Effects of Simulated Acidic Mist on Germination of *Alternaria solani* and *Phytophthora infestans* in vitro and their Infection Efficiency and Sporulation on Potato

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ABSTRACT

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Effects of acidity of simulated mist on germination of *Alternaria solani* and *Phytophthora infestans* in vitro and their infection efficiency and sporulation on potato were studied in the laboratory and greenhouse. Acidic mist solutions (pH 2.8, 3.4, 4.0, and 4.6) contained sulfuric and nitric acids in a 2:1 mass ratio (2.6:1 ratio on an H^+ equivalent basis) and background ions similar to those in ambient rain. In vitro germination of conidia of *A. solani* and sporangia of *P. infestans* decreased curvilinearly with increasing acidity, with maximum germination at pH 4.0 and 3.4, respectively, of the original solution, corresponding to pH 6.0 and 5.2 of the spore suspensions. Rooted potato cuttings of the cultivars Norchip, Monona, and Katahdin were exposed to acidic mist (pH 2.8, 3.4, 4.0, or 4.6) for 24 hr before and after inoculation with spore suspensions of either pathogen. After 48–72 hr in a greenhouse, the plants were exposed again to simulated mist at the same acidity levels to induce sporulation. When simulated mist was applied at the same acidity before and after inoculation, infection efficiency, lesion area, and sporulation of both pathogens decreased linearly or curvilinearly with increasing acidity of simulated mist. In the case of curvilinear relationships, maxima were obtained at pH 4.0. Exposure of potato leaves to simulated mist at pH 2.8 before inoculation resulted in higher infection levels than exposure to mist at pH 4.0, both before and after inoculation. However, exposure to mist at pH 2.8 after inoculation drastically reduced infection levels compared with pH 4.0, regardless of preinoculation treatment. The postinoculation acidity effect was more important than the preinoculation acidity effect. The acidity of droplets of simulated acidic mist solution on potato leaf surfaces, after termination of exposure of the leaves to simulated acidic mist, decreased rapidly to final pH levels of 6.3–7.7, when initial pH levels of simulated mist and droplets were 3.4 or 4.0. At pH 2.8 the neutralizing capacity of the leaves was reduced after exposure to simulated acidic mist and the pH rose only to 4.0–5.0, depending on the duration of exposure.

Additional key words: acid mist, early blight, late blight, neutralizing capacity, predisposition, *Solanum tuberosum*.

A major effort has been made to determine direct effects of simulated acidic precipitation on crop growth and development but, so far, the results have been contradictory (2,11,22). Indirect effects of simulated acidic precipitation through changes in plant disease development have not been studied intensively (8,18,20,27,28).

Although the importance of water in plant disease epidemiology is well known, relatively little is known of the role that dissolved components of rain have on the interaction between host and pathogen. Subtle alterations of the leaf surface by simulated acidic rain have been observed (10,17), but effects of such changes on infection processes are not clear. Nevertheless, increases and decreases of disease severity induced by simulated acidic rain have been reported, depending on the host-pathogen association (3,5,6,18,27,28).

To date, there are only a few reports on effects of simulated acidic precipitation on early and late blight. Campbell et al (7) reported a decrease in spore germination of *Phytophthora infestans* (Mont.) de Bary in acidic solutions. Martin et al (18) observed a decrease in infection efficiency on potato when sporangia of *P. infestans* were suspended in acidic 'rain' solutions, but late blight development was unaffected by simulated acidic rain under field conditions (8). Leben (15) found that the development of early blight caused by *Alternaria solani* Sorauer on tomato was restricted after the foliage had been sprayed with acidic buffers. However, various types of acidic precipitation may affect disease development in different ways, depending on their influences on dispersal and germination of the propagules and on susceptibility of the host. Thus, effects of acidic rain on disease development may be different from those of acidic mist. Effects of exposure of foliage to acidic mist on development of early and late blight have not been reported thus far.

