Influence of Pine Bark, Matric Potential, and pH on Sporangium Production by *Phytophthora cinnamomi*

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ABSTRACT

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Sporangium production on mycelial mats of *Phytophthora cinnamomi* was compared in pine bark and in a Cecil clay soil at controlled matric potentials of 0 to −150 mb. Optimum sporangium production occurred between −15 and −25 mb in both substrates. Sporangium production was markedly less at −10 mb and at −150 mb in both pine bark and soil. Matric potential, not water content of the medium, regulated sporangium production. Maximum numbers of sporangia were produced 1 day after mats of mycelium were placed in the tensiometers at a constant matric potential of −25 mb. Incubation of mats for an additional 1–3 days did not increase sporangium production. Pine bark at pH 3.7 suppressed sporangium production. At pH 3.5, no sporangia were observed on mats incubated at a constant matric potential of −25 mb. Air-drying pine bark prior to saturation in Büchner funnel tensiometers completely suppressed sporangium production by *P. cinnamomi*.

Additional key words: moisture retention curve, physical properties, summation curve.

Hardwood bark compost suppresses root rot caused in ornamental plants by *Phytophthora cinnamomi* Rands (8,9,10). Chemical factors (17) and biological components (11) of the hardwood bark compost were implicated in disease suppression. Pine bark, a widely used substrate in nursery mixes in the southeastern and western United States, also has been associated with suppression of *Phytophthora* (15). Phytophthora root rot of lupine was less in pine bark and in hardwood bark compost than in a peat moss:sand medium (15). Spencer and Benson (15) also observed undefined stimulators and inhibitors of sporangium formation by *P. cinnamomi* in water extracts of pine bark.

The nature of disease suppressiveness associated with pine bark-based media is unknown. The factor(s) may be biological, chemical, physical, or a combination of these. For instance, soil matric potential is a key edaphic factor regulating the production of sporangia by *Phytophthora* spp. (5). Low soil pH is another factor known to inhibit sporangium production (2,15). However, it is not known whether matric potential and pH have the same effect with pine bark as observed with soil (2), U.C. mixes (2), and various extracts (15,16).

The physical properties of soil-less nursery media for growing ornamental plants have received more attention from horticulturalists and soil scientists in the past few years (4). As the physical properties of nursery media that govern plant growth and development are better understood, manipulation of physical properties may improve disease suppression by the media.

This report focuses on the influence of pine bark, matric potential, and pH on sporangium production by *P. cinnamomi*.

MATERIALS AND METHODS

Physical properties of pine bark and soil. A commercial grade of pine bark (Kamlar Corp. Rocky Mount, NC 27801, pH 5.0) that passed a 1.3-cm screen was stored in outdoor bins until needed. Soil material from the B horizon (the A horizon had been removed as fill dirt several years prior to establishment of the nursery site) of a poorly-drained, Cecil clay soil (pH 5.8) was collected from a nursery bed and air-dried on a greenhouse bench. Large clods were broken up and the soil was sieved through a 1.3-cm screen to remove remaining clods and rocks prior to use.

The particle size distribution of the pine bark and soil was determined by sieving four 50-g air-dried samples of each medium for 3 min on a shaker at 160 cycles per min (1). Nested sieves with openings (followed by logarithm) of 6.40 (0.8061), 4.75 (0.6766), 2.00 (0.3010), 1.00 (0.000), 0.60 (−0.2218), 0.25 (−0.6020), and 0.11 (−0.9746) mm were used. The amount of medium on each sieve and the amount that passed all sieves and was collected on the pan was weighed. The percent of medium passing each sieve was calculated and plotted as a summation curve (3, Fig. 1). About 66% of the soil and 81% of the pine bark passed the 4.75-mm sieve. The soil had more fine particles than the pine bark since 14% more soil particles passed the 0.25-mm sieve than pine bark particles.

