

## The Effect of Osmotic Potential and Specific Ions on Growth of *Phytophthora cinnamomi*

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### ABSTRACT

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To establish the response of the root pathogen *Phytophthora cinnamomi* to osmotic potential, growth of the fungus was studied in a liquid minimal medium amended with eight osmotica. The isolate used in the experiments had a high radial growth rate on agar, 196  $\mu\text{m}/\text{hour}$ , but a low specific growth rate in liquid culture, 0.027/hour. The amount of growth reduction varied with the osmoticum. Common osmotica like NaCl and KCl reduced growth 50% at approximately -9 bars. Comparable reductions by CaCl<sub>2</sub> and MgCl<sub>2</sub> were at -11 and -16 bars, respectively, and MgSO<sub>4</sub> at -4 bars. Osmotic solutions of polyethylene glycol 6000 caused 50% reductions in growth at -13.3 bars. The relative activity of Na<sup>+</sup> in NaCl that reduced growth 50% was

0.092. All other cations affected growth at lower activities; Mg<sup>2+</sup> in MgSO<sub>4</sub> was most toxic with 50% reduction at 0.009. Polyethylene glycol solutions acted like NaCl and KCl and these three osmotica influenced growth only as they altered osmotic potential. Collectively, the data argue for specific ion toxicities rather than a general osmotic effect of some salt solutions on growth. The osmotic potential that severely limited colonial radial growth rate of *P. cinnamomi* on agar media was twice as low as that that severely limited specific growth rate in liquid media. The data with solid media agree with responses previously reported and illustrate that culture methods directly affect osmotic potential responses of *P. cinnamomi*.

The components of water potential (primarily matrix and osmotic potential) have been studied in relation to the growth of plant pathogenic fungi (6, 8), but there are few data for species of *Phytophthora*. In 1958, Bingham et al. (4) evaluated the effects of the nutrition of avocado seedlings on severity of root rot caused by *Phytophthora cinnamomi* Rands and they noted that solution cultures with the lowest osmotic potential ( $\approx -3$  bars) had the lowest rates of disease development. Sommers et al. (16) and Adebayo and Harris (1) reported that *P. cinnamomi* can grow at potentials as low as -30 bars in agar systems amended with different solutes and in petri dishes of soil. The radial growth rate of the fungus was highest between -10 to -15 bars; growth trends in their experiments were more closely related to osmotic potential than to specific ion toxicity. Jones and Jennings (12) studied the effects of cations on the growth of several fungi in liquid media amended with salts. Because monovalent and divalent cations interacted to affect growth, the authors concluded that factors other than osmotic potential were responsible for growth reductions. To determine if specific ions affect *P. cinnamomi*, our experiments focused on mycelial growth when different osmotica were used to regulate osmotic potential in a minimal growth medium.

### MATERIALS AND METHODS

**Inoculum.**—Isolate Pc40 (*Phytophthora* Culture Collection, Department of Plant Pathology, University of California, Riverside) of *P. cinnamomi* was used in all experiments. Flasks of liquid media and agar plates were inoculated with 4-mm diameter disks cut from mycelial mats grown in a minimal medium (see below) and washed three times with demineralized water.

**Media.**—The minimal medium used in all experiments was modified from that of Bartnicki-Garcia (2). The composition (molar concentration) was: KH<sub>2</sub>PO<sub>4</sub>,  $1 \times 10^{-2}$ ; CaCl<sub>2</sub>,  $3.1 \times 10^{-5}$ ; FeCl<sub>3</sub>·6H<sub>2</sub>O (EDTA disodium salt),  $4 \times 10^{-6}$ ; ZnSO<sub>4</sub>·7H<sub>2</sub>O,  $1.1 \times 10^{-3}$ ; MnSO<sub>4</sub>·H<sub>2</sub>O,  $2 \times 10^{-6}$ ; CuSO<sub>4</sub>·5H<sub>2</sub>O,  $2 \times 10^{-6}$ ; (NH<sub>4</sub>)<sub>6</sub> Mo<sub>7</sub>O<sub>24</sub>·H<sub>2</sub>O,  $2 \times 10^{-7}$ ; MgSO<sub>4</sub>·7H<sub>2</sub>O,  $2 \times 10^{-6}$ ; thiamine HCl,  $3 \times 10^{-6}$ ; and NaNO<sub>3</sub>,  $1.59 \times 10^{-3}$ . All ingredients were dissolved in 900 ml of demineralized H<sub>2</sub>O, pH was adjusted to 6.0, 20 g of glucose was added, and the volume was brought to 1 liter. For solid medium, 15 g/liter Noble agar was added. In one experiment, yeast extract (0.10 g/liter) was added to the solid medium.

