Influence of Simulated Acidic Rain on *Phytophthora cinnamomi* and Phytophthora Root Rot of Blue Lupine

S. R. Shafer, R. I. Bruck, and A. S. Heagle

Research plant pathologist, USDA-ARS, and assistant professor, Departments of Plant Pathology and Soil Science; associate professor, Departments of Plant Pathology and Forestry; and research plant pathologist, USDA-ARS, and professor, Departments of Plant Pathology and Crop Science; North Carolina State University, Raleigh 27695.

Use of trade names in this publication does not imply endorsements by the North Carolina Agricultural Research Service or the U.S. Department of Agriculture of the products named or criticism of similar ones not mentioned.

This study was supported in part by a sub-contract from the EPA-NCSU Acid Precipitation Program, EPA Cooperative Agreement CR806912 between the U.S. Environmental Protection Agency and North Carolina State University. It has not been subjected to EPA's required peer and policy review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.

Technical assistance by Rita Neill, Robert Philbeck, and Virginia Lesser is appreciated.

Paper No. 9357 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695.

Accepted for publication 5 April 1985.

ABSTRACT

Shafer, S. R., Bruck, R. I., and Heagle, A. S. 1985. Influence of simulated acidic rain on *Phytophthora cinnamomi* and Phytophthora root rot of blue lupine. Phytopathology 75:996-1003.

Effects of acid deposition on *Phytophthora cinnamomi* were examined with simulated rain solutions (pH 5.6, 4.0, 3.2, or 2.4), Lakeland sand, and blue lupine seedlings. Infected radicles were buried in soil that was subsequently saturated for 15 min with solutions and then drained to −3.0 to −2.3 kPa. The number of sporangia formed on radicles decreased linearly with increasing solution acidity (47% fewer sporangia formed in soil treated with pH 2.4 solution than with pH 5.6 solution). Release of zoospores from sporangia incubated in soil extracts was unaffected by the acidity of solutions used to prepare extracts. Seedlings grown in soil, inoculated with zoospores, and exposed to simulated rainfall (2.4 cm, 1 hr) at pH 2.4 had 44% fewer infection sites on roots than did seedlings exposed to rain at pH 5.6. Effects of rain acidity on onset of disease symptoms and rate of disease increase were not consistent among seedlings maintained for 28 days in infested soil and repeatedly exposed to simulated rains. Although simulated acidic rain significantly affects epidemiologically important steps in the life cycle of *P. cinnamomi*, gradual deposition of H^+ in rain probably has little short-term effect on Phytophthora root rot if plants remain exposed to inoculum.

Additional key words: acid deposition, *Lupinus angustifolius*, soil microorganisms.

Increased acidity of precipitation during recent decades has prompted interest in the potential effects of acidic rain on ecosystems (e.g., 13,14,29,48). Effects of simulated rains at various acidity levels on vegetation and soil microorganisms have been described (e.g., 16,24,47). However, few studies have addressed the impact of increased acidity in rain on host-parasite interactions. Results of studies with simulated acidic rains and foliar and stem pathogens have varied with the host-parasite combination and patterns of exposures (6,36,44). Severities of the diseases that have been examined were quantified only once, and possible differences in the rates of disease increase attributable to rain acidity have not been considered.

Information on effects of acid deposition on root-infecting organisms is limited. Investigations have demonstrated effects of simulated acidic rain on: nodulation of leguminous plants by *Rhizobium* spp. (11,35,45); chlamydospore production by an endomycorrhizal fungus associated with soybean [*Glycine max* (L.) Merr.] (8); formation of ectomycorrhizae on loblolly pine seedlings (*Pinus taeda* L.) (42); severity of root knot caused by *Meloidogyne hapla* Chitwood on kidney bean (*Phaseolus vulgaris* L.) (44); and mortality of sugar maple (*Acer saccharum* Marsh.) seedlings caused by a root rot (possibly induced by a bacterium) (37). To date, effects on soilborne pathogenic fungi are undescribed.

Soil chemical factors influence diseases caused by soilborne *Phytophthora* spp., and manipulation of the soil chemical environment could affect efforts to manage these diseases (39). This sensitivity of *Phytophthora* spp. to soil chemistry and the availability of soil water (15) suggests that these fungi are sensitive to acid deposition. Various stages in the life cycles of *Phytophthora* spp. exhibit varying sensitivities to acid or alkaline conditions in vitro (9,19,32,50) and in soil or soil mixes (3,7,34,46). However, such studies provide limited insight into possible responses of these fungi to atmospheric deposition. Pegg (34) evaluated elemental S for soil acidification in control of root and heart rot caused in pineapple by *Phytophthora cinnamomi* Rands. However, the smallest quantity of S added to plots in those experiments (600 $kg \cdot ha^{-1}$) vastly exceeded the S deposition in precipitation (<50 $kg \cdot ha^{-1}$) for all of 1980 in areas that received the most acidic precipitation in the United States (30). The 0.1% H_2SO_4 used to adjust the acidity of container media to control *P. cinnamomi* (3,7) also differs in composition and concentration from the very dilute mixture of acids in precipitation (30). Results of several experiments (23,47) show that effects of accelerated H^+ loads on microbiological properties of soil can greatly differ from effects of equivalent H^+ loads applied gradually. Other studies (4) indicate that the anionic composition of rain affects microbial responses. Hence, studies that involve rapid shifts in soil acidity induced by large additions of concentrated acid, lime, or elemental S may be of limited value for assessment of effects of acidic precipitation on *Phytophthora* spp. in soil. In vitro investigations on effects of chemical factors on these fungi (such as those reviewed by Schmitthenner and Canaday [39]) are also of limited value to assessment of acidic rain effects due to difficulties in interpretation with respect to interactions among rainfall chemistry, soil properties, plant roots, and the fungus.

Acid deposition occurs in some areas coincidental to the geographic range of *P. cinnamomi* (30,52), and this species was selected for detailed evaluation of effects of simulated acidic rain on a soilborne pathogenic fungus. Results of preliminary experiments

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

(40,41) demonstrated that, although pathogenic activities of *P. cinnamomi* were only partially suppressed, a small number of exposures of infested soil to simulated acidic rain affected population densities in infested soil and inhibited infection of plant roots.

Experiments described here were performed to evaluate the influence of simulated acidic rain on asexual reproduction of *P. cinnamomi*, infection of roots by zoospores of this fungus, and the rate of disease progress among host plants in infested soil.

MATERIALS AND METHODS

Characteristics of soil and culture of *P. cinnamomi*. All experiments were performed with Lakeland sand (siliceous thermic, coated; typic quartzipsamments; analyses of field sample: mineral fraction 91% sand, 7% silt, 2% clay; organic matter, 0.6%; cation exchange capacity, 4.7 meq/100 cc; base saturation, 66%; pH 5.9; major exchangeable cations [meq/100 cc]: acidity, 1.6; Ca^{++}, 2.2; K^{+}, 0.2; Mg^{++}, 0.7)] collected from the Claridge State Nursery near Goldsboro, NC. Soil was steamed for 2 hr at 80 C and stored in covered polyethylene cans.