The moisture retention curve (water content by volume) for the soil and pine bark were determined with a porous pressure plate apparatus (6). Briefly, a 345.5-ml aluminum cylinder was packed uniformly with the test medium, then placed on a 600-ml Büchner funnel with a porous plate and saturated with water for 12–24 hr by adding the water between the cylinder and the funnel perimeter. An air-tight lid was placed on top of the funnel then positive air pressure was applied to each funnel and the system was allowed to equilibrate for 24 hr as the outflow water was collected and measured. The air pressure was then increased and the system again was allowed to re-equilibrate an additional 24 hr as the outflow water was again collected and measured. This sequence was repeated until pressures equivalent to −4, −10, −20, −40, −50, −75, −100, −200, and −300 mb (1 cm H_2O approximately equals −1 mb) had been applied. There were eight replications of each medium. After measurement at −300 mb, cores were removed from the funnels and the amount of shrinkage was measured for each one prior to calculation of the moisture retention curve. At matric potentials higher than −10 mb, pine bark held about 5 to 24% more water by volume than the soil (Fig. 2). At −10 mb water content in soil and pine bark was about equal. At matric potentials between −15 and −150 mb, the soil held 3 to 8% more water by volume than the pine bark.

Preparation of fungal mats. Isolate 101 (ATCC 46292) of *Phytophthora cinnamomi* was cultured on cornmeal agar prior to transfer of three, 5-mm-diameter agar disks to a petri dish. Lima bean extract broth (50 g frozen lima bean per liter) was added to the level of the top surface of the agar disks. The disks were incubated

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at ambient temperature for 1 day, after which mycelium extended 5–10 mm from the edge of the disk. Two - three sterile, 1 cm^2 nylon screens (100 μm; Tetko, Inc., Elmsford, NY 10523) were placed on the mycelium and the screen and fungus were incubated 1 or 2 additional days. During this time, hyphae grew across and through the screen, forming a visible layer of mycelium on the top surface. The right-side-up orientation was maintained in subsequent tests. The mats of screen and mycelium were cut free from the rest of the mycelium in the culture by using a sterile scalpel. The lima bean extract was removed with suction, and the mats were rinsed three times with sterile deionized water. Mycelial mats were then incubated for 2 hr in a modified Chen-Zentmyer (13) salt solution to activate hyphae for sporangium production. The salt solution was removed by suction and the mats were rinsed three times with sterile deionized water. The mats were immediately placed on pine bark or soil in tensiometers or were left in sterile deionized water in the petri dish as a control. No sporangia were observed on the mycelium following the 2-hr soak in salt solution.

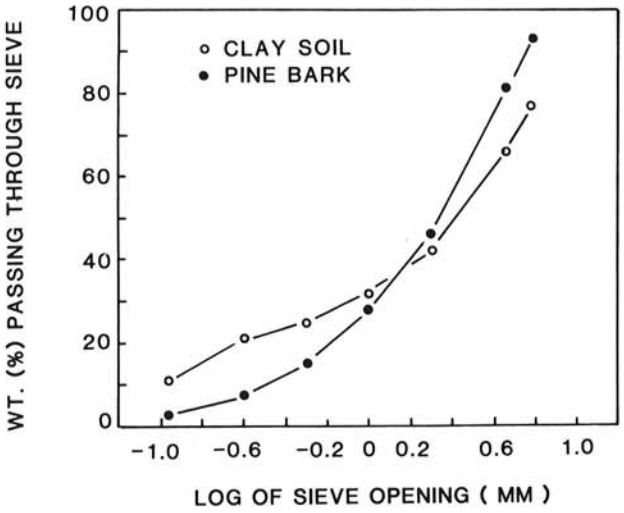

Fig. 1. Summation curve for particle size distribution in the pine bark and soil as determined by screening through sieves of various sizes.