**Amended media.**—The osmotic potential of liquid or solid media was controlled by adding either NaCl, KCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>, Na<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, MgSO<sub>4</sub>, or polyethylene glycol 6000 (PEG 6000, Carbowax 6000). Polyethylene glycol is a high molecular weight osmoticum used to control water potential (15) and the effects of this non-

ionized polymer can be compared to ionized species that control osmotic potential. The osmotic potential of all amended media (three replications) was determined with both a vapor pressure osmometer and an isopiestic psychrometer. The pH of the culture solutions was readjusted to 6.0 prior to inoculation and measured again when the fungus cultures were harvested. The pH dropped in all cultures, but never more than 0.9 units in any one treatment.

**Fungal growth measurements.**—Figure 1 shows the results of an experiment to determine the optimum growth period in the liquid cultures. From the results of that experiment, 21 days was chosen for exposure time in all experiments. Growth in liquid culture was measured by harvesting from flasks (three replicates per treatment) with a nylon screen pad in a filter. The mycelial mats were washed twice with 25 ml of distilled water, dried 48 hours at 70 C, and weighed. Growth on agar was measured as the distance from the circumference of the inoculum to the growing edge of the colony. The specific growth rate ( $\alpha_s$ ) for the isolate of *P. cinnamomi* in these experiments was determined according to the formula:  $\alpha_s = \ln 2/td$ , in which  $td$  = the doubling or mean effective generation time (18). Flasks (three replicates inoculated with *P. cinnamomi*) were harvested every 4 hours for 96 hours and the dry weight values were used to compute doubling time. The colonial radial growth rate ( $K_r$ ,  $\mu\text{m}/\text{hour}$ ) on solid media was calculated from measurements of the expanding colony edge.

**Activities of cations in amended media.**—The ionic strength of the minimal medium was obtained by entering the molar concentrations of the components into a computer program. The relative activity of a cation in an

aqueous solution is the product of an activity coefficient for the cation ( $\gamma$ ,  $M^{-1}$ ) and the molar concentration ( $M$ ). Relative activity does not have units. Values of  $\gamma$  were computed from the modified Davies' equation (17). Before determining  $\gamma$ , the ionic strengths of the amended solutions were corrected by adding the ionic strength of the minimal medium.

## RESULTS AND DISCUSSION

**Influence of osmotic potential on growth of *Phytophthora cinnamomi* in liquid culture.**—Under the conditions of this study, the specific growth rate for the *P. cinnamomi* isolate was .027/hour, a figure lower than all the rates in Trinci's (18) study of fungal growth kinetics. The radial growth rate ( $K_r$ ) on minimal agar was 196  $\mu\text{m}/\text{hour}$ . A ratio  $K_r/\alpha_s$  of 7,259 indicated a rapid radial growth rate relative to a slow specific growth rate. Concerning fungal growth kinetics, Trinci (18) stated that, "although an organism may be able to synthesize protoplasm at a comparatively low rate (i.e., it has a low specific growth rate) it may be able to sustain a rapid radial rate of colony growth." Specific growth rate reflects the ability of the fungus to synthesize protoplasm, but radial growth rate mainly can measure the ability to transport protoplasm to hyphae at the colony edge. In experiments in which nutrient concentration varied, Trinci found that radial growth rate was not a reliable parameter of specific growth rate in submerged culture. To study the effects of specific salts, we chose a liquid culture system with a defined minimal medium, because we believed this system would best reflect changes in growth rate.

Figure 2 shows the growth response of *P. cinnamomi* to several of the osmotica tested. The data for KCl and  $K_2SO_4$  were not plotted because they were similar to NaCl and  $MgSO_4$ , respectively. Growth in the minimal-medium control ( $-3.5$  bars osmotic potential) was taken as the 100% value in all the experiments, and variation among controls in the different tests was not significant at 0.05. Table 1 gives the osmotic potentials associated with 50% growth reductions for the osmotica in Fig. 2.