P. cinnamomi was isolated on pimaricin-chloramphenicol-hymexazol (PCH) agar (43) from roots of a two-year-old Fraser fir [*Abies fraseri* (Pursh) Poir.] seedling collected from the Linville River Nursery at Crossnore, NC. The isolate was maintained on blue lupine plants (*Lupinus angustifolius* L. 'Tiftblue' grown from seeds provided by H. D. Wells, Coastal Plain Experimental Station, Tifton, GA) grown in a greenhouse in pots containing Lakeland sand. The fungus was reisolated from diseased lupine plants to initiate each experiment.

Preparation of simulated acidic rain. Rain solutions were prepared with deionized water in glass flasks or polyethylene tanks. Background ion concentrations characteristic of rain in the eastern United States (8,13) added to all solutions were (μeq/l): Ca^{++}, 21.0; K^{+}, 2.2; Mg^{++}, 9.8; Na^{+}, 5.1; NH_4^{+}, 7.8; Cl^{-}, 12.0; NO_3^{-}, 12.0; and $SO_4^{=}$, 22.0. Rain solutions prepared in this manner were not amended (pH 5.6 $\pm$ 0.2) or were adjusted to pH 4.0, 3.2, or 2.4 (all $\pm$0.1) with a mixture of H_2SO_4 and HNO_3 (1N: 70 meq $SO_4^{=}$:30 meq NO_3^{-}). Rain acidity was determined with a pH meter. These four solutions represented the treatments in all experiments.

Effects of simulated rain solutions on sporangium production. Lupine seeds were planted in vermiculite moistened with deionized water. After 48 hr, the radicle tip (1.0 cm) of each seedling was excised and incubated in a suspension of zoospores (22) in deionized water (30,000 zoospores per milliliter) for 12 hr at 20 C in the dark. Radicles were then rinsed in deionized water, and each was placed between two 1.5 $\times$ 1.5-cm squares of nylon mesh (100-μm openings). The edges of the mesh squares were melted together with a heated dissecting needle. Lakeland sand (dried for 48 hr at 105 C) was wetted to 3% moisture (w/w) with a 2% extract of Lakeland sand in which noninfected lupines had grown for 4–6 wk. Moistening with non-sterile soil extract was intended to re-introduce microflora to the oven-dried soil. Moistened soil was incubated for 24 hr at 20 C. Moistened soil (15 $\pm$ 0.01 g) was then placed on preweighed coarse-grade filter paper in the detachable top of a tared polypropylene Büchner funnel (5.5-cm diameter). Three radicles in individual nylon mesh enclosures were weighed, placed on the soil, and covered with an additional 15 $\pm$0.01 g of soil. Radicles were thus in the center of a layer of soil approximately 1 cm thick. The soil was then saturated from beneath with rain solution (five funnels per treatment) for 15 min. The top of each funnel was replaced onto the stem, and excess solution was removed by suction through the stem until the moisture content of the soil was 12–15% (−3.0 to −2.3 kPa). Soil moisture content in each funnel was determined by the difference in weights between the funnel containing filter paper, soil, and radicles before saturation and after removal of excess solution. (Moisture content was verified at the end of the experiment by gravimetric determination of moisture in a subsample from each funnel. This procedure demonstrated that soil water content in the funnels was consistently between 12 and 15%.) Each funnel top was then placed in a 400-ml beaker, which was sealed with Parafilm® and incubated for 48 hr at 20 C in the dark. After incubation, mesh enclosures containing radicles were removed from the soil, rinsed by gentle agitation in 0.7% aqueous Tween 20, and stained in 0.1% aqueous crystal violet for 1 min. Sporangia visible at $\times$100 in all focal planes at the periphery of each radicle were counted, and the mean sporangium count for the three radicles from each funnel was calculated. The test was performed twice. Data from both tests were combined into a single data set and were analyzed by analysis of variance (ANOVA).

Acidity conditions in the soil during the initial saturation period were determined with a pH meter. A slurry of soil in each rain solution (30 g each) was prepared in a paper cup (six replicates). The slurry was stirred vigorously for 15 sec and was allowed to settle for 15 min prior to measurement. Acidity conditions during the incubation period were determined from soil prepared in funnels as described previously except that radicles in the nylon mesh enclosures were omitted. The pH value of the soil from funnels from each treatment was determined with deionized water 0.5, 24, and 48 hr after adjustment of soil moisture content to 12–15%.

Effect of simulated rain solutions on zoospore release. Cultures of *P. cinnamomi* for production of zoospores were prepared as described previously (22) (one cornmeal agar culture plug per plate). Formation of sporangia was induced over a 7-day period in a 2% extract of soil collected from the Linville River Nursery. Three mycelial mats were transferred to each of twelve 10-cm-diameter petri plates. Extracts of Lakeland sand were prepared with the four rain solutions as follows. Equal quantities (300 g) of air-dried soil and rain solution were combined in 1-L flasks. The slurries were placed on a rotary shaker for 15 min at 180 rpm and were allowed to settle for 15 min. Supernatants were centrifuged for 15 min at 20,000 *g*, and the supernatants after centrifugation were decanted into glass flasks. Small quantities of floating organic matter were removed by aspiration. These extracts simulated the soil solution for Lakeland sand exposed to rain at each acidity. Rinsed mycelial mats were covered with 20 ml ($\pm$0.01 ml) of extract (three replicate plates per treatment), and zoospore release was induced by incubation for 1 hr at 4 C. One hour after cultures were returned to room temperature (approximately 22 C), mycelial mats were removed from each plate. Five 10-μl drops of zoospore suspension from each plate were placed on solidified 1.5% agar in petri plates. After the drops had dried, each spot was stained with 0.1% aqueous crystal violet. The zoospore count in each plate was expressed as the mean of the counts from the five drops. The test was performed three times. Data were combined into a single set and subjected to ANOVA.

Effect of simulated rain on root infection by zoospores. Lupine seeds were planted 1 cm deep in Lakeland sand in plastic pots (10-cm diameter, 10 seeds per pot). The pots were placed on a greenhouse bench, and seedlings received deionized water daily. One week after planting, abnormally small seedlings were removed, and 40 pots (four to eight seedlings per pot) were selected. Drainage holes of the pots were plugged, and the soil was saturated with deionized water. A suspension of 30,000 zoospores in 10 ml deionized water was pipetted onto the surface water in each pot. Fifteen minutes later, the drainage holes were unplugged and water drainage drew the inoculum into the soil. The pots of seedlings were exposed 15 min later to simulated rain in the greenhouse. The four rain solutions were simultaneously pumped at 83 kPa from polyethylene tanks through independent delivery systems (constructed of epoxy, neoprene, nylon, polyallomer, polyethylene, polyvinyl chloride, stainless steel, and Tygon® plastic components). Each solution was applied from a stainless steel solid-cone nozzle (Fulljet 1/8 G2.8W, Spraying Systems Co., Wheaton, IL; median volume diameter = 1,160 μm) suspended 1.2 m above greenhouse benches. Pots of seedlings were exposed to a single 1-hr application of simulated rain (10 pots per acidity level). Deposition was monitored with beakers placed among the pots.

