Influence of Container Medium pH on Sporangium Formation, Zoospore Release, and Infection of Rhododendron by *Phytophthora cinnamomi*

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ABSTRACT

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The effect of pH on the asexual life cycle of *Phytophthora cinnamomi* was studied in pH-adjusted U.C. mix (UCM) and UCM extracts. Most sporangia formed on colonized leaf disks in unadjusted UCM with a pH of 5.5–6.0 when maintained at the near-optimum matric potential of −10 mb. Sporangium formation was greatly reduced in UCM or Yolo fine sandy loam (YFSL) soil adjusted to pH 3.8–4.0 with H_2SO_4. The upper pH limit on sporangium formation in K_2CO_3- or $CaCO_3$-adjusted UCM and KOH-adjusted YFSL was 8.5. When colonized leaf disks bearing sporangia were removed from UCM and placed in extracts of known pH, the maximum release and motility of zoospores occurred at pH 5.6. Zoospore release and motility were not affected at pH 3.8 but were almost nil at pH 3.3. Although zoospores were not released at pH 3.3, they did form, encyst, and germinate within sporangia, and mycelial growth from colonized leaf disks was not significantly reduced. Susceptible rhododendrons grown in UCM infested with *P. cinnamomi* at pH 3.4–3.7 or 5.7–6.0 developed severe disease in the high-pH treatments but no detectable disease in the low-pH treatments after 44 days, although viable *P. cinnamomi* was still present in the medium. This work confirms an earlier report that low pH can control Phytophthora root and crown rot of rhododendron and indicates that it may do so by severely reducing sporangium formation, zoospore release, and zoospore motility.

Phytophthora cinnamomi Rands causes a serious root and crown rot of rhododendrons that can result in losses both in nursery and landscape plantings (11,16). This disease occurs primarily on cuttings and 1- to 3-yr-old plants, although older plants may be infected and killed during periods of excessive rainfall in poorly drained soils (16). In 1937, White (25) reported that Phytophthora root and crown rot of rhododendrons could be controlled if soil was adjusted to a pH ≤4.0. Since then, other researchers have demonstrated control of *P. cinnamomi* on avocado (3) and pineapple (21) at pH 3.0 and 3.8, respectively, although use of low pH has limited application in disease control because few agronomic or horticultural plants can tolerate such soil acidity. Even though rhododendrons are considered acid-tolerant plants (16), the pH recommended for cultivation is 4.5–5.5 (5,16) and lower pH values are not used routinely for *Phytophthora* control. Chemical soil drenches have provided limited control of root and crown rot in the nursery but may only temporarily suppress disease. Resistant cultivars likewise appear limited in their ability to resist disease and can be severely infected following brief periods of flooding or drought stress (4). In recent years, composted tree bark media have come into wide use in the nursery industry because they have been found to effectively suppress several important root pathogens (13). However, the mechanism by which *P. cinnamomi* is suppressed in bark media is not fully known. In uncomposted pine bark media, suppression has been attributed partly to effects of low pH (23).

In his early report on the control of Phytophthora root and crown rot of rhododendron, White (25) assumed that control was achieved by inhibition of mycelial growth at low pH. More recently, researchers using solution culure techniques and working with *P. cinnamomi* and other species of *Phytophthora* have reported inhibition of both zoospore motility (2,9,12) and sporangium formation (6,10,21,23,26) at pH values near 5.0 and 3.8, respectively. Although studies in solution culture are revealing and are important tools in understanding the behavior of *Phytophthora* spp., they may not fully simulate the soil environment where complex physical-chemical interactions can greatly influence microorganism activity. Data from pH-adjusted soil extracts and buffer solutions (2,9,12) seem to suggest that zoospores of *Phytophthora* would not be motile in soil at pH 4.0–5.0, where disease is well known to occur. Unfortunately, there are no quantitative studies that describe the influence of pH on the behavior of *P. cinnamomi* in soil or artificial media. The purpose of this study was to determine the effect of container medium pH on sporangium formation, indirect germination, and zoospore motility, and to evaluate the possible role of pH in the control of Phytophthora root and crown rot of rhododendrons.