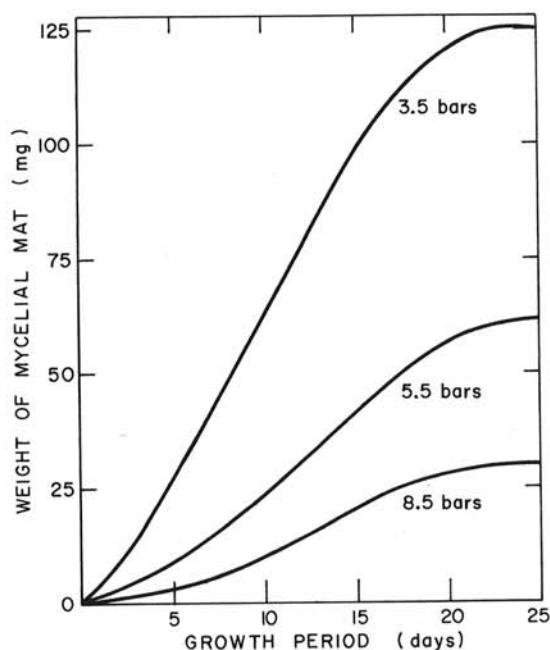


Fig. 1. Growth curves for *Phytophthora cinnamomi* grown in a minimal medium adjusted to three osmotic potentials with NaCl. Dry weights of mycelium from three replicates of each treatment were determined every 72 hours.

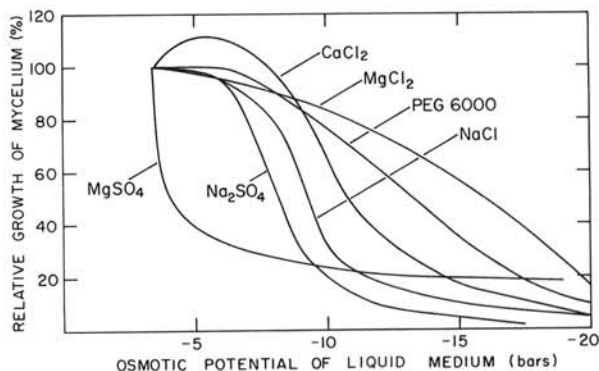


Fig. 2. The effect of osmotic potential on growth of *Phytophthora cinnamomi* in minimal medium amended with several osmotica. The 100% value pertains to growth in nonamended minimal medium ( $\psi\pi = -3.5$  bars). Polyethylene glycol (MW = 6,000-7,500) is PEG 6000.

*Phytophthora cinnamomi* did not grow at osmotic potentials of  $-20$  to  $-30$  bars, nor did optima occur at  $-10$  to  $-15$  bars as previously reported (1, 16). In fact, the range of tolerances we recorded was similar to those noted for many economic plants, namely  $-2$  to  $-8$  bars (3, 11).

Table 1 shows that the NaCl treatment reduced growth of *P. cinnamomi* by 50% at an osmotic potential of approximately  $-9$  bars. The 50% growth decrement for  $\text{CaCl}_2$  and  $\text{MgCl}_2$  corresponded to osmotic potentials of  $-11$  and  $-16$  bars, respectively. When we plotted growth vs. chloride concentration of the treatments (curves not shown), NaCl and KCl were similar in their effects; both monovalent salts were more restrictive of growth than was  $\text{CaCl}_2$  or  $\text{MgCl}_2$ . Growth restrictions did not parallel the chloride-ion concentration of the medium, but were related to the associated cation. The osmotic potentials associated with 50% growth decrements (Table 1) were lower for the sulfate salts;  $\text{MgSO}_4$  was the most toxic of any salt tested. From a study of the effects of PEG 6000 solutions on three mycorrhizal fungi, Mexal and Reid (14) concluded that PEG 6000 was not metabolized or toxic to the fungi and that the polymer might be used to approximate soil moisture stress. Their data suggest that the growth response to PEG 6000 can be interpreted as the effect of water potential alone. Since all the amendments in the present study were in the same minimal medium, the growth trends were not related specifically to nutrition as Sommers et al. (16) observed. Generally, it is held that higher plants respond to osmotic potential independent of the salt species involved (10, 13), but incidences of specific salt effects have been observed (7, 19). Taken collectively, our data show that some salts were more toxic to *P. cinnamomi* and the degree of toxicity varied with the cation and anion of the salt.