Roots were removed from each pot 48 hr after exposure to simulated rain, washed on a sieve (2-mm openings) under running tap water, placed in deionized water amended with 10 μg/ml of penicillin and 5 μg/ml of pimaricin (Delvocid Instant 50%; Enzyme

Development Corp., New York, NY), and assayed for *P. cinnamomi* as follows. Each root system was blotted dry, air-dried for 10 min, and weighed (to nearest 0.01 g). The entire root system was spread onto PCH agar in petri plates and pressed into the medium. Plates were incubated for 48 hr at 20 C in the dark. The number of colonies of *P. cinnamomi* that developed was expressed as colonies per gram (CPG) of root fresh weight, and a mean CPG value was calculated for each pot. The test was performed three times. Data were combined and subjected to ANOVA.

Effect of simulated rain on Phytophthora root rot of lupine. Lupine seedlings in pots of Lakeland sand were inoculated with zoospores as described above. Plants were maintained in a greenhouse and watered with deionized water. After inoculum density had increased 4–6 wk, the infested soil was diluted with steamed soil until the inoculum density was approximately one to two propagules per gram (ppg) of dry soil as determined with PCH agar (21,43). Infested soil was placed (9 cm deep) on a single layer of cheesecloth in each of 16 plastic containers (25 × 17 × 11 cm deep, with six 1-cm-diameter drainage holes in the bottom).

Lupine seeds were sown in vermiculite moistened with deionized water. Three days later, 24 seedlings were transplanted into each container of infested soil such that the cotyledons were 5 mm beneath the soil surface. Plants and soil were exposed for 30 min immediately after transplanting to simulated rain (four containers per acidity level). Deposition of rain was monitored with beakers placed among containers. Seedlings that did not fully emerge were removed from each container three days after transplanting. Each container of plants and soil was exposed to eight additional applications of simulated rain (30 min each) over 28 days. Except during exposure to rain (a total of 4.5 hr over 28 days), containers were arranged in a Latin square on a bench in the same greenhouse as the simulator. Each container occupied the same bench position throughout the experiment. No fertilizer or water in addition to simulated rain was applied during the experiment.

Plants in each container were inspected daily for 28 days for symptoms of Phytophthora root rot (26,46). The number of plants with symptoms was expressed as a proportion of the total number of plants in each container. Disease progress in each container was expressed as the proportion of plants with symptoms, Y, (arithmetic data) or as Y transformed as $\log_e[1/(1-Y)]$ (logarithmic data) (51) as a function of time (days since transplanting and the first exposure to rain). For $Y = 0.00$ or $Y = 1.00$ in any container, a value of 0.01 or 0.99 was substituted, respectively. Values of Y ($n = 16$) on each day were subjected to ANOVA. All days were identified for which significant ($P < 0.10$) differences occurred for mean values of Y associated with different acidity levels. Logarithmic data for each acidity level were regressed on days within a defined time interval to examine the rate of disease increase (slope ± standard error) associated with each acidity level. The first day selected for this interval was the first day on which at least one seedling exhibited symptoms among all seedlings exposed to rain at each acidity level (i.e., mean $Y > 0.01$ for all acidity levels). The last day selected for the interval was the day after the last day on which significant differences in mean Y occurred among acidity levels.

Twenty-nine days after transplanting, three soil samples were collected from the center and two diagonally opposite corners of each container. Each sample consisted of three bulked soil cores (each 1.5 cm diameter × 9 cm deep) and was quantitatively assayed for *P. cinnamomi* on PCH agar. The inoculum density in each container was estimated by the mean propagules per gram value for the three samples. An additional soil core (7.5 cm diameter × 9 cm deep) removed from each container was air-dried and chemically analyzed by the North Carolina Department of Agriculture (Agronomic Division, Blue Ridge Road, Raleigh).

The test was performed three times. Initial inoculum densities for the three tests were 1.7, 1.4, and 0.9 ppg. The number of plants per container was 15–24 (mean = 21) in the first test, 20–24 (mean = 23) in the second test, and 19–24 (mean = 22) in the third test. In the third test, simulated rain was applied on 12 days to compensate for increasing drying of the soil due to excessive heat in the greenhouse.

RESULTS

Effect of simulated rain solutions on sporangium production. Numbers of sporangia on lupine radicle tips decreased linearly with increasing rain acidity (Tables 1 and 2). An ANOVA (Table 2) indicated that significantly more sporangia were produced in the second test (185 versus 304, averaged across all four acidity levels for the two respective tests). However, the test × pH interaction was not significant and indicated a consistent relative effect of solution acidity on sporangium formation in the independent tests. Sporangium production on radicles buried in soil adjusted to −3.0 to −2.3 kPa moisture with rain at pH 2.4 averaged 47% less than production on radicles buried in soil moistened with rain at pH 5.6 (163 versus 306, respectively).

Soil pH values varied little despite a wide range of solution acidities. Only simulated rain solution at pH 2.4 had a pronounced effect on soil pH values throughout the experiments (Fig. 1). Within 0.5 hr after moistening soil with simulated rain solution at

TABLE 1. Effect of simulated acidic rain on *Phytophthora cinnamomi*: sporangium production, zoospore release, and root infection by zoospores

Parameter	Test	Rain acidity (pH)[a] 5.6	4.0	3.2	2.4
Sporangia per radicle[b]	1	230	221	166	122
	2	382	342	290	204
	$\bar{X}$	306	282	228	163
Zoospores per 10-μl drop[c]	1	340	333	270	329
	2	109	111	112	121
	3	208	229	188	261
	$\bar{X}$	219	224	190	237
Colonies per gram of root tissue[d]	1	13.8	13.7	6.7	7.7
	2	17.3	10.3	15.3	5.9
	3	14.5	15.2	16.7	12.0
	$\bar{X}$	15.2	13.1	12.9	8.5

[a] Adjusted with $H_2SO_4 + HNO_3$.
[b] Mean number of sporangia on the excised terminal centimeter of an infected radicle of a blue lupine seedling after incubation for 48 hr in Lakeland sand moistened to approximately −2.6 kPa with simulated rain solution. The tabulated value associated with each rain acidity within each test is the mean for five funnels; the value for each funnel was the mean for three radicles.
[c] Mean number of zoospores in a 10-μl drop sampled from 20 ml of extract of Lakeland sand (prepared with simulated rain solution) into which zoospores had been released from sporangia on three mycelial mats. The tabulated value associated with each rain acidity within each test is the mean for three plates; the value for each plate was the mean for five drops.
[d] Number of colonies of *Phytophthora cinnamomi* formed on selective medium from roots of lupine seedlings grown in Lakeland sand, inoculated with zoospores, and exposed to simulated rain (1 hr, 2.4 cm deposition). The tabulated value associated with each rain acidity within each test is the mean for 10 pots; the value for each pot was the mean for from four to eight seedling root systems.