MATERIALS AND METHODS

Sporangium formation. An isolate of *P. cinnamomi* (ATCC No. 28381) pathogenic to rhododendron was obtained from H. A. J. Hoitink (Ohio Agricultural Research and Development Center, Wooster). Rhododendron leaf disks (8-mm-diam.) were surface-disinfested for 5 min in 0.5% NaOCl and placed for 48 hr on the advancing margin of 2- to 3-day-old colonies of *P. cinnamomi* growing on clear V-8 juice agar (20) at 25 C. After colonization, the leaf disks were removed from the culture plates and buried in U.C. mix (UCM, Delta peat and sand; 1:1, v/v) (19) contained in Büchner funnel tension plates (8). Disks were placed on the surface of a 1-cm layer of UCM and, after covering with an additional 1 cm of medium, saturated by sprinkling with distilled water. The matric potential (ψ_m) of the medium was set at values between 0 and −300 mb by adjusting the height of tension plates above a water reservoir. To determine the optimum ψ_m for sporangium formation, four disks were removed from each ψ_m treatment daily for 8 days. Each disk was rinsed gently with distilled water to remove adhering medium, fixed and stained with acid fuchsin in lactophenol, and viewed at ×100 magnification to count sporangia around the perimeter.

The pH of the UCM was adjusted to

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different values by adding 125 ml of 0.1–3.0% H_2SO_4 or 0.1–3.0% KOH solution to 500 cm^3 of medium and incubating for 3 wk with daily mixing. The pH of the treated UCM was determined at the beginning and end of each experiment by measuring the pH of extracts obtained by vacuum filtration of saturated pastes. Colonized leaf disks were buried in pH-adjusted UCM and maintained at −10 mb ψ_m and 25 C for 4 days, after which four leaf disks were removed from each pH treatment and examined for sporangia. The number of sporangia formed in pH-adjusted media was expressed as a percentage of the number formed in unadjusted UCM, which served as a control. In other experiments, sporangium formation was examined in Yolo fine sandy loam (YFSL), which also was adjusted to various pH levels in the manner described.

Effect of low pH on sporangium discharge and zoospore motility. Colonized leaf disks were buried in unadjusted UCM on tension plates at −10 mb ψ_m to obtain a large, homogeneous population of sporangia. After 4 days, 15 leaf disks bearing sporangia were removed from the medium and placed together in petri dishes containing 10 ml of soil extract to provide optimum conditions for formation and release of zoospores (18). Soil extracts were obtained by vacuum filtration of saturated pastes of unadjusted UCM (pH = 5.6) and two pH-adjusted samples. These samples were adjusted to low pH values (3.8 and 3.3) using H_2SO_4 as described, after which they were leached thoroughly with four volumes of distilled water to remove ions displaced from exchange sites by the acid treatment. Zoospore release and motility in soil extracts was observed over a 26-hr period by examination at ×100 magnification. The relative numbers of motile zoospores released during that period were rated on an arbitrary scale of 0–4 in which 0 represented no motile zoospores and 4 represented the maximum, as observed in extracts made from unadjusted UCM (about 5×10^4 zoospores per milliliter).