Effects of air pollutants on host-pathogen interactions may be the result of a direct influence on the pathogen, a change in host susceptibility or a change in antagonistic microflora (12,14). A direct effect on the pathogen could come about on exposed parts of the pathogen, e.g., when the pollution stress coincides with the early part of the infection cycle, or at the time of reproduction of the pathogen. Growth and penetration of a pathogen could be affected via a change in antagonistic microflora, if the pollution stress occurs before arrival of the pathogen on the host surface. Indirect effects on disease development via changes in host susceptiblity could take place, regardless of the timing of the pollution stress. In studies on effects of simulated acidic precipitation on early and late blight of potato (8,15,18), no distinction was made between pre- and postinoculation acidity stresses.

The objectives of the research reported herein were to assess the direct effect of acidity of simulated precipitation solutions on spore germination of *A. solani* and *P. infestans* and to test whether infection of potato leaves by *A. solani* and *P. infestans* and subsequent disease development were affected by acidity of simulated mist, applied before and/or after inoculation.

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MATERIALS AND METHODS

Plant culture. Rooted single-leaf cuttings were prepared from 2–4-mo-old greenhouse-grown potato (*Solanum tuberosum* L.) plants as described previously (29) and transplanted to 7-cm-diameter pots containing Cornell peat-lite mix 'A' (4). They were placed in a greenhouse with a 16-hr photoperiod and day/night temperatures of approximately 25/18 C. Supplementary lighting was provided by multivapor lamps (MV400/U, General Electric, Bridgeport, CT). Three potato cultivars were compared, viz., Norchip, Monona, and Katahdin. Norchip is highly susceptible to both late and early blight, Monona is highly susceptible to late blight and moderately susceptible to early blight, and Katahdin is moderately susceptible to both blights (25).

Production of simulated mist. Simulated precipitation solutions were acidified to pH 2.8, 3.4, 4.0, and 4.6 as described previously (29). The pH of the precipitation solutions was checked with a pH meter. Separate plastic mist chambers ($1.3 \times 0.6 \times 0.6$ m) were set up for each pH level inside a controlled environment chamber. Each mist chamber contained a humidifier (Model 250, DeVilbiss Co., Somerset, PA) producing simulated mist of the desired pH at a deposition rate of 0.29 ± 0.05 mm/hr. The pH of the mist was measured after 6 and 24 hr and did not change over time. The temperature in the main chamber was kept at 18 C for the late blight experimants and at 24 C for the early blight experiments; there was no light in the chamber.

Acidity of simulated mist droplets on leaf surfaces. To estimate the acidity of moisture on leaf surfaces exposed to acidic precipitation, the pH of simulated mist droplets on potato leaves was measured. Rooted potato cuttings of Norchip and Monona were exposed to simulated acidic mist (pH 2.8, 3.4, and 4.0) for various durations (0, 1, 5, or 20 hr). Because we were not able to measure the pH directly in the film of moisture on the leaf surface, the leaves were allowed to dry, and a 50-μl drop of simulated precipitation solution was placed on each cutting. The initial pH of the drops was the same as that of the mist applied previously. The plants were placed in a Plexiglas box ($91 \times 61 \times 91$ cm^3) with water on the bottom to insure a relative humidity of at least 90%. Flat-bottom electrodes (No. 476216, Corning Glass Co., Corning, NY) were placed in the drops. pH meters and recorders were situated outside the box. The pH in each drop was measured continuously for 1 hr. During this time the drops did not dry out. Similar drops were placed on polyethylene strips as a control. They did not show any change in pH for several hours.