TABLE 2. Analysis of variance for effect of simulated acidic rain on sporangium production by *Phytophthora cinnamomi*[a]

Source of variation	Degrees of freedom	Sum of squares	Mean square	F[b]
Corrected total	39	431,726.0		
Tests	1	143,520.4	143,520.4	68.83[c]**
pH	3	120,248.6	40,082.9	19.22[c]*
linear	1	104,233.1	104,233.1	50.00[c]**
non-linear	2	16,015.5	8,007.8	3.84[c]
Test × pH	3	6,255.0	2,085.0	0.41[d]
Funnels (test × pH)	32	161,702.0	5,053.2	

[a] Data for experiment summarized as "Sporangia per radicle" in Table 1.
[b] Asterisks * or ** indicate that the probability of obtaining a larger F value is less than 0.05 or 0.01, respectively.
[c] The mean square for the test × pH term used as error mean square.
[d] The mean square for the funnels (test × pH) term used as error mean square.

pH 5.6, 4.0, or 3.2, soil was characterized by pH 5.5–6.0, while soil moistened with solution at pH 2.4 was at pH 4.8. After 48 hr of incubation, soil moistened with the three least-acidic solutions was at approximately pH 6.0, while soil treated with pH 2.4 solution was at pH 5.4.

Effect of simulated rain solutions on zoospore release. The number of zoospores per 10-μl drop (averaged across all three tests) was 219, 224, 190, or 237 from mycelial mats incubated in soil extracts at pH 6.1, 6.1, 5.9, or 4.7 prepared with simulated rain solutions at pH 5.6, 4.0, 3.2, or 2.4, respectively. The ANOVA for the entire data set (*unpublished*) indicated no significant effect attributable to the acidity of rain solutions used for extract preparation.

Effect of simulated rain on root infection by zoospores. Average deposition of simulated rain during 1-hr applications was 2.4 cm. Mean CPG values (averaged across three tests) for seedling root systems generally declined with increasing rain acidity (Table 1). Values of CPG associated with an exposure of plants and soil to rain at pH 2.4 averaged 44% less than those associated with rain at pH 5.6 (8.5 versus 15.2, respectively). Despite this trend, the ANOVA for the entire data set indicated no significant differences among treatment means. However, single-degree-of-freedom contrasts suggested that, although a significant test × acidity interaction occurred, CPG values associated with the most acidic rain were significantly less than those associated with other treatments (Table 3).

Effect of simulated rain on Phytophthora root rot of lupine. Mean deposition of simulated rain was 1.3 cm per application in all three tests. Foliage of plants exposed to rains at pH 2.4 exhibited bifacial, tan, necrotic lesions. Initially, lesions were 1 mm in diameter or less but these enlarged and coalesced with other lesions after additional exposures. Occasionally, leaves located distally from lesions on petioles wilted and died. After nine exposures over 28 days, approximately 15–25% of the foliar area of these plants was necrotic. Foliar injury was not evident on plants exposed to rains at other acidities.

In the first test, symptoms of Phytophthora root rot appeared last among plants exposed to rain at pH 2.4 (Fig. 2A). However, once symptoms began to develop among those plants, the apparent rate of disease increase during the next 3 days was greater than that for any other treatment (Table 4). By day 15, the incidence of disease symptoms did not differ among plants regardless of the acidity of simulated rain.

In the second test, symptoms of root rot again appeared last among plants exposed to rain at pH 2.4 (Fig. 2B). The rate of disease increase among plants exposed to rains at pH 2.4 or 3.2 was less than that for plants exposed to rains at pH 5.6 or 4.0 (Table 4). By day 24, the incidence of symptoms was unrelated to rain acidity.

In the third test, symptoms of root rot appeared last among plants exposed to rains at pH 3.2 (Fig. 2C). The incidence of symptoms increased slowly. After 28 days, less than half of the plants in all treatments exhibited symptoms. The rate of disease increase was slightly greater among plants exposed to rains at pH 3.2 than for plants exposed to other treatments (Table 4). By day 27, the incidence of symptoms was again unrelated to rain acidity.

Propagule densities of *P. cinnamomi* at the end of each test did not vary significantly with acidity of rain, nor was any general trend evident. Soil pH values, Mg^{++} concentrations, and base saturation decreased with increased rain acidity. Exchangeable acidity and $SO_4^{=}$ concentrations increased (Table 5). No consistent trends occurred for soil cation exchange capacity or concentrations of K^+, Ca^{++}, Na^+, Mn^{++}, Zn^{++}, Cu^{++}, NH_4^+, NO_3^-, or humic material (data not

TABLE 3. Analysis of variance for effect of simulated acidic rain on infection of roots of blue lupine seedlings by zoospores of *Phytophthora cinnamomi*[a]

Source of variation	Degrees of freedom	Sum of squares	Mean square	F[b]
Corrected total	119	6,240.70		
Tests	2	348.06	174.03	1.64[c]
pH	3	705.55	235.18	2.22[c]
2.4 versus others	1	605.28	605.28	5.72[c](*)
2.4 versus 5.6	1	668.47	668.47	6.31[c]*
Test × pH	6	635.21	105.86	2.51[d]*
Pots (test × pH)	108	4,551.89	42.15	

[a] Data for experiment summarized as "Colonies per gram of root tissue" in Table 1.
[b] Asterisks (*) or * indicate that the probability of obtaining a larger F value is less than 0.10 or 0.05, respectively.
[c] The mean square for the Test × pH term used as error mean square.
[d] The mean square for the pots (test × pH) term used as error mean square.

