agar medium amended with KCl are similar to those reported here. Although many parameters determine the ecological niches and activities of plant-pathogenic fungi, their distribution and activities in nature may reflect, at least in part, their abilities to grow at various Ψ values. In this regard, it is noteworthy that *Phytophthora* spp. are most often found in aquatic habitats or in soils and host plants that have been subjected to relatively wet conditions, but this distribution is probably caused more by the water requirements of sporangia and zoospores than by the water requirements for mycelial growth (9). In contrast to *P. cryptogea, F. moniliforme* can be pathogenic under very diverse conditions, including conditions of water stress in cornstalks (22).

A complete analysis of Ψ effects on growth kinetics is desirable (26), but complete time-course studies of growth with all the osmotica and Ψ values used in the present study would require very large experiments. The few experiments that were done to describe the kinetics of growth, however, suggest that the reported measurements of net growth in the time periods used are adequate to illustrate relative effects of various osmotica and Ψ values on mycelial growth. Although in the present study it was observed only with *F. moniliforme*, low Ψ values have been previously found to prolong the lag phase of growth by other fungi (11,12,17,27). In most of the treatments reported here, the addition of osmotica influenced the maximum slope of the growth curve more than the duration of the lag phase or growth period (28).

The fresh and/or dry weights of mycelia from liquid media can be confounded by the presence of extracellular water and solutes in the capillary spaces between hyphae (11,12,19). Mycelial mats have commonly been washed with water or $CaCl_2$ solutions to remove extracellular solutes, yet prolonged washing can remove cytoplasmic solutes and, in the case of water relations studies, even brief contact with a solution that differs from the substrate Ψ is likely to affect hyphal Ψ, ψ_p, and water content (12,13,19). Furthermore, even after washing, extracellular liquid must be removed in a consistent manner if the fresh weights and water relations parameters of mycelia are to be estimated accurately. The methods commonly used to remove extracellular solutions from mycelial mats include blotting and/or draining on paper, sometimes with suction (13), or centrifuging at low speeds on mesh supports (23). These methods often lack precision and repeatability or are too slow to be useful in many physiological studies. Use of tension plates to drain extracellular liquids from mycelium in a rapid, yet repeatable manner proved to be of value in determining the fresh weights and Ψ components of intact mycelial mats grown on broth. When precautions were taken to avoid evaporative water loss from mycelium and when the medium did not contain molecules, such as PEG 6000, that increased viscosity greatly, drainage on tension plates gave consistent fresh weights (28). Although some extracellular liquid was retained by mycelial mats after drainage at 200 mbar tension, the volume retained appeared to be reasonably small and constant relative to the volume of the hyphae.

The manner in which growth is measured can influence the results of experiments describing the effects of substrate Ψ on the growth of mycelium by fungi. For example, although there are reports that growth in dry weight on liquid medium and growth in colony diameter on agar medium are affected similarly by decreases in Ψ (11), both the present (Fig. 1) and previous studies (9,25) found decreases in Ψ to have differential effects on these two measures of growth. Luard (16,18) found that the fresh/dry weight ratios in mycelia of *Phytophthora cinnamomi* and *Penicillium chrysogenum* tended to decrease with decreasing Ψ in the growth medium. The results presented here, especially those for *F. moniliforme* (Fig. 1), show that growth in fresh weight was generally decreased much more by small decreases in medium Ψ than was growth in dry weight. An increased density of the cytoplasm from a lower water content, increased wall synthesis, and a net acquisition of solutes by mycelia grown in media of higher solute concentrations may explain the relatively large difference between the effects of Ψ on fresh and dry weights (3,11,12,16,18). For example, enhanced uptake of K^+ may have contributed to the stimulatory effects of KCl on growth of *F. moniliforme* measured as dry weight in P-3 broth at Ψ at values between −25 and −120 bars, a stimulation that was very small in the terms of fresh weight (Fig. 3D).