Inoculum preparation. One isolate of each pathogen was used, viz., isolate R-4 of *A. solani* and isolate 128 of *P. infestans* (race 0), both obtained from the Department of Plant Pathology, Cornell University, Ithaca, NY. Both pathogens were routinely cultured on V-8 medium (200 ml of V-8/$CaCO_3$ [5 g of $CaCO_3$ per 12-oz can of V-8], 800 ml of deionized water, and 15 g of Bacto agar). Three plugs of agar plus mycelium were transferred to V-8/lima bean agar (lima bean broth from 80 g of lima beans in 200 ml of deionized water added to 100 ml of clarified V-8/$CaCO_3$, and 15 g of Bacto agar; pH 6.0). The plates were inverted at 18 C for 11–15 days to induce sporulation of *P. infestans*. Sporangial suspensions were prepared by adding 10 ml of simulated mist solution per culture plate and rubbing gently to release sporangia. The suspension was poured through two layers of cheesecloth, shaken vigorously to disperse the propagules, and sporangia were counted in a hemacytometer. To induce sporulation of *A. solani*, single 6-mm plugs of agar plus mycelium were transferred to plates of V-8 agar, which were then covered with autoclaved nylon netting and incubated at 24 C in darkness for 7 days. The netting, intertwined with mycelium, was placed on moist sterile filter paper in a petri dish sealed with Parafilm and placed alternatively under fluorescent lights and in the dark (8/16 hr) at 24 C. After about a week, the Parafilm was removed so that the filter paper dried. Dishes with conidial mats were stored at 10 C in darkness. Conidial suspensions were prepared by withdrawing conidia by suction from the netting into an acidified precipitation solution of the desired pH. To quantify inoculum, conidia were counted in 10-μl droplets on an object glass.

Spore germination in vitro. Conidia of *A. solani* and sporangia of *P. infestans* were suspended at concentrations of 10^4–10^5 spores per milliliter in simulated mist solutions at pH 2.8, 3.4, 4.0, and 4.6. For each fungus and pH level, 10-μl drops were placed on three or four microslides with a circular depression (Corning Glass Works, Corning, NY), and covered with a cover glass. The slides were placed on moist filter paper in petri plates, and incubated at 24 or 18 C, for *A. solani* and *P. infestans*, respectively. After 4 hr, the germinated and ungerminated conidia of *A. solani* and directly germinated, ungerminated, and empty sporangia (zoospore release) of *P. infestans* were counted. Before and after incubation the pH's of the spore suspensions were measured with a pH meter. These experiments were repeated at least three times.

Inoculation and mist treatment. Before inoculation, all plants were exposed to acidic mist for 24 hr at pH levels ranging from 2.8 to 4.6. Three leaflets per cutting were inoculated with sporangia of *P. infestans* or conidia of *A. solani*. Sporangia or conidia were suspended in simulated mist solutions acidified to the desired pH levels as described above. Each leaflet received 50 μl of inoculum (10^4–10^5 sporangia or conidia per milliliter) administered with an Eppendorf pipette. In the case of *P. infestans*, 50-μl drops were spread over the adaxial leaf surface. In the case of *A. solani*, a 50-μl suspension was pipetted into an inverted DeVilbiss sprayer and sprayed through a hole (2.5 cm diameter) in a petri dish lid onto the adaxial leaf surface. Similarly, 50 μl of conidial suspension were sprayed on three agar plates to count the number of conidia deposited. Inoculated plants were subjected immediately to acidic mist for 24 hr, and then placed on a greenhouse bench for 48–72 hr. To test the effect of acidity of simulated mist on sporulation, inoculated plants were returned to the mist chambers for 30 hr continuously to induce sporulation by *P. infestans* or during 16 hr at night for 4 days to stimulate sporulation by *A. solani* (during the daytime these plants remained in the greenhouse).

Measurements of disease development. Inoculated plants were checked daily for symptom expression. Lesions were counted 3–4 days after inoculation. Production of sporangia and conidia was determined by washing three inoculated leaflets in vials with 10 ml of water containing a surfactant (0.1% Tween 80). The vials were placed on a rotary shake for 2 hr and shaken vigorously on a mixer (Super-mixer, Lab-line Instruments, Melrose Park, IL) for 1 min before spores were counted in a hemacytometer (*P. infestans*) or in 10 or 100 μl droplets (*A. solani*). Lesion areas were estimated by means of a calibrated grid with dots at a spacing of 0.5 cm printed on a transparent plastic sheet (31).