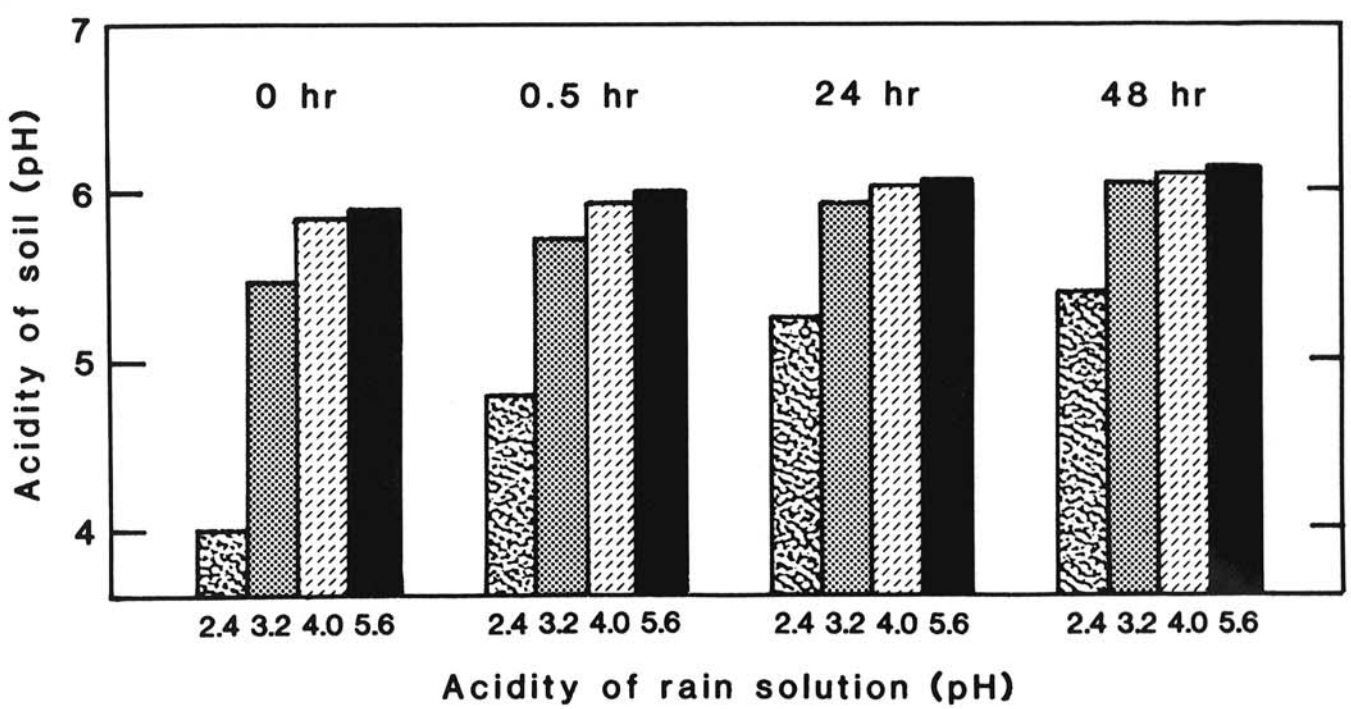

Fig. 1. Effect of acidity of simulated rain solutions on soil acidity during the sporangium production experiment. Each bar is the mean of six replicates. All standard errors are ±0.08 pH units or less. The initial soil acidity was pH 5.9.

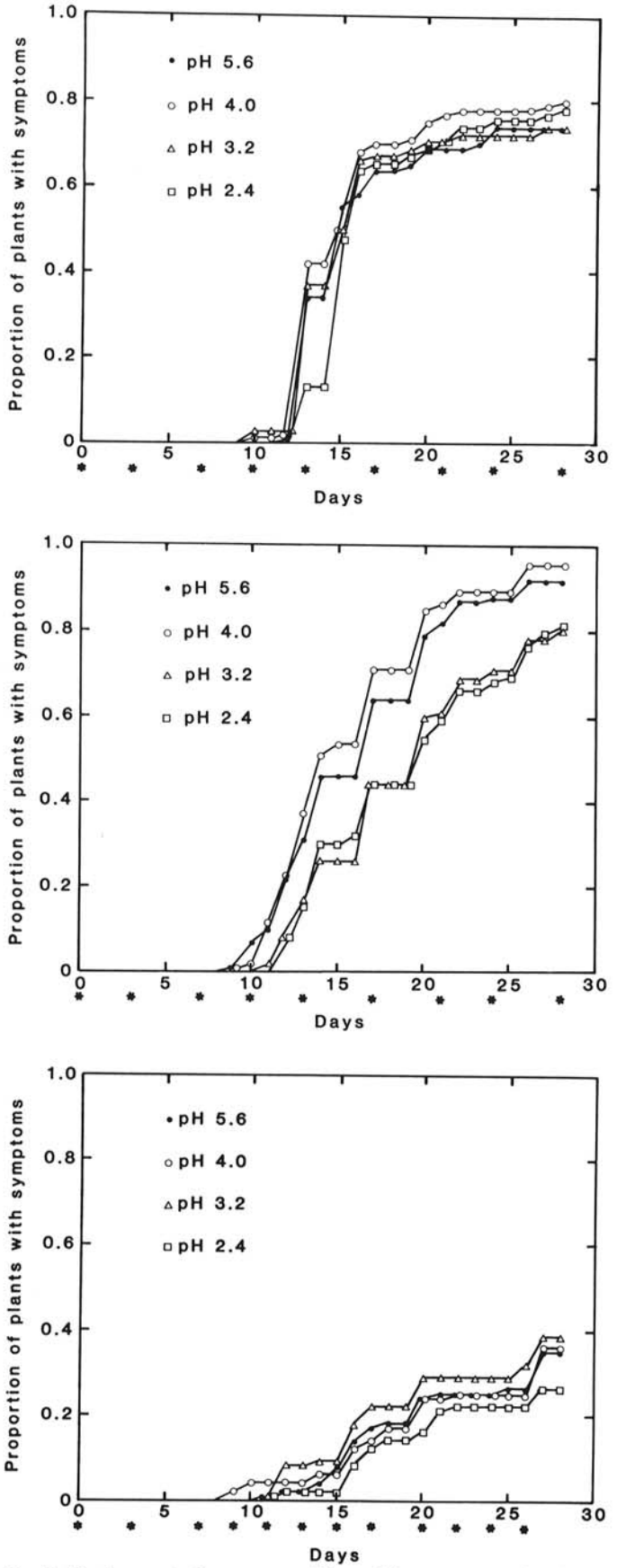

Fig. 2. Development of symptoms of Phytophthora root rot of lupine at four rain acidity levels. On day 0, seedlings were transplanted into infested Lakeland sand (inoculum density = 1–2 propagules per gram of dry soil) and exposed to a 30-min application of simulated acidic rain. Four replicate containers of soil and seedlings were exposed to each acidity level. Days on which rains were applied are indicated by an asterisk (*). **A,** test 1, **B,** test 2, and **C,** test 3.

presented). Inoculum densities of *P. cinnamomi* at the end of each test were not consistently correlated with any soil chemical factor.

DISCUSSION

Experiments described here are the first to examine possible effects of acidic rain on a soilborne pathogenic fungus. *P. cinnamomi* infects many plant species in areas that currently receive acid deposition. Acidity levels selected for simulated rain treatments encompass the range of acidity typical of ambient rain (16,30), and results are expressed in terms of rainfall acidity, a parameter documented by several rainfall chemistry monitoring networks (30).