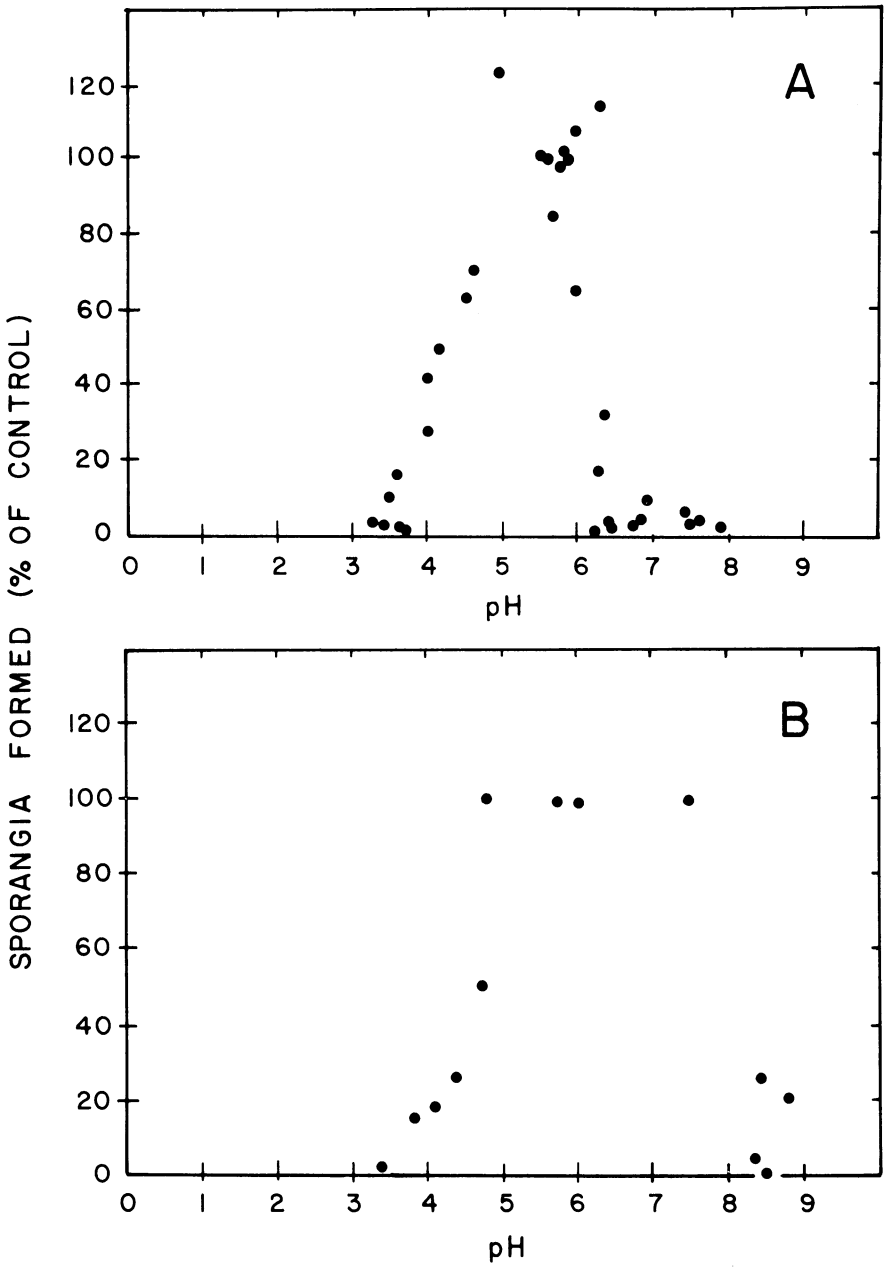

Fig. 1. Effect of pH on sporangium formation by *Phytophthora cinnamomi* on colonized leaf disks in **(A)** U.C. mix (UCM) and **(B)** Yolo fine sandy loam (YFSL) at −10 mb ψ_m. Samples of UCM and YFSL were amended with dilute solutions of H_2SO_4 or KOH to obtain a range of pH. Sporangium formation at various pH values is expressed as a percentage of the number formed (410 ± 68 per disk in UCM, 28 ± 5 per disk in YFSL) in unamended UCM and YFSL (pH 5.6 and 7.4, respectively).

Effect of pH on disease development. One-year-old rooted cuttings of the rhododendron cultivar Boule de Neige, known to be highly susceptible to Phytophthora root and crown rot (14), were obtained from a commercial nursery. The plants were potted in 15-cm-diameter plastic pots containing steam-pasteurized UCM (pH 5.6) and maintained under greenhouse conditions for 10 wk before use. Plants were watered daily with deionized water and fertilized every 8 wk with a commercial fertilizer formulated for ericacious plants (Best, Rhododendron, Azalea, and Camellia Food, Occidental Chemical Co., Houston, TX 77027). After 10 wk, one group of 18 plants was repotted in a U.C.-type medium made from Canadian sphagnum peat (CSP) and quartz sand (1:1, v/v; pH 3.7) and a second group in H_2SO_4-amended UCM (pH 3.4–3.7). A third group of plants was left in the unadjusted UCM for a high-pH (pH 5.6) control.