Experimental design and analysis. Separate experiments were conducted for early blight and late blight. There were two types of experiments concerning effects of acidic mist on disease development: those in which the same acidity of mist was used before and after inoculation (pH 2.8, 3.4, 4.0, and 4.6) and those in which the acidity of the mist before and after inoculation varied (pH 2.8 or 4.0). Experiments of the first type had split-plot designs with pH levels in main plots in randomized complete blocks (three replications over time) and potato cultivars in subplots (five to six plants each of two or three cultivars). The pH treatments were rerandomized over the mist chambers for each replication over time. In experiments of the second type, only one cultivar (Norchip) was used. These experiments were conducted in four randomized complete blocks, with four to five plants per plot. The data were analyzed using regression and analysis of variance, respectively (26). For experiments of the first type, sums of squares for the pH effects were partitioned into linear, quadratic, and cubic components. Analyses of the data for individual plants resulted in residual values that were not normally distributed. To obtain normally distributed residual values, the analyses were performed on the means of four to five plants per plot. In the case of conidial production by *A. solani*, a logarithmic (base e) transformation of the means was needed to normalize residual values.

pH measurements of drops on leaf surfaces were made in separate experiments for two cultivars (Norchip and Monona). These experiments had split-plot designs, with duration of mist in the main plot (the Plexiglas box) and initial pH of drops in subplots (pH electrodes). The durations of exposure to mist (main

plots) were randomized over time within each block. The measurements for each block were completed in 2 days, and there were four blocks over time. The measured pH values for each treatment were regressed on the natural logarithm of the time period since the simulated mist drop was placed on the leaflet. Subsequently, the estimated values for the slopes were regressed on duration of exposure to mist and pH of mist in a split-plot analysis, with duration of exposure to mist in the main plot and pH of mist in the subplot.

RESULTS

Spore germination in vitro. The percentage of conidia of *A. solani* that germinated in vitro remained constant at pH 4.0 and 4.6 and decreased with increasing acidity (Fig. 1A). Similarly, the percent sporangia of *P. infestans* that released zoospores remained constant between pH 3.4 and 4.6 and decreased at pH 2.8 (Fig. 1B). Direct germination of sporangia decreased slightly with increasing acidity. Regression of the natural logarithm of the percentages of germination and zoospore release on pH showed that there were significant linear and quadratic pH effects for germination of conidia of *A. solani* ($P < 0.01$ for both effects) and zoospore release by *P. infestans* ($P < 0.01$ and $P = 0.02$, for the linear and quadratic effects, respectively). For direct germination of the sporangia only the linear trend was significant ($P < 0.01$).

TABLE 1. pH of spore suspensions of *Alternaria solani* and *Phytophthora infestans*, 0 and 4 hr after preparation of the suspensions

	A solani pH of suspension after		*P. infestans* pH of suspension after	
Initial pH	0 hr	4 hr	0 hr	4 hr
2.8	3.0 (±0.2)[a]	3.3 (±0.4)	5.5 (±0.1)	6.0 (±0.2)
3.4	3.7 (±0.2)	4.3 (±0.2)	6.0 (±0.3)	6.4 (±0.2)
4.0	5.2 (±0.7)	5.5 (±0.4)	6.4 (±0.4)	6.7 (±0.1)
4.6	5.8 (±0.8)	5.8 (±0.6)	6.6 (±0.6)	6.7 (±0.2)

[a] Standard deviation.