Sporangium production in soil was suppressed by simulated rain solutions at acidities similar to that of ambient precipitation for parts of the eastern United States (30). Although not all infected roots are near the soil surface, the microenvironment of a deeper root might be similar to that of a root nearer the surface with respect to rain chemistry if rainwater moved by mass flow through cracks, root channels, and other macropores and failed to chemically equilibrate with the soil (49). Incubation of infected radicle tips in soil saturated for a short time with rain solutions and subsequently drained simulated conditions for roots in such conditions. In this experiment, sporangium production decreased linearly over the range of rain acidity from pH 5.6–2.4. However, this 1,585-fold difference in H^+ activities characteristic of the rain solutions did not occur in the soil layers in funnels. Even in the most-acidic treatment, soil pH values remained above 4.0 and were greater than 5.0 for at least half of the incubation period. Thus, sporangium formation was partially suppressed across a narrow range of soil acidity conditions. Results suggest that, in this soil, inhibition of sporangium formation could occur in soil exposed once to rain at pH $\leq$4.0, even though the pH of soil saturated with simulated rain at pH 4.0 or 3.2 was pH 5.5–6.0. Such conditions have been previously demonstrated as inconsequential to sporangium formation in soil or potting mixes (3,7). Differences in acidification procedures, soil characteristics, or conditions other than acidity induced by rain solutions could contribute to this discrepancy. Effects of rain acidity on sporangium production may vary with soil type, rain chemistry, and frequency and duration of precipitation.

Although sporangium formation was affected by acidified simulated rain solutions, release of zoospores into soil extracts prepared with similar solutions was unaffected. These results support the suggestion (7) that sporangium formation is more sensitive than zoospore release to acidity.

The number of zoospore-caused infection sites (as CPG) on lupine roots was suppressed by a single 1-hr exposure to simulated rain at pH 2.4. Values of CPG were variable, particularly those associated with less-acidic rains, and prevent clear definition of a dose-response relationship. Such variation arose largely from the occasional loss of small lateral roots during the washing procedure and from an inability to distinguish between lesions that coalesced during the 48-hr postinoculation period. Although the occurrence of precipitation characterized by pH $<$3 is unusual (16), results reported here indicated that, in a poorly buffered soil, chemical characteristics of a single rainfall can affect infection by zoospores. Suppression of infection by zoospores may have been due to chemical environment shifts which can impair zoospore movement (1,5,18,19) and tactic responses (1,10,12,20). Reduced zoospore motility can result in a lower disease severity than would otherwise be expected (27). Zoospore germination in soil may also have been suppressed, although zoospores within sporangia of *P. cinnamomi* germinated in water extracts of acidified (pH 3.3) peat-sand mix (7).

Lupine seedlings transplanted into infested soil and exposed repeatedly to simulated acidic rain exhibited symptoms typical of Phytophthora root rot. Although no seedlings in noninfested soil were included in these tests, noninfected plants exposed repeatedly to simulated acidic rain in preliminary unreported tests exhibited only foliar lesions. Hence, the systemic wilt and damping-off symptoms that developed during the experiment described here are

attributed to Phytophthora root rot. Symptoms always appeared last among plants exposed to one of the two most acidic solutions, so partial suppression of sporangium formation and infection by zoospores demonstrated in other experiments reported here may have been sufficient to delay slightly the onset of disease symptoms in soil with a low inoculum density. Once disease began to develop, however, rain acidity had no consistent effect on the rate of increase of symptoms apparently because the suppression of asexual reproduction and infection of roots was incomplete. The inoculum density continued to increase during the experiment, and rain acidity was apparently of minor consequence when plants remained constantly exposed to inoculum for 28 days. In the third test of this series, air temperature in the greenhouse exceeded 37 C on 8 of the first 14 days. High temperature inhibits activities of *P. cinnamomi* (52) and probably accounts for the slow increase of disease in that test. After 28 days, fewer plants from all treatments

TABLE 4. Effect of repeated simulated rains of different pH values on disease progress among blue lupine seedlings[a] planted in Lakeland sand infested with *Phytophthora cinnamomi*

Test	Days inclusive[b]	Rain acidity (pH)	Regression equation[c]	Standard error of the estimate of slope for regression equation	r[d]
1	13–15	5.6	$Y' = 0.178\ X - 1.913$	0.058	0.43
		4.0	$Y' = 0.077\ X - 0.481$	0.018	0.75**
		3.2	$Y' = 0.110\ X - 0.987$	0.031	0.39
		2.4	$Y' = 0.291\ X - 3.742$	0.085	0.69*
2	12–24	5.6	$Y' = 0.170\ X - 1.871$	0.011	0.91**
		4.0	$Y' = 0.220\ X - 2.271$	0.024	0.59**
		3.2	$Y' = 0.105\ X - 1.224$	0.007	0.87**
		2.4	$Y' = 0.112\ X - 1.305$	0.012	0.66**
3	11–27	5.6	$Y' = 0.024\ X - 0.260$	0.002	0.73**
		4.0	$Y' = 0.023\ X - 0.244$	0.002	0.72**
		3.2	$Y' = 0.028\ X - 0.264$	0.002	0.72**
		2.4	$Y' = 0.020\ X - 0.219$	0.001	0.78**

[a] Seedlings were placed in four containers for each acidity level in each test.
[b] See Materials and Methods for definition of the time period considered for regressions in each test.
[c] $Y' = \log_e [1/(1 - Y)]$, in which Y = the mean proportion of plants in each container that exhibited symptoms of disease; X = the number of days since day 0. On day 0, seedlings were transplanted into infested soil and exposed to the first simulated rain.
[d] Correlation coefficient for Y' versus X. Values followed by asterisks * or ** are significantly greater than zero at $P < 0.05$ or $P < 0.01$, respectively.

TABLE 5. Characteristics of Lakeland sand in containers[a] before each test and after 28 days

Parameters	Test	Initial analysis	Final analyses (after exposures to rain) Rain acidity (pH) 5.6	4.0	3.2	2.4	F[b]
Inoculum density[c]	1	1.7	14.9	25.6	11.4	15.6	1.28
	2	1.4	15.5	18.8	15.1	23.8	0.72
	3	0.9	13.3	13.0	14.1	3.5	3.16
Acidity (pH)	1	6.20	6.38	6.33	6.12	5.05	239.58**
	2	6.17	6.17	6.21	6.06	5.62	5.86*
	3	5.98	6.14	6.16	5.92	4.96	78.16**
Exchangeable acidity (meq/100 cc)	1	0.50	1.10	1.10	1.20	2.10	9.13*
	2	0.81	0.82	0.78	0.89	1.16	35.51**
	3	0.91	0.98	0.84	1.11	1.39	6.07*
Mg (meq/100 cc)	1	0.87	0.59	0.54	0.56	0.26	63.48**
	2	0.73	0.60	0.60	0.62	0.49	23.82**
	3	0.76	0.54	0.54	0.58	0.20	73.74**
Base saturation[d] (Percentage of CEC)	1[e]	85.0	64.5	62.8	62.0	41.0	37.43**
	2	77.3	74.2	76.0	73.5	65.6	33.24**
	3	73.8	67.0	71.0	66.8	49.2	34.64**
SO_4-S (mg/1,000 cc)	1	...[f]	...	...	...	...	...
	2	17.0	13.3	14.9	23.8	135.0	250.11**
	3	14.8	0.0	0.3	9.3	114.0	258.21**

[a] Lupine seedlings were grown in each container.
[b] F values from analyses of variance for each parameter in each test. Each analysis of variance was performed for a Latin square design (four treatments, four replicates per treatment). Exceptions to this are F values for Mg and base saturation in test 2. In test 2, data for these two parameters from one container were not available. Means for these parameters in test 2 are based on three replicates for treatment pH 4.0. F tests for significant variation were calculated in test 2 according to an unbalanced design with the General Linear Models procedure of the Statistical Analysis System (see reference 38). F values followed by asterisks * or ** indicate that significant variation at $P < 0.05$ or $P < 0.01$, respectively, occurred among the four means for posttreatment soil analysis.
[c] Propagules of *Phytophthora cinnamomi* per gram of soil.
[d] Calculated as $[(k + Ca + Mg + Na)/CEC] \times 100$.
[e] Does not include contribution by Na.
[f] Not determined.

in the third test exhibited symptoms than plants exposed to rains at pH 2.4 in the other tests. Thus, high but naturally occurring temperatures apparently had more effect on disease progress than did unusually high acidity of repeated rains.