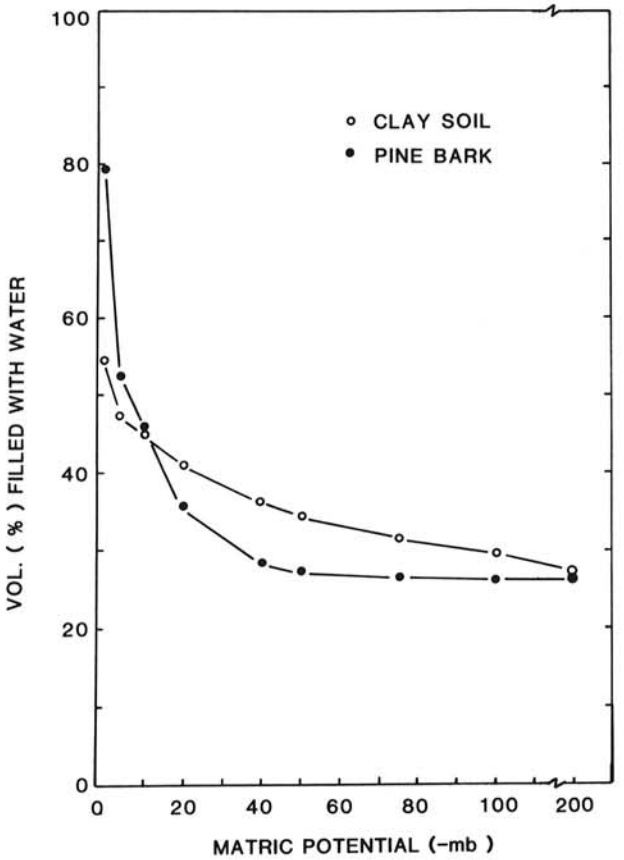

Fig. 2. Moisture retention curves for pine bark and soil as determined with a porous pressure plate apparatus.

Tensiometers. Büchner funnel tensiometers as described by Duniway (5) were filled with a 1-cm layer of air-dried pine bark or soil and saturated from below with deionized water. In later experiments, the pine bark was placed in 15-cm-diameter clay pots in a greenhouse and watered daily so it became thoroughly moistened prior to placement in the funnels. Two sterile nylon screens (2 cm^2) were placed about 3 cm apart on the surface of the saturated medium. A 1 cm^2 "activated" mat was placed on each screen followed by a second layer of 2 cm^2 screen. The sandwich of larger nylon screens aided in subsequent recovery of the mats from the funnels. A 0.5-cm layer of medium was added over the screens and became saturated from below within 20–30 min . The funnels were adjusted to potentials between 0 mb (saturated medium) and −150 mb by using the porous glass plate in the funnel and the top of the water reservoir as reference points. A loose-fitting plastic lid was placed on top of the funnel to prevent moisture loss from the medium surface.

After 1 day, the mats were removed from each funnel, rinsed gently in deionized water, and stained in a 0.025% aqueous solution of crystal violet for several seconds. The mats were then rinsed in deionized water to remove excess stain and mounted between microscope slides. The entire area and edges on the top side of the mat were examined (×100) and sporangia of *P. cinnamomi* were counted. Sporangia appeared to be randomly distributed over the entire inner mat surface; however, sporangia were clustered along