**Plots of cation activity versus growth.**—To help reveal the effects of the different salts we plotted growth vs. the relative activity of cations added as different salts to the minimal medium (Fig. 3). The relative activity reflects the thermodynamic state of the cation. Table 2 shows the activities of cations from different salt sources associated with 50% growth reductions. For  $\text{Na}^+$  in NaCl, 50% growth occurred at an activity of 0.092, whereas  $\text{Ca}^{2+}$  in  $\text{CaCl}_2$  and  $\text{Mg}^{2+}$  in  $\text{MgCl}_2$  reduced growth by 50% at 0.036 and 0.068, respectively. A slight stimulation by calcium ions was noted at low activities. Jones and Jennings (12) observed a similar response with *Dendryphiella saline*

TABLE 1. Osmotic potential associated with a 50% growth reduction of *Phytophthora cinnamomi* by several osmotica

Osmoticum	Osmotic potential for 50% growth reduction <sup>a</sup> (bars)
$\text{MgCl}_2$	-16.0
PEG 6000 <sup>b</sup>	-13.0
$\text{CaCl}_2$	-11.0
NaCl	-9.3
$\text{Na}_2\text{SO}_4$	-8.2
$\text{MgSO}_4$	-4.0

<sup>a</sup>Cultures were grown in minimal medium amended with various osmotica, at 24 C, in the dark for 21 days.

<sup>b</sup>Polyethylene glycol, a non-ionized polymer for comparison with the ionized osmotica.

and postulated that calcium altered membrane permeability and reduced an efflux of stimulatory ions. Examination of the curves for the sulfate salts shows that they were in the same relative positions as in the growth-osmotic potential curves and that  $\text{MgSO}_4$  was most effective in reducing growth. The curve for PEG 6000 was plotted from concentrations unchanged by activity coefficients, because the activity coefficient of an uncharged molecule in a dilute electrolyte solution usually is equal to unity (5). Any correction to the coefficient would increase its value above one, and move the PEG 6000 curve closer to the NaCl curve in Fig. 3. If NaCl, KCl, and PEG 6000 do exert similar effects when expressed as activities, then all three might be used as nontoxic osmotica. The cation activity data support the interpretation that growth restrictions in amended liquid cultures were related to the particular combination of cation and anion used to control osmotic potential.

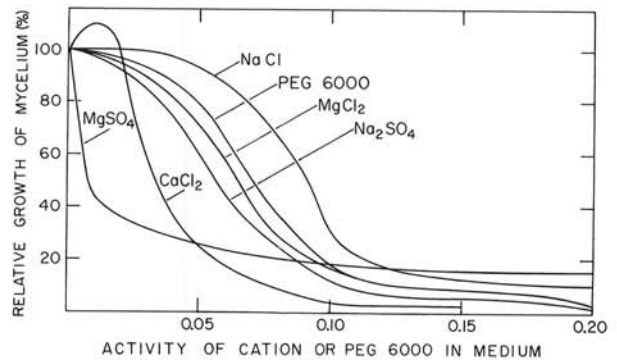


Fig. 3. The effect of relative activity of cations or PEG 6000 (polyethylene glycol, MW 6,000-7,500) on growth of *Phytophthora cinnamomi* in amended minimal medium. The relative activity of a cation in an osmoticum was the product of the molar concentration of the cation (M) and an activity coefficient ( $\gamma$ ,  $\text{M}^{-1}$ ). The 100% value pertains to growth in nonamended minimal medium. Polyethylene glycol (PEG 6000) is a non-ionized polymer for comparison with the ionized osmotica, and the activity coefficients for PEG 6000 solutions were assumed to be unity.

TABLE 2. Relative activity<sup>a</sup> of cations in osmotica associated with a 50% growth reduction of *Phytophthora cinnamomi*

Cation/osmoticum	Relative activity for 50% growth reduction <sup>b</sup>
$\text{Na}^+$ /NaCl	.092
PEG 6000 <sup>c</sup>	.070
$\text{Mg}^{2+}$ /MgCl <sub>2</sub>	.068
$\text{Na}^+$ /Na <sub>2</sub> SO <sub>4</sub>	.056
$\text{Ca}^{2+}$ /CaCl <sub>2</sub>	.036
$\text{Mg}^{2+}$ /MgSO <sub>4</sub>	.009

<sup>a</sup>Relative activity of a cation is its molar concentration (M) multiplied by an activity coefficient ( $\gamma$ ,  $\text{M}^{-1}$ ).