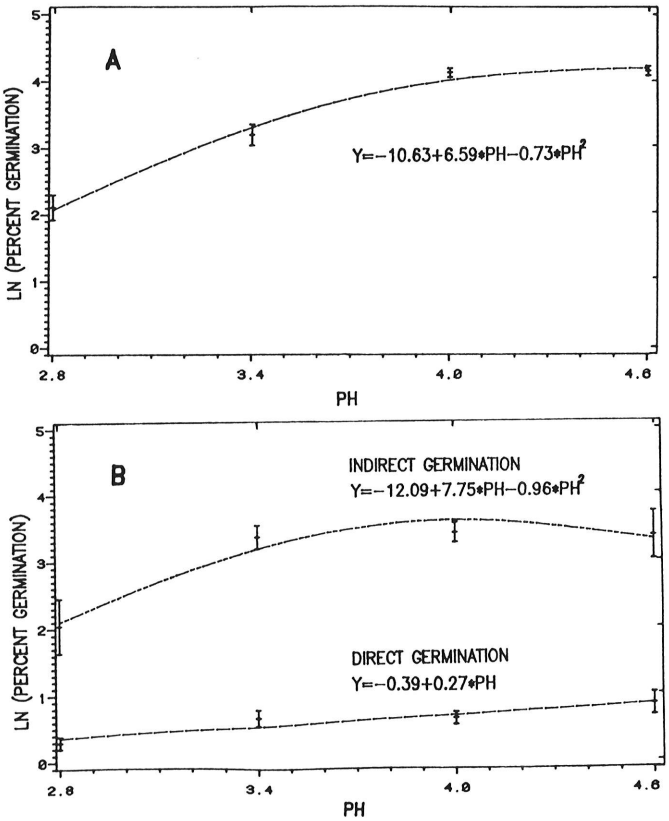

Fig. 1. Relationship between germination of conidia of *Alternaria solani* **(A)** and of sporangia of *Phytophthora infestans* **(B)** on microslides and pH of 'simulated mist' solution used to prepare spore suspensions.

The pH of the simulated mist solutions increased when conidia or sporangia were added to the solutions and continued to rise during germination (Table 1). With *A. solani*, the increase was smaller than with *P. infestans*, and the differences among pH levels remained distinct during incubation of conidia of *A. solani*. In the case of *P. infestans*, the pH of the simulated mist solutions rose to about 6.0 as soon as sporangia were added to the acidic solutions and increased only slightly during germination.

Infection and sporulation on potato. The infection efficiency of *A. solani* on potato leaves exposed to simulated acid mist for 24 hr before and after inoculation increased between pH 2.8 and 4.6 for all cultivars (Fig. 2A). Regression analysis for all cultivars showed that only the linear term for pH was significant (Table 2). Similarly, total lesion area per plant increased linearly with pH of simulated acidic mist (Fig. 2B and Table 2). When the natural logarithm of infection efficiency and lesion area were regressed on