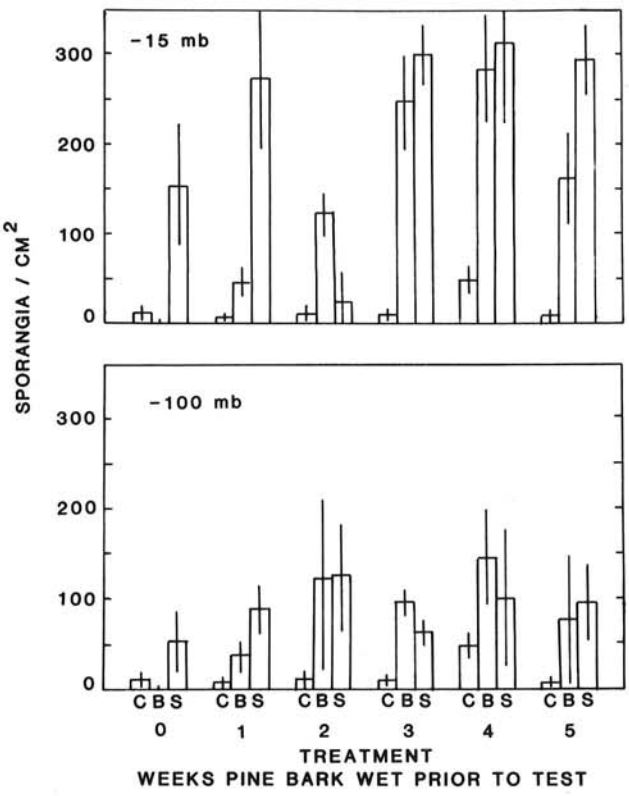

Fig. 3. Sporangium production by *Phytophthora cinnamomi* in the control (C) incubated in deionized water following a 2-hr soak in salt solution, and in pine bark (B) and soil (S) in Büchner funnel tensiometers at constant matric potential. Pine bark was potted in 15-cm-diameter clay pots and watered daily in the greenhouse for various time intervals prior to placing on the Büchner funnel tensiometers. **A,** −15 mb and **B,** −100 mb. Vertical line in each bar is the standard deviation of two observations over two replications.

the cut edge of the mat possibly due to stimulation of hyphae that were cut as the mat with mycelium was separated from the culture. In some experiments, mats were removed at times up to 4 days after equilibration on the funnel to assess the effect of incubation period at a constant matric potential on sporangium production. There were two funnels at each tension for a given run of the experiment and experiments were run at least three times. Data from all experiments were combined for presentation.

Adjustment of pine bark pH. The effect of pine bark pH on sporangium production was assessed in tensiometers at −25 mb by incubating pine bark moistened in either 0.1% H_2SO_4 or a 30-mM solution of $CaCO_3$ (2) for at least 2 days prior to placing the medium on the tensiometers. Initially, a 0.1% KOH solution was used to raise pH, but this resulted in discolored water below the tension plate and an inhibition of sporangium production similar to that reported by Blaker and MacDonald (2).

RESULTS

Effect of matric potential on sporangium production in pine bark and soil. Sporangium production by *P. cinnamomi* was completely suppressed in pine bark that was air-dried prior to placement on the funnel. For instance, at −15 mb and −100 mb, sporangia numbered 0.3 ±0.3/cm^2 and 0 in the pine bark medium, respectively, and 302 ±166/cm^2, and 121 ±62/cm^2 in the soil, respectively. Since pine bark is normally stored in the open in nurseries and thus is subject to wetting during rain storms as well as irrigation during production of the crop, several 15 cm-diameter clay pots of pine bark were set up and watered daily in the greenhouse. At 0, 1, 2, 3, 4, and 5 wk, subsamples were placed on the funnels to assess sporangium production. Within 1 wk, the number of sporangia produced at −15 and −100mb on the pine bark medium increased (Fig. 3). At 2 wk, there was no difference between number of sporangia in pine bark and in the soil at a given matric potential; however, more sporangia per square centimeter were observed at −15 mb than at −100 mb regardless of medium. In subsequent experiments, pine bark was premoistened in the greenhouse for at least 2 wk prior to use in the funnels.

Sporangium production by *P. cinnamomi* increased at the lower matric potentials tested up to an optimum between −15 and −2 5 mb (Fig. 4). Sporangium production in soil decreased only slightly between −15 and −75 mb, while in pine bark a sharp decrease occurred at −25 and −50 mb, respectively. Sporangium production in soil at −100 mb was only about 4% of the value at −25 mb. At −10 mb sporangium production in both pine bark and soil was only 17–27% of that observed for soil and pine bark at −15 mb, respectively (Fig. 4). On mats maintained in sterile deionized water following the salt soak, sporangium production was 56 ±52 sporangia per square centimeter. The coefficient of variation (c.v.) between replications within a given run of the experiment ranged from 2 to 141% depending on medium and matric potential with an overall c.v. of 49%. Variation in sporangium production was greater between runs of the experiment (range 2 to 129% with an overall c.v. of 76%) than between replications within a given run.