<sup>b</sup>Cultures were grown in minimal medium amended with various osmotica, at 24 C, in the dark for 21 days.

<sup>c</sup>Polyethylene glycol, a non-ionized polymer for comparison with the ionized osmotica. Activity coefficients for PEG solutions were assumed to be unity.

**Influence of osmotic potential on growth rate of *Phytophthora cinnamomi* in amended agar.**—Our results in solution culture related poorly to the data Sommers et al. (16) and Adebayo and Harris (1) obtained on osmotically controlled agars. Therefore, we designed experiments to test the effects of osmotic potential on *P. cinnamomi* when the minimal medium, which was amended with NaCl or KCl, was prepared as 1.5% agar medium. In one experiment, yeast extract was added at the concentration used by Sommers et al. (0.1 g/liter). Compared with the growth-osmotic potential curves in Figure 2, the growth curves for the salt-amended agar (Fig. 4) extended to potentials approximately two times lower. In fact, when yeast extract was added to the medium, the growth trends approximated the responses reported by Sommers et al. (16) and also reflected the nutrient-osmotic potential interaction they observed. The growth optima we observed on solid media (0 to -10 bars) were still lower than those previously reported (-10 to -15 bars), but the differences may relate to additional nutrient stimulation by malt extract (1 g/liter) in the other systems. The increased tolerance of *P. cinnamomi* to osmotic potential when grown on amended solid media suggests that radial growth rate was more a measure of stress on hyphal extension than impaired synthetic ability of the fungus.

### CONCLUSIONS

As Griffin noted (9), agar culture systems and amended soil systems can be used to evaluate total water potential effects on the growth of fungi. But Sommers et al. (16) and Adebayo and Harris (1) discussed the problem of partitioning potential energy between a matrix and osmotic component in agar systems or amended soils and concluded that separating the component effects is impossible at that time. It might be argued that

experimental results for fungi growing in solution cultures cannot easily be applied to fungi living in a complex soil environment. Nevertheless, our experiments showed that accurately defined liquid cultures can be used to evaluate both the osmotic component of water potential and the effects of specific salts. We also showed that an osmoticum like PEG 6000 can be used to obtain an estimate of the degree of ion toxicity relative to the effects of osmotic potential alone.

Throughout this paper we discussed the differences between previous data and our own concerning osmotic potential responses of *P. cinnamomi*. We also pointed out that most of the differences can be attributed to the different culture methods (agar or liquid culture) used to study the same variable; i.e., osmotic potential. The two most important differences in results and interpretations are summarized as follows. First, Sommers et al. (16) and Adebayo and Harris (1) found a broad range (0 to -30 bars) of osmotic potential tolerance for the fungus, with optimum growth at -10 to -15 bars. In our study, 50% growth decrements occurred at potentials higher than -10 bars for most osmotica, and the tolerance range approximated that of many economic plants (-2 to -8 bars). Second, our work indicated that different salts used to adjust osmotic potential caused different growth decrements at the same level of osmotic stress; i.e., specific ions were more toxic to *P. cinnamomi* than was osmotic potential alone. The earlier studies (1, 16) did not reveal specific ion toxicity and reported that growth was affected by osmotic potential alone, with possible nutritional influences. The differences point out that different culture methods should be considered when designing experiments to relate fungal growth to a component of water potential, to nutrient imbalances, or to specific ions.

At this time, we can draw no conclusions about how specific ions might affect fungi, but we agree with Sommers et al. (16) that understanding osmoregulation and ion uptake by fungi is primary to comprehending total soil water influences. Our experiments indicate that liquid culture methods serve well for investigating both osmotic and specific salt effects on the growth of fungi. Information from such studies can be used to guide further research and to help interpret results from soil systems. In fact, a sequence of experiments progressing from liquid cultures to soil seems a proper approach for investigating the relationship of water potential to the total ecology of a soil fungus.

### LITERATURE CITED

1. ADEBAYO, A. A., and R. F. HARRIS. 1971. Fungal growth responses to osmotic as compared to matrix water potentials. *Soil Sci. Soc. Am. Proc.* 35:465-469.
2. BARTNICKI-GARCIA, S. 1966. Chemistry of hyphal walls of *Phytophthora*. *J. Gen. Microbiol.* 42:57-69.
3. BERNSTEIN, L., and H. E. HAYWARD. 1958. Physiology of salt tolerance. *Annu. Rev. Plant Physiol.* 9:25-46.
4. BINGHAM, F. T., G. A. ZENTMYER, and J. P. MARTIN. 1958. Host nutrition in relation to *Phytophthora* root rot of avocado seedlings. *Phytopathology* 48:144-148.
5. BUTLER, J. N. 1964. Page 439 in *Ionic equilibrium: a mathematical approach*. Addison-Wesley, Menlo Park, California. 547 p.