Low-pH treatments in the H_2SO_4-amended UCM were watered daily with 0.1 or 0.05% H_2SO_4 to maintain a pH of 3.4–3.7. Plants were allowed 2 wk to acclimate to the pH shift, after which half of the plants in each treatment were inoculated with *P. cinnamomi* by carefully unpotting them, mixing 5% (v/v) washed, colonized vermiculite (20) with the remaining medium, and repotting them. At 7 and 21 days after inoculation, all plants were flooded with distilled water by placing the pots in watertight containers and adding enough water to maintain 0.5 cm on the medium surface for 24 hr. Otherwise, plants were irrigated as described and allowed to drain freely. Root and crown rot development were rated on a subjective scale of 0–5 in which 0 = no visible symptoms, 1 = youngest flush of leaves slightly wilted, 2 = both youngest and older leaves wilted, 3 = leaves wilted and stem canker <2 cm above the soil line, 4 = plant severely wilted and stem canker ≥2 cm above soil line, and 5 = dead plant.

The experiment was terminated 44 days after inoculation and root and crown

tissues from all plants with symptoms were cultured on a modified $P_{10}VP$ medium (20) to confirm the presence of *P. cinnamomi*. To detect pathogen survival in soil, pieces of vermiculite inoculum were recovered and cultured on $P_{10}VP$ medium.

RESULTS

Sporangium formation. Sporangium formation reached its peak 3–5 days after colonized leaf disks were buried in unadjusted UCM (pH 5.6) held at constant ψ_m values of 0 and −10 mb. Numbers of sporangia decreased sharply with decreasing ψ_m so that only 10% of the number formed at $\psi_m = 0$ were formed at −300 mb ψ_m. Because large populations of sporangia were needed for meaningful comparisons of formation or indirect germination, all subsequent experiments used sporangia formed in media at −10 mb ψ_m.

When colonized leaf disks were buried in pH-adjusted UCM, the pH change from the start to the end of the experiments was consistently ≤0.2 pH units. Relative to the number of sporangia (410 ± 68 per disk) formed at pH 5.6, sporangium formation decreased sharply as pH approached 4 and very few formed at pH 3.5–4.0 (Fig. 1A). In CSP, which had a naturally low pH of 3.7, few sporangia formed but large amounts of mycelium grew from the colonized leaf disks, indicating that *P. cinnamomi* was still capable of vegetative growth at low pH.

The abrupt decline in sporangium formation when UCM was adjusted to pH values of 6.5 or higher with KOH (Fig. 1A) was unexpected but may have resulted from solubilization of humus under alkaline conditions (evidenced by a black discoloration of water below the sintered glass plate in the Büchner funnels) and the release of toxic materials (22). When this high-organic-matter medium was adjusted to pH 6.5–6.7 with a 30-mM solution of $CaCO_3$ or K_2CO_3, no solubilization of humus was evident and sporangium formation was equivalent to that of unadjusted UCM at pH 5.6. To examine sporangium formation at higher pH, YFSL (which contains only about 3% organic matter [24]) was adjusted to various pH values as described, using H_2SO_4 and KOH. Although the absolute numbers of sporangia formed in YFSL (28 ± 5 per disk) were much less than in UCM, there were similar low-pH limits on formation (Fig. 1B) and a much broader range for formation at high pH.

Effect of pH on sporangium discharge and zoospore motility. When leaf disks bearing sporangia were placed in soil extract made from unadjusted UCM, maximum discharge occurred after 1–2 hr and the number of motile zoospores remained high for 10 hr (Fig. 2). During the next 12 hr, there was a gradual decrease in the number of motile zoospores until a relatively constant, low number remained, coinciding with the formation and discharge of fresh sporangia. When sporangia were placed in extracts made from pH-adjusted UCM, sporangium discharge and zoospore motility at pH 3.8 was not significantly reduced relative to controls at pH 5.6 (Fig. 2) but was severely restricted at pH 3.3. The abrupt reduction in zoospore release at pH 3.3 probably resulted from a dysfunction of the discharge process. In the first few hours after sporangia were placed in the extract, zoospores formed within the sporangia, but were not released; instead, they encysted within the sporangia and germinated by germ tubes, which subsequently penetrated the sporangial wall (Fig. 3).