A second set of experiments was performed with pine bark at 0, −25, −50, −75, and −100 mb to characterize the sharp decrease in sporangium production observed between −25 and −50 mb in earlier experiments. A response curve similar in shape to that

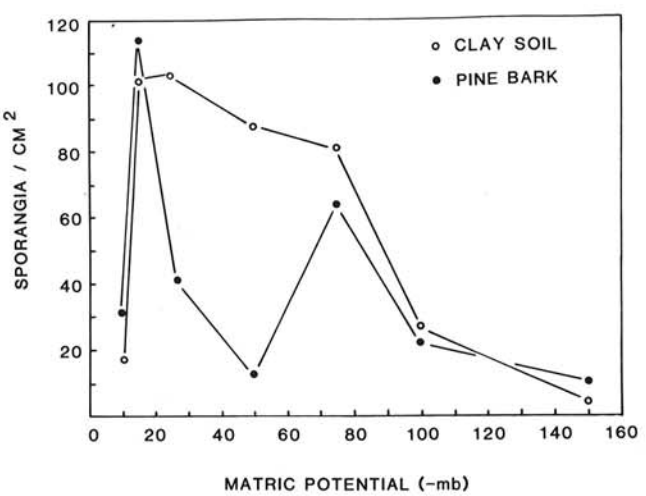

Fig. 4. Effect of matric potential on sporangium production by *Phytophthora cinnamomi* in pine bark and soil. Each point represents the mean number of sporangia per square centimeter for two tensiometers (two observations per tensiometer) per run averaged over two to six runs per matric potential.

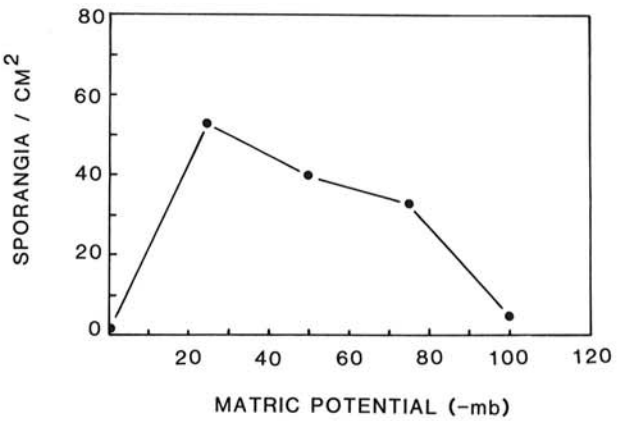

Fig. 5. The influence of matric potential on sporangium production by *Phytophthora cinnamomi* in pine bark. Each point represents the mean number of sporangia per per square centimeter for two tensiometers (two observations per tensiometer) per run averaged over two to four runs per matric potential.