Changes in soil chemistry during experiments that involved single applications of rain solutions were probably small and, if similar to soil pH, transient (Fig. 1). Significant effects nevertheless occurred under such conditions, and these results support the hypothesis that soilborne pathogens might be affected by short-term changes in the soil solution in the absence of measurable changes in the soil mass (44). Effects might be due to ions other than H^+ that are mobilized by simulated acidic rain and are inhibitory to fungi (2,17). Furthermore, effects may vary with the chemical composition of rain (4).

Still in question are possible effects of acid deposition on soilborne pathogens caused by changes in chemical characteristics of soil after long-term exposure. In the 28-day lupine experiments, increased soil acidity (i.e., both lowered pH value and increased exchangeable acidity), increased SO_4-S concentrations, and decreased Mg^{++} concentrations were detected. Several investigations have demonstrated that changes of these types, and others, are associated with suppression of diseases caused by *Phytophthora* spp. (25,28,33,34). Such changes did not occur quickly enough to alter disease progress in the present study. Evaluation of interactions among soilborne pathogens, host plants, soil chemical characteristics, and long-term acid deposition will be difficult. Studies that involve inducement of immediate and marked shifts in chemical characteristics of infested soil with S or lime applications (e.g., 25,34) or acidification of soil prior to infestation (e.g., 7) are successful for evaluation of management techniques but would be inappropriate for long-term acid deposition questions. The latter questions would be best addressed by rain simulation studies with infested field plots that allow natural drainage and leaching. In the absence of such experiments, documented effects of soil chemical factors on *Phytophthora* spp. (25,28,33,34) and hypothetical effects of acid deposition on soils (31) suggest that decades of acid deposition on poorly buffered infested soils could eventually cause detectable changes in the incidence or severity of diseases caused by *Phytophthora* spp.

Shriner (44) found that simulated rains at pH 3.2 inhibited development of several plant diseases, particularly if the parasites were exposed. Results of experiments reported here suggest that acidity levels in precipitation occurring in the eastern United States, when applied as simulated rain, affect epidemiologically important stages in the life cycle of *P. cinnamomi* in soil. Short-term variations in disease incidence attributable to current ambient levels of rainfall acidity are probably small and difficult to detect. Effects on the pathogen in soil exposed to acid deposition over many years, however, remain in question.

LITERATURE CITED

1. Allen, R. N., and Harvey, J. D. 1974. Negative chemotaxis of zoospores of *Phytophthora cinnamomi*. J. Gen. Microbiol. 84:28-38.
2. Babich, H., and Stotzky, G. 1982. Nickel toxicity to microbes: effect of pH and implications for acid rain. Environ. Res. 29:335-350.
3. Benson, D. M. 1984. Influence of pine bark, matric potential, and pH on sporangium production by *Phytophthora cinnamomi*. Phytopathology 74:1359-1363.
4. Bewley, R. J. F., and Stotzky, G. 1983. Anionic constituents of acid rain and microbial activity in soil. Soil Biol. Biochem. 15:431-437.
5. Bimpong, C. E, and Clerk, G. C. 1970. Motility and chemotaxis of zoospores of *Phytophthora palmivora* (Butl.) Butl. Ann. Bot. 34:617-624.
6. Bisessar, S., Palmer, K. T., Kuja, A. L., and Linzon, S. N. 1984. Influence of simulated acidic rain on bacterial speck of tomato. J. Environ. Qual. 13:18-22.
7. Blaker, N. S., and MacDonald, J. D. 1983. Influence of container medium pH on sporangium formation, zoospore release, and infection of rhododendron by *Phytophthora cinnamomi*. Plant Dis. 67:259-263.
8. Brewer, P. F., and Heagle, A. S. 1983. Interactions between *Glomus geosporum* and exposure of soybeans to ozone or simulated acid rain in the field. Phytopathology 73:1035-1040.
9. Cameron, H. R., and Milbrath, G. M. 1965. Variability in the genus *Phytophthora*. I. Effects of nitrogen sources and pH on growth. Phytopathology 55:653-657.
10. Cameron, J. N., and Carlile, M. J. 1980. Negative chemotaxis of zoospores of the fungus *Phytophthora palmivora*. J. Gen. Microbiol. 120:347-353.
11. Chang, F. -H., and Alexander, M. 1983. Effects of simulated acid precipitation on growth and nodulation of leguminous plants. Bull. Environ. Contam. Toxicol. 30:379-387.
12. Chang-Ho, Y., and Hickman, C. J. 1970. Some factors involved in the accumulation of phycomycete zoospores on plant roots. Pages 103-108 in: Root Diseases and Soilborne Pathogens. T. A. Toussoun, R. V. Bega, and P. E. Nelson, eds. University of California Press, Berkeley.
13. Cogbill, C. V., and Likens, G. E. 1974. Acid precipitation in the northeastern United States. Water Resour. Res. 10:1133-1137.
14. Dochinger, L. S., and Seliga, T. A. (eds.). 1976. Proc. First Int. Symp. on Acid Precipitation and the Forest Ecosystem. U.S. Dep. Agric., For. Serv. Gen. Tech. Rep. NE-23. Exp. Stn., Upper Darby, PA. 1074 pp.
15. Duniway, J. M. 1983. Role of physical factors in the development of Phytophthora diseases. Pages 175-187 in: *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. The American Phytopathological Society, St. Paul, MN.
16. Evans, L. S. 1982. Biological effects of acidity in precipitation on vegetation: A review. Environ. Exp. Bot. 22:155-169.
17. Firestone, M. K., Killham, K., and McColl, J. G. 1983. Fungal toxicity of mobilized soil aluminum and manganese. Appl. Environ. Microbiol. 46:758-761.
18. Goode, P. M. 1956. Infection of strawberry roots by zoospores of *Phytophthora fragariae*. Trans. Br. Mycol. Soc. 39:367-377.
19. Ho, H. H., and Hickman, C. J. 1967. Asexual reproduction and behavior of zoospores of *Phytophthora megasperma* var. *sojae*. Can. J. Bot. 45:1963-1981.
20. Ho, H. H., and Hickman, C. J. 1967. Factors governing zoospore responses of *Phytophthora megasperma* var. *sojae* to plant roots. Can. J. Bot. 45:1983-1994.
21. Kenerley, C. M., and Bruck, R. I. 1983. Overwintering and survival of *Phytophthora cinnamomi* in Fraser fir and cover-cropped nursery beds in North Carolina. Phytopathology 73:1643-1647.
22. Kenerley, C. M., Papke, K., and Bruck, R. I. 1984. Effect of flooding on development of Phytophthora root rot in Fraser fir seedlings. Phytopathology 74:401-404.
23. Killham, K., and Firestone, M. K. 1982. Evaluation of accelerated H^+ applications in predicting soil chemical and microbial changes due to acid rain. Commun. Soil. Sci. Plant Anal. 13:995-1001.
24. Killham, K., Firestone, M. K., and McColl, J. G. 1983. Acid rain and soil microbial activity: Effects and their mechanisms. J. Environ. Qual. 12:133-137.
25. Kincaid, R. R., Martin, F. G., Gammon, N., Jr., Breland, H. L., and Pritchett, W. L. 1970. Multiple regression of tobacco black shank, root knot, and coarse root indexes on soil pH, potassium, calcium, and magnesium. Phytopathology 60:1513-1516.
26. Kirby, H. W., and Grand, L. F. 1975. Susceptibility of *Pinus strobus* and *Lupinus* spp. to *Phytophthora cinnamomi*. Phytopathology 65:693-695.
27. Kliejunas, J. T., and Ko, W. W. 1974. Effect of motility of *Phytophthora palmivora* zoospores on disease severity in papaya seedlings and substrate colonization in soil. Phytopathology 64:426-428.
28. Lee, B. S., and Zentmyer, G. A. 1982. Influence of calcium nitrate and ammonium sulfate on Phytophthora root rot of *Persea indica*. Phytopathology 72:1558-1564.
29. Likens, G. E., Wright, R. F., Galloway, J. N., and Butler, T. J. 1979. Acid rain. Sci. Am. 241:43-51.
30. Linthurst, R. A., and Altshuller, A. P. (eds.). 1984. The acidic deposition phenomenon and its effects: Critical assessment review papers. Vol. I.—Atmospheric Sciences, U.S. Environmental Protection Agency Rep. 600/8-83-016AF. US-EPA, Washington, DC. 750 pp.
31. Linthurst, R. A., and Altshuller, A. P. (eds.). 1984. The acidic deposition phenomenon and its effects: Critical assessment review papers. Vol. II. Effect Sciences. U.S. Environmental Protection Agency Rep. 600/8-83-016 BF. US-EPA, Washington, DC. 700 pp.
32. Mircetich, S. M., Zentmyer, G. A., and Kendrick, J. B., Jr. 1968. Physiology of germination of chlamydospores of *Phytophthora cinnamomi*. Phytopathology 58:666-671.
33. Muchovej, J. J., Maffia, L. A., and Muchovej, R. M. C. 1980. Effect of exchangable soil aluminum and alkaline calcium salts on the pathogenicity and growth of *Phytophthora capsici* from green pepper. Phytopathology 70:1212-1214.
34. Pegg, K. G. 1977. Soil application of elemental sulfur as a control of