Effect of pH on root and crown rot of rhododendron. The differences in disease severity between plants grown 44 days in

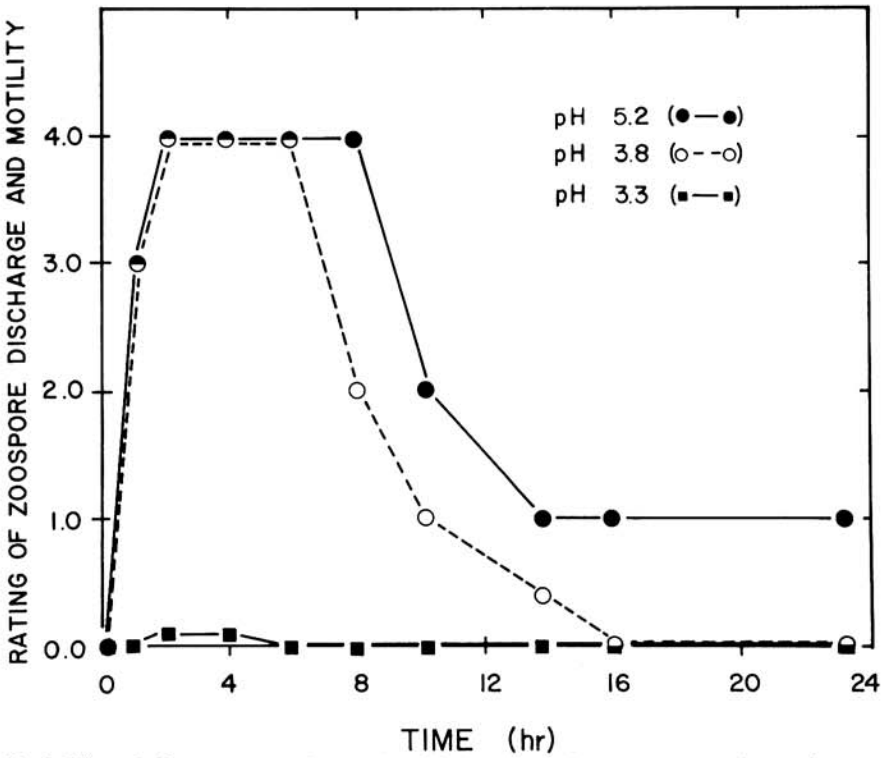

Fig. 2. Effect of pH on zoospore release and motility by *Phytophthora cinnamomi*. Sporangia were formed on colonized leaf disks in U.C. mix (UCM, pH 5.6) at −10 mb ψ_m and placed in water extracts of UCM previously adjusted to pH 3.8 and 3.3 with H_2SO_4. Zoospore release and motility was rated on a scale of 0–4 in which 0 = no motile zoospores and 4 = the maximum observed (about 5×10^4 zoospores/ml).

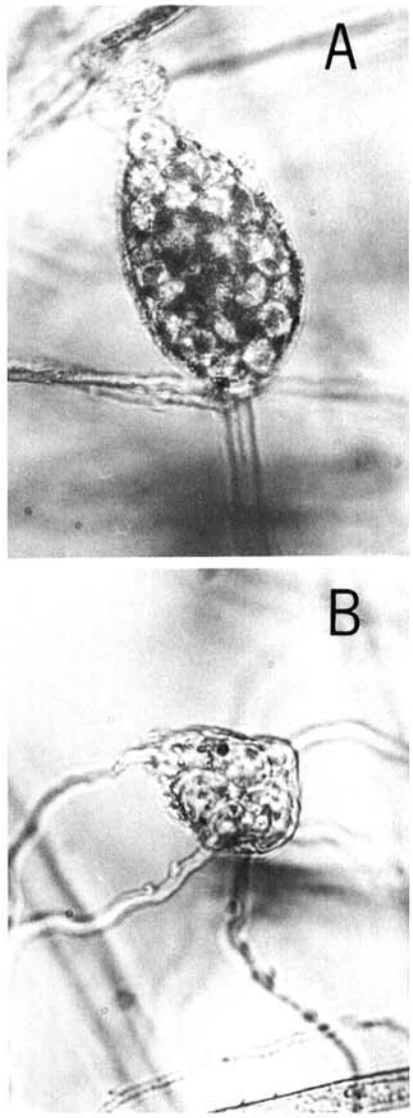

Fig. 3. Zoospore encystment and germination within sporangia of *P. cinnamomi* at low pH. Sporangia were formed on leaf disks in U.C. mix (UCM, pH 5.6) at −10 mb ψ_m and placed in a water extract of pH-adjusted UCM (pH 3.3). **(A)** Zoospore encystment within sporangia 1–2 hr after sporangium placement in UCM extract. **(B)** Germ tube penetration of the sporangium wall 2–3 hr after placement in UCM extract.