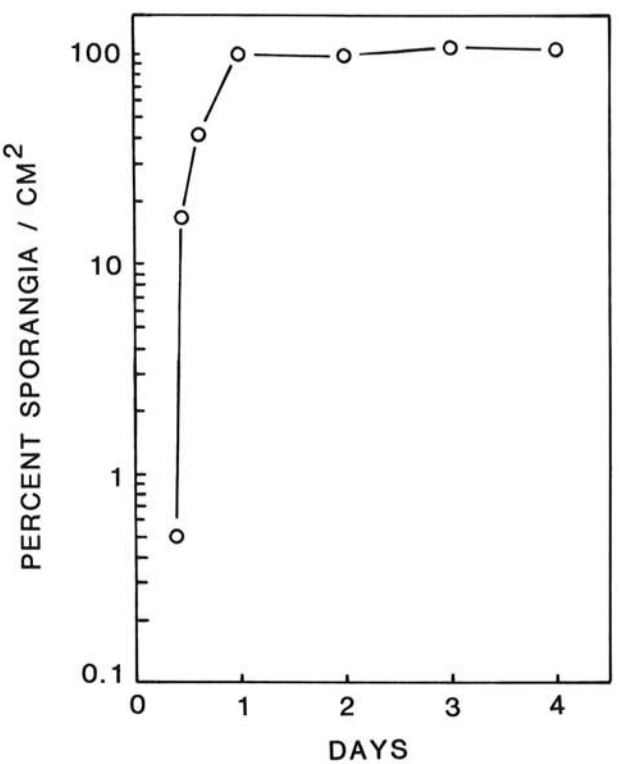

Fig. 6. Production of sporangia by *Phytophthora cinnamomi* at a constant matric potential of −25 mb as influenced by the time mats of mycelium were allowed to incubate in the pine bark on the tensiometers. Ordinate represents percent of sporangia formed at the various times compared to the number formed at 24 hr (average 148 sporangia per square centimeter). Each point represents the mean number of sporangia per square centimeter formed compared to the number at 24 hr for two tensiometers (two observations per tensiometer) per run averaged over four runs.

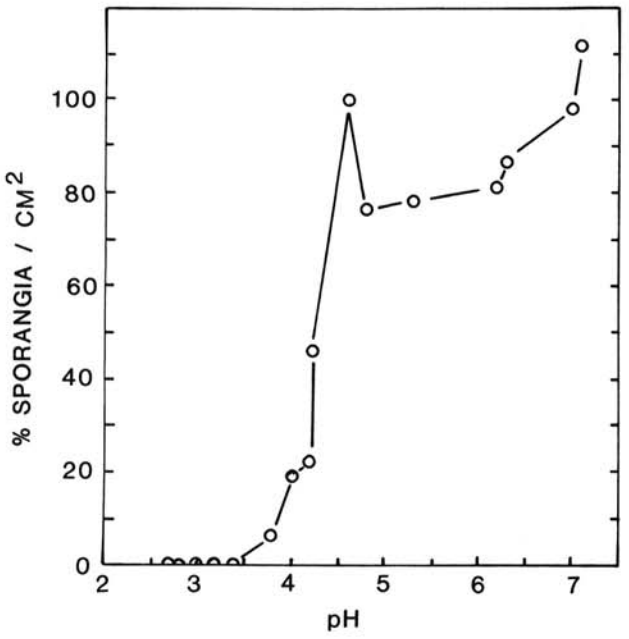

Fig. 7. Effect of pine bark pH on sporangium production by *Phytophthora cinnamomi* at a constant matric potential of −25 mb. Ordinate represents percent of sporangia formed at the various pHs compared to the number formed at a pH of 4.6 (average 383 sporangia per square centimeter). The pH of the pine bark (pH 4.6) was adjusted with either sulfuric acid or calcium carbonate in the greenhouse prior to placement on the tensiometers. Each point represents the mean number of sporangia per square centimeter formed at a given pH compared to the number formed at pH 4.6 for two tensiometers (two observations per tensiometer) per run averaged over one to three runs per pH level.

observed for soil (Fig. 4) was found; however, sporangium production was about 50% of that found in the first set of experiments. Sporangium production was maximum at −25 mb and declined slightly between −25 and −75 mb (Fig. 5). At −100 mb sporangium production by *P. cinnamomi* was about 10% of that at −25 mb.

Effect of time on sporangium production. At a constant matric potential of −25 mb, sporangium production reached a maximum level within 1 day (Fig. 6). A few sporangia were observed within 8 hr (includes 2 hr in salt solution). Between 8 and 11 hr, production increased to 17% of the maximum number observed after 24 hr (average 148 sporangia per centimeter over four experiments). At 13 hr, production was 41% of the maximum.