Previous studies, which are reviewed elsewhere (3,9,11,12), suggest that the growth of fungi at low Ψ generally increases with the complexity or nutritional value of the medium. Presumably, complex media may aid growth at low Ψ by providing solutes that can be used for internal osmotic adjustments (16,18), and they may alleviate nutritional limitations that arise at low Ψ (12). In general, the results obtained with *P. cryptogea* confirm the value of increased nutrition to growth at low Ψ in that equivalent reductions in Ψ reduced growth less on LBB than on P-3 broth (Fig. 1, Table 1). It is not clear why *F. moniliforme* showed the opposite result of greater sensitivity to lower Ψ values in LBB than in P-3 broth. However, to an extent, the relative data shown in Figure 1B,D may not illustrate the influence of nutrition because the actual growth of *F. moniliforme* was greater on LBB than on P-3 broth at $\Psi > -100$ bars (e. g., nearly 2.5 fold greater at $\Psi = -5$ bars) and P-3 broth was the superior medium only at very low Ψ values.

Differential effects of specific solutes on fungal processes have been noted at low Ψ (12), and sulfate salts caused larger reductions in mycelial growth by *P. cinnamomi* than did many other solutes (25). However, the results of the present study show that although growth by mycelium of *P. cryptogea* was somewhat more sensitive to Na_2SO_4 than to other salts, it did not show a clearly differentiated response to sulfate salts. Growth on P-3 broth amended with $MgSO_4$ was similar to that on P-3 broth amended with KCl, and Ψ effects on growth on LBB were fairly uniform among the solutes tested (Table 1). The slight enhancement of growth by *P. cryptogea* at Ψ values greater than −25 bars when sucrose or mannitol was added to broth media may reflect the additional energy source provided by these compounds or their use as or conversion to solutes for osmotic adjustment. In general, however, the differential effects of specific solutes on growth of *P. cryptogea* in P-3 broth were small, and in LBB, they were negligible relative to the effects of medium Ψ. Therefore, it appears that the effects of added solutes on growth by *P. cryptogea* were due largely to their effects on medium Ψ (11). The same conclusion can be made for the growth of *F. moniliforme* on P-3 agar amended with KCl or NaCl; in broth media at equivalent Ψ values, however, growth by the same fungus was sometimes considerably less in the presence of NaCl than with KCl (Fig. 1). Many fungi preferentially take up K^+ when both K^+ and Na^+ are present externally (23), and uptake of K^+ as a compatible solute may have contributed to the significantly greater growth of *F. moniliforme* at moderately low Ψ values when KCl rather than NaCl was added to the medium. Furthermore, in substrates where some component of Ψ other than ψ_s predominates, such as a significantly negative matric potential (ψ_m) in drained soil, the effects of Ψ on growth may be different from those described here and elsewhere on liquid or agar media (1,4,9,11,12). In addition, temperature can modify the effects of Ψ on fungal growth (4,11).

Although expansive growth by fungal mycelia is confined largely to hyphal tips, maintenance of a positive turgor is essential for tip growth, and fungal growth at low Ψ values requires effective mechanisms for osmoregulation (2,9,11,12,21,26). Both *P. cryptogea* and *F. moniliforme* appear to maintain large turgors throughout the entire Ψ range that permits growth (Figs. 1 and 2); a reduction or lack of turgor per se, therefore, does not appear to have limited growth at low Ψ values. This conclusion agrees with the one reached by Luard and Griffin (19) and by Luard (16,18), who found that a variety of fungi have the capacity to maintain positive turgors when grown at low Ψ. For example, when grown on agar medium overlain with cellophane, the mycelium of *P. cinnamomi* maintained turgors of 6–13 bars at Ψ values of −5 to −35 bars and *F. equisesti* maintained turgors of 6–20 bars at Ψ values of −5 to nearly −200 bars, even though growth by both fungi was very much reduced at the lower Ψ values used (18,19). In the only other study that quantified turgor of mycelia grown at a