P. cinnamomi-infested media (Fig. 4) were significant ($P = 0.01$), according to Student's t test of independent means. Plants grown in UCM at pH 5.8 had an average symptom rating of 3.6, whereas those grown in UCM at pH 3.4–3.7 or CSP (pH 3.5, *unpublished*), showed no symptoms of disease. Although the plants showed no symptoms of disease, *P. cinnamomi* survived in the soil and was easily cultured from recovered pieces of vermiculite inoculum. Uninoculated plants in the low-pH treatments showed no symptoms of nutrient deficiency, toxicity, or root injury throughout the experimental period.

DISCUSSION

Our greenhouse experiments (Fig. 4) confirmed the earlier report of White (25) that pH ≤4 can successfully control root and crown rot of rhododendron caused by *P. cinnamomi*. Our results, however, did not support his hypothesis (25) that control was achieved by inhibition of mycelial growth at low pH. We found that compared with other stages in the life cycle of *P. cinnamomi*, mycelial growth was relatively tolerant of low pH. Hyphae from colonized leaf disks in UCM at pH 3.3–3.5 grew vigorously and germ tube growth from zoospore cysts was not inhibited at pH as low as 3.3 (Fig. 3). Our results showed instead that sporangium formation was the process most affected by low pH; it was greatly reduced at pH 4 and almost nonexistent at pH 3.4 (Fig. 1A, B). In this respect, our results agreed with earlier reports (6,10,21,23) based on solution-culture methods. The mechanism for low-pH inhibition of sporangium formation by *Phytophthora* spp. is not known. Certainly in the case of *P. cinnamomi*, low pH could act indirectly by inhibiting bacteria that stimulate sporangium formation (26) or by altering the synthesis or chemical nature of "sporangium-inducing compounds" (1). In addition, at pH ≤4, hydrogen ions are reported to affect membrane permeability and other physiological processes in fungi (7,17) and could interfere directly with sporangium formation.

The inability of sporangia to form in large numbers at pH ≤4 made it impossible to quantitatively compare zoospore release and motility in undisturbed samples of UCM over a wide range of pH. For that reason, colonized leaf disks were placed in media at near-optimum ψ_m and pH to promote development of large, homogeneous populations of sporangia. These sporangia then were placed in soil extracts of known pH to observe the formation and release of zoospores. With this method, we found that zoospores of *P. cinnamomi* were much less sensitive to low pH than earlier reports (2,9,12) suggested. The pH limit of 3.3 (Fig. 2) contrasts sharply with reports that describe loss of motility and even disintegration of zoospores at pH 4.4–6.0 (2,9,12). This difference may be due to inherent differences between species or differences in methodology.

We used a method whereby zoospores were formed and released directly in the solutions of desired pH, whereas earlier workers (2,9,12) transferred swimming zoospores from a solution of one pH to another. A sudden shift in pH that typically would not occur in soil could injure motile zoospores and shorten their period of motility. We felt that such effects would be minimized by allowing zoospores to differentiate and release directly into the solutions. Although we cannot exclude the possibility that sporangia were injured by shifts in pH, our limited observations with undisturbed sporangia formed in UCM at low pH showed that they responded similarly. Our experiments thus showed that zoospore release and motility were no more sensitive to pH than sporangium formation and may actually be somewhat less sensitive. It was unclear whether the inability of sporangia to release zoospores at pH 3.3 (Fig. 3) resulted from a disruption of the release mechanism or a rapid encystment of zoospores within the sporangium, but a similar effect has been observed in *P. capsici* at pH 4.8–5.0 (15).