Phytophthora cinnamomi root and heart rot of pineapple. Aust. J. Exp. Agric. Anim. Husb. 17:859-865.
35. Porter, J. R., and Sheridan, R. P. 1981. Inhibition of nitrogen fixation in alfalfa by arsenate, heavy metals, fluoride, and simulated acid rain. Plant Physiol. 68:143-148.
36. Raynal, D. J., Leaf, A. L., Manion, P. D., and Wang, C. J. K. 1980. Actual and potential effects of acid precipitation on a forest ecosystem in the Adirondak Mountains. New York State Energy Res. Devel. Author., Res. Rep. 80-28.
37. Raynal, D. J., Roman, J. R., and Eichenlaub, W. M. 1982. Response of tree seedlings to acid precipitation. II. Effect of simulated acidified canopy throughfall on sugar maple seedling growth. Environ. Exp. Bot. 22:385-392.
38. SAS Institute, Inc. 1982. SAS User's Guide: Statistics, 1982 edition. SAS Institute, Inc., Cary, N.C. 584 pp.
39. Schmitthenner, A. F., and Canaday, C. H. 1983. Role of chemical factors in development of Phytophthora diseases. Pages 189-196 in: *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao (eds.). The American Phytopathological Society, St. Paul, MN.
40. Shafer, S. R., Bruck, R. I., and Heagle, A. S. 1982. Survival of *Phytophthora cinnamomi* in soil columns exposed to simulated acid rain. (Abstr.) Phytopathology 72:361.
41. Shafer, S. R., Bruck, R. I., and Heagle, A. S. 1982. Tissue infection by *Phytophthora cinnamomi* zoospores released from naturally infested soil into simulated acid rain solutions. Proc. 1982 South-Wide Forest Disease Workshop. Virginia Polytechnic Institute and State University, Blacksburg, VA.
42. Shafer, S. R., Grand, L. F., Bruck, R. I., and Heagle, A. S. 1985. Formation of ectomycorrhizae on *Pinus taeda* seedlings exposed to simulated acidic rain. Can. J. For. Res. 15:66-71.
43. Shew, H. D., and Benson, D. M. 1982. Qualitative and quantitative soil assays for *Phytophthora cinnamomi*. Phytopathology 72:1029-1032.
44. Shriner, D. S. 1978. Effects of simulated acidified rain on host-parasite interactions in plant diseases. Phytopathology 68:213-218.
45. Shriner, D. S., and Johnston, J. W. 1981. Effects of simulated acidified rain on nodulation of leguminous plants by *Rhizobium* spp. Environ. Exp. Bot. 21:199-209.
46. Spencer, S., and Benson, D. M. 1982. Pine bark, hardwood bark compost, and peat amendment effects on development of *Phytophthora* spp. and lupine root rot. Phytopathology 72:346-351.
47. Strayer, R. F., and Alexander, M. 1981. Effects of simulated acid rain on glucose mineralization and some physicochemical properties of forest soils. J. Environ. Qual. 10:460-465.
48. Tamm, C. O. 1976. Acid precipitation: Biological effects in soil and on forest vegetation. Ambio 5:235-238.
49. Tamm, C. O. 1977. Acid precipitation and forest soils. Water, Air, Soil Pollut. 7:367-369.
50. Turner, G. J. 1969. Effects of hydrogen ion concentration on *Phytophthora palmivora* from *Piper nigrum*. Trans. Br. Mycol. Soc. 52:419-423.
51. Vanderplank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York. 349 pp.
52. Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Phytopathological Monograph 10. The American Phytopathological Society, St. Paul, MN. 96 pp.