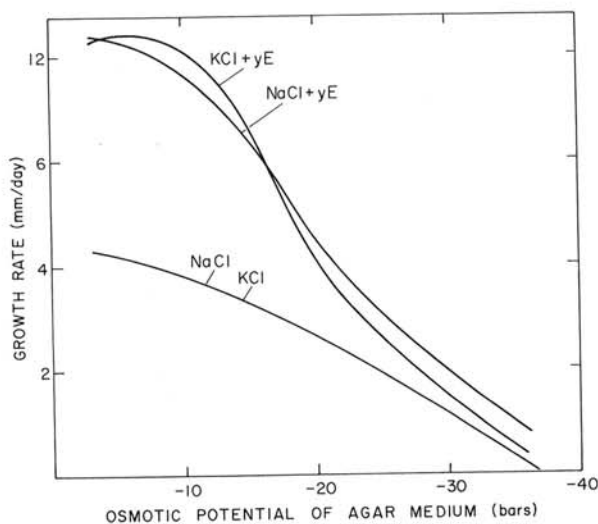


Fig. 4. The effect of osmotic potential on growth of *Phytophthora cinnamomi* on minimal medium prepared as 1.5% agar. The osmotic potential of the medium was controlled with either NaCl or KCl, and in one experiment, the minimal medium was supplemented with yeast extract (0.10 g/liter).

6. COOK, R. J., R. I. PAPENDICK, and D. M. GRIFFIN. 1972. Growth of two root rot fungi as affected by osmotic and matric water potentials. *Soil Sci. Soc. Am. Proc.* 36:78-82.
7. GAUCH, H. G., and C. H. WADLEIGH. 1944. Effects of high salt concentrations on the growth of bean plants. *Bot. Gaz.* 105:379-387.
8. GRIFFIN, D. M. 1963. Soil physical factors and the ecology of fungi. III. Activity of fungi in relatively dry soil. *Trans. Br. Mycol. Soc.* 46: 373-377.
9. GRIFFIN, D. M. 1972. Page 90 in *Ecology of soil fungi*. Syracuse Univ. Press, Syracuse, N.Y. 193 p.
10. HAYWARD, H. E., and E. M. LONG. 1943. Some effects of sodium salts on the growth of the tomato. *Plant Physiol.* 18:556-569.
11. HOFFMAN, G. J., and S. L. RAWLINS. 1971. Growth and water potential of root crops as influenced by salinity and relative humidity. *Agron. J.* 63:877-880.
12. JONES, E. B. G., and D. H. JENNINGS. 1965. The effect of cations on the growth of fungi. *New Phytol.* 64:86-100.
13. MAGISTAD, O. C., A. AYERS, C. WADLEIGH, and H. GAUCH. 1943. The effect of salt concentration, kind of salt, and climate on plant growth in sand cultures. *Plant Physiol.* 18:151-166.
14. MEXAL, J., and C. P. P. REID. 1973. The growth of selected mycorrhizal fungi in response to induced water stress. *Can. J. Bot.* 51:1579-1588.
15. MICHEL, B. E., and M. R. KAUFMANN. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiol.* 51:914-916.
16. SOMMERS, L. E., R. F. HARRIS, F. N. DALTON, and W. R. GARDNER. 1970. Water potential relations of three root-infecting *Phytophthora* species. *Phytopathology* 60:932-934.
17. STUMM, W., and J. J. MORGAN. 1970. Page 83 in *Aquatic chemistry*, John Wiley & Sons, New York. 583 p.
18. TRINCI, A. P. J. 1969. A kinetic study of the growth of *Aspergillus nidulans* and other fungi. *J. Gen. Microbiol.* 57:11-24.
19. WADLEIGH, C. H., and H. G. GAUCH. 1944. The influence of high concentrations of sodium sulfate, sodium chloride, calcium chloride, and magnesium chloride on the growth of guayule in sand culture. *Soil Sci.* 58:399-403.