Sporangium formation and zoospore release is important for the epidemic spread of Phytophthora root rot diseases (26). The impairment of sporangium formation at pH ≤4 and the resulting lack of zoospore inoculum probably explains the control of Phytophthora root and crown rot of rhododendron reported by White (25) and confirmed in our greenhouse experiment (Fig. 4). Although disease from mycelial infection could still occur, this is considered less important in the epidemiology of Phytophthora root rots (26), and certainly in the time frame of our greenhouse experiment, it did not result in any detectable disease symptoms.

Utility of low pH as a disease control for Phytophthora root rot of rhododendron, as originally suggested by White (25), depends on the ability of plants to tolerate long exposure to low pH and remain horticulturally acceptable. Rhododendrons have been reported to grow well in soils with a pH as low as 2.9 (25), and we found *Rhododendron macrophyllum* specimens in native stands along the northern coast of California growing well in soil at pH 3.4. Therefore, while the ability of rhododendrons to grow normally for prolonged periods at low pH might vary with the species or hybrid grown, chemical characteristics of the container medium or soil, and the presence or absence of mycorrhizae it does appear that in some cases they can tolerate a pH low enough to severely restrict the processes of sporangium

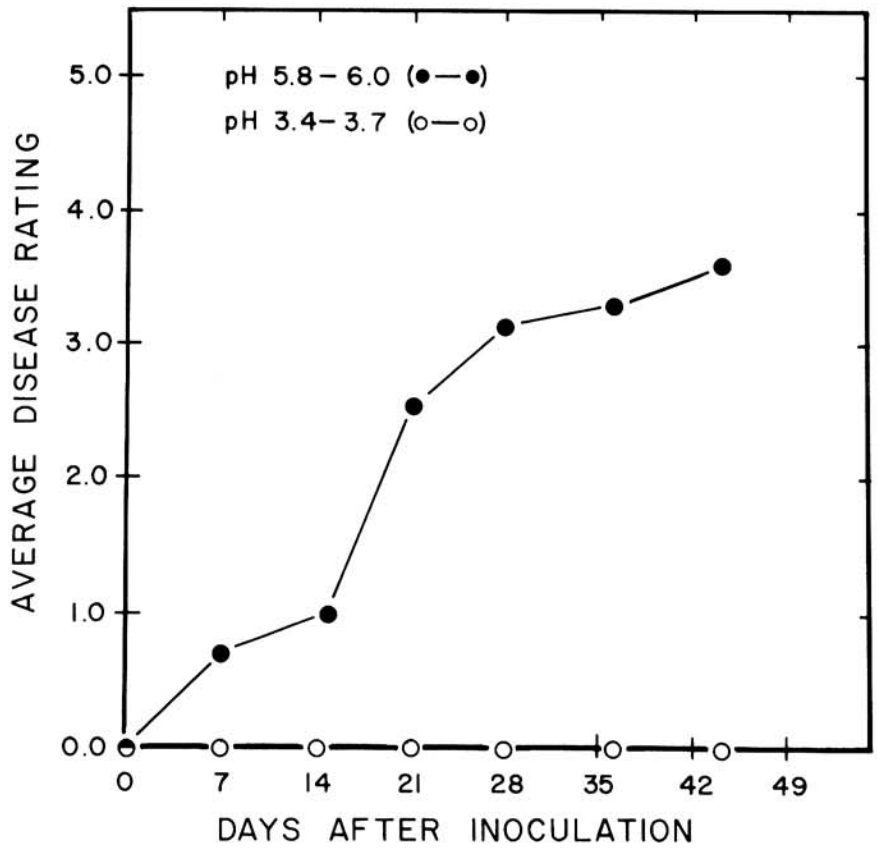

Fig. 4. Development of Phytophthora root and crown rot on the rhododendron cultivar Boule de Neige in U.C. mix (UCM, pH 5.8–6.0) and pH-adjusted UCM (pH 3.4–3.7). Root and crown rot development was rated on a scale of 0–5 in which 0 = no visible symptoms and 5 = dead plant. After 44 days, means based on seven replicates were analyzed using Student's t test and were significantly different at $P = 0.01$.

formation, zoospore release, and zoospore motility by *P. cinnamomi*.

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