number of Ψ values, Adebayo et al (2) removed mycelium of *Aspergillus wentii* and *Mucor hiemalis* from agar media amended with KCl and found turgors quite similar to the values reported here. In particular, *A. wentii* resembles *F. moniliforme* in that both are relatively xerophytic fungi and both maintained a turgor of about 15 bars over a wide range in Ψ. The growth of *M. hiemalis* declined more than did the growth of *A. wentii* as medium Ψ declined from −1 to −41 bars; yet the estimated turgor of *M. hiemalis* increased from 4 to 11 bars, results that are similar to those obtained with *P. cryptogea* (Figs. 1C and 2A). Whereas Luard and Griffin (19) found that turgors in several fungi tended to increase with decreases in medium Ψ, Luard (18) found the turgor of *P. cinnamomi* to be nearly constant. Transfers of mycelia to media of higher or lower ψ_s have shown that *Aphanomyces euteiches* has a limited capacity to adjust osmotically within 5 hr (13) and that *Penicillium chrysogenum* and *Chrysosporium fastidium* can regain normal turgors within 8 hr (17).

The studies cited above used thermocouple psychrometers to estimate mycelial ψ_s and ψ_p. The bursting, growth, and branching of hyphal tips in various solutions is related to water uptake and osmosis (21), but studies of those phenomena are not likely to measure turgor quantitatively (19). Although the turgor values obtained for mycelia in psychrometers appear to be as accurate as any available, they are only estimates. The measurements of mycelial ψ_s reported here did not separate the individual contributions of the growing apical tips and older nongrowing regions of hypha, but limited data on *P. chrysogenum* do suggest that turgor is uniform in various parts of a mycelial mat (16). Furthermore, though the use of tension plates reduced errors caused by adhering media, the exact effects of external solution and cell wall volume on the measured ψ_s values remain largely unknown. Most psychrometer estimates of hyphal ψ_p are made on the assumption that the Ψ of the mycelium and medium are equal, but there is some risk, especially on agar media, that evaporation may depress the Ψ of aerial hyphae below that of the medium. It is also possible that the solute composition of mycelium may change during the time required for psychrometer measurements.

Maintenance of turgor requires the concentration of solutes within the mycelium to reduce the ψ_s of hyphal cells below the Ψ of the medium, and some portion of the acquired internal osmotica cannot be obtained by passive transport and must be acquired through active uptake or metabolic synthesis (e.g., 3,12,16–18). Energy for the active acquisition or production of solutes for osmoregulation in fungi must come from respiration. Therefore, though other factors might also influence respiration rate, increased energy required for osmotic adjustment might be expected to increase respiration rates at low Ψ values (11,12,27). In experiments reported here, the respiration rate of *P. cryptogea* was nearly sevenfold greater in medium at −23 bars than than in the unamended medium at −5 bars, whereas the respiration rate of *F. moniliforme* at −90 bars was nearly twice that in the unamended medium (Table 2). There have been few previous measurements of respiration on mycelial fungi subjected to various Ψ values. Wilson and Griffin (27) investigated relationships between decreased Ψ of agar medium and respiration in four species of fungi and obtained results analogous to those reported here in that respiration rates were greater at low Ψ. Furthermore, Wilson and Griffin found that the increase in respiration rate at lower Ψ values was greater in aquatic fungi such as *P. cinnamomi* than in more xerotolerant fungi such as *Penicillium canescens*. Although low Ψ has been reported to reduce respiration rates in *Verticillium albo-atrum* (20) and *Ophiobolus graminis* (6), in *Phytophthora cryptogea* and *F. moniliforme* respiration rates were greater at low Ψ values, even though growth was decreased (Table 2, Fig. 3). Additionally, results obtained when 5-day-old cultures of *P. cryptogea* were transferred to fresh media indicate that a depletion of nutrients to levels below those initially present in P-3 broth did not limit growth rate at the time respiration was measured. However, respiration was measured as the rate of CO_2 efflux and the metabolic pathways used and the amounts of energy derived from reactions leading to CO_2 release were not determined. Furthermore, respiration was examined in detail only on one medium amended to low Ψ with NaCl. Although more definitive research is needed on the metabolic costs of mycelial growth at low Ψ, the results (Fig. 3) suggest that such costs are much higher and more limiting for *P. cryptogea* than for *F. moniliforme*.

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