Effect of pH of pine bark on sporangium production. At a constant matric potential of −25 mb, maximum numbers of sporangia were produced between a pH of 4.5 to 7.2 (Fig. 7). A sharp decrease in sporangium production occurred between pH 4.6 and 3.5. No sporangia were observed at pHs of 3.5 or less in the pine bark.

DISCUSSION

Sporangial production by *P. cinnamomi* in pine bark at constant matric potentials was similar to that observed in a Cecil clay soil. Optimum sporangium production occurred between −15 and −75 mb in both media. This optimum range for sporangium production by *P. cinnamomi* is considerably higher (−160 mb) than previously reported for this fungus (7). However, for other species of *Phytophthora* such as *P. cactorum* (14), *P. cyptogea* (5), and *P. megasperma* (12) the optimum matric potential for sporangium production was in the same range as that observed for *P. cinnamomi* in the present study. The reasons for the rather large difference in optimum matric potential for sporangium production reported here and by Gisi et al (7) are unknown. Gisi et al (7) prepared mats of *P. cinnamomi* beginning with blended mycelium which probably resulted in less hyphae per disk and thus a lower total number of sporangia per disk than found in the present study. In addition, a 4-hr soak in salt solution followed by 2–3 hr saturation with the second soil layer in the tensiometer may have promoted formation of sporangia prior to equilibration of the soils to the various matric potentials. Variation in response to matric potentials between isolates of *P. cinnamomi* also may occur.

Large differences in water content of the pine bark and soil at the same matric potential were noted, and this supports the hypothesis of Gisi et al (7) that matric potential, rather than water content, regulates sporangium production in *P. cinnamomi*. Thus, comparisons of sporangium production in future studies should be based on the use of controlled matric potential and not on gravimetric measures of water content.

At a matric potential of −25 mb, sporangia were present as soon as 8 hr after the mat of mycelium was soaked in the salt solution, rinsed, and transferred to pine bark in the tensiometer. This length of time at favorable matric potential could easily be met following either irrigations or rainfall unless the medium rapidly drained to unfavorable matric potentials. Sporangium production in the upper levels of a container may be suppressed by rapid drainage of pine bark media following irrigation or rainfall, however, inoculum in the lower levels of a container would be at favorable matric potentials until the water was removed by plant uptake or evaporation. Physical properties of pine bark media (such as particle size) that influence drainage patterns may play a key role in the enhancement of medium suppression through direct effects on matric potential (1,4). The rather limited time required for sporangium production and the even shorter time reported for zoospore release (2) imply that a well-defined pine bark-based medium is needed that will drain rapidly to tensions near −100 mb to suppress sporangia, but still supply moisture adequate for optimum plant growth.

DeBoodt and Verdonck (4), describing the ideal conditions for growing ornamental plants, stated that the water potential of the growing medium in the containers should be maintained between −10 and −100 mb. At matric potentials lower than −100 mb, growth of azaleas and other ornamentals may be inhibited because in a coarse medium like pine bark the large pores have drained (Fig. 2) and there is no reserve of water for plant growth held in smaller pores. Thus, matric potential can drop rapidly to very low levels that may stress plant growth as the plant continues to extract water from the pine bark. Unfortunately, the optimum range of matric potentials for sporangium production by *P. cinnamomi* lies within the range recommended by DeBoodt and Verdonck (4) and could lead to enhanced disease development if crops were maintained within this matric potential range.

The inhibition of sporangium production in pine bark at a pH of 3.7 and lower was similar to the inhibition reported by Blaker and MacDonald (2) for U.C. mixes and Canadian sphagnum peat. Because pine bark has a pH of 4.5 to 5.0, its normal pH is not low enough to inhibit sporangium production. Use of an amendment that would lower medium pH below 3.7 might be useful in suppressing disease development if plant growth was not adversely affected.

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