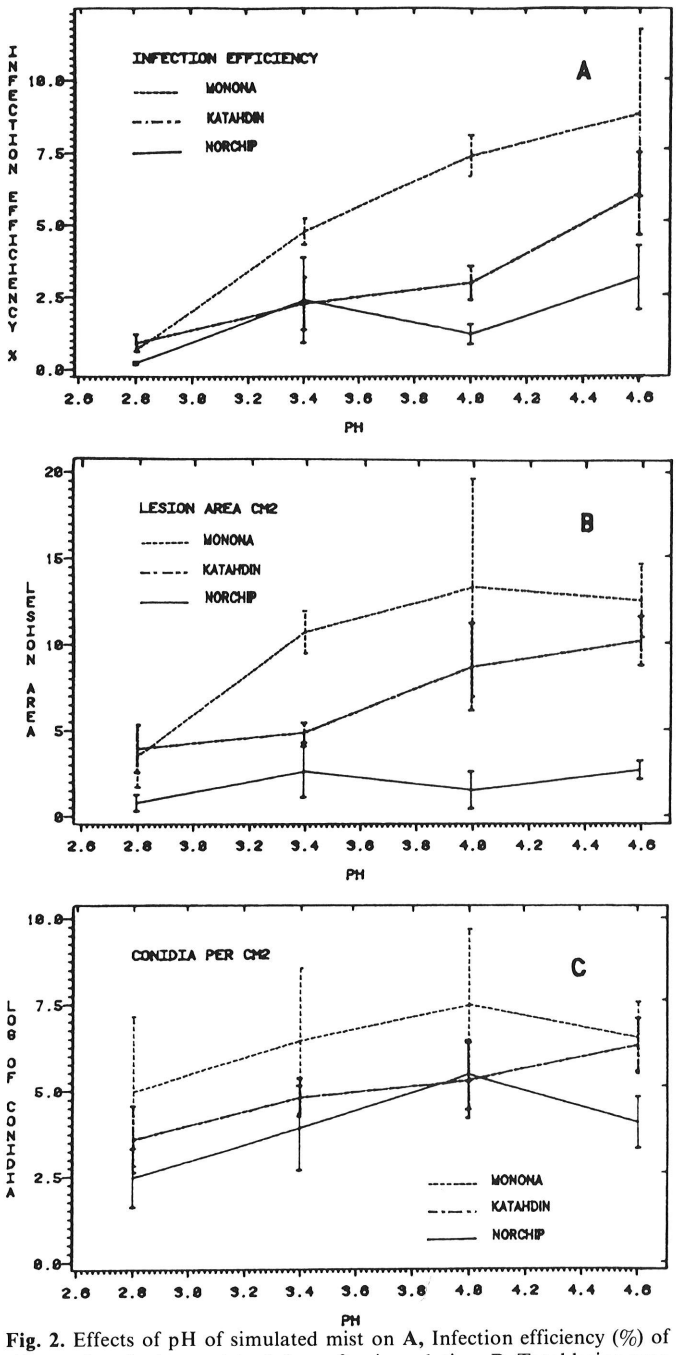

Fig. 2. Effects of pH of simulated mist on **A,** Infection efficiency (%) of *Alternaria solani* on potato 4 days after inoculation; **B,** Total lesion area (square centimeter) per plant; and **C,** The logarithm of numbers of conidia per square centimeter of infected area 10 days after inoculation (standard error indicated by vertical bars).

pH for each cultivar separately, the quadratic terms for pH were significant for Monona only ($P < 0.01$ in both cases). A combined analysis for all cultivars showed that the logarithm of numbers of conidia per square centimeter increased curvilinearly with pH of simulated mist (Fig. 2C and Table 2). Although there was no significant interaction between pH and cultivar when the data for all cultivars were analyzed together, regression analysis for the individual cultivars indicated that quadratic terms of pH were almost significant for Monona and significant for Norchip ($P = 0.06$ and $P = 0.03$, respectively). In all cases when the relationships between measurements of early blight and pH were nonlinear, maximum values were observed at pH 4.0 (Fig. 2).

Infection efficiency of *P. infestans*, blighted area per plant, and numbers of sporangia per square centimeter of blighted area were all curvilinearly related to pH of simulated acidic mist (Fig. 3). Regression analyses for all cultivars combined indicated that quadratic terms of pH were significant (Table 3). When the data were analyzed for each cultivar separately, quadratic terms of pH were only significant for spore production per square centimeter on Norchip ($P = 0.03$), and almost significant for infection efficiency on Monona ($P = 0.06$). Maxima in late blight measurements were reached at pH 4.0 (Fig. 3).

When effects of simulated mist before and after inoculation were compared, the numbers of early and late blight lesions were significantly fewer at a lower pH applied after inoculation, regardless of acidity level before inoculation (Tables 4 and 5). A lower pH level after inoculation did not affect lesion expansion or sporulation of *A. solani*, but significantly decreased the lesion area caused by *P. infestans* (Tables 4 and 5). There was a significant interaction between pre- and postinoculation acidity of simulated mist for number of lesions induced by *A. solani*. An analysis of least square means (26) showed that this interaction was due to an increase in number of lesions applied by mist at pH 2.8 before inoculation on plants that were exposed to mist at pH 4.0 after inoculation ($P = 0.08$). There were no significant interactions

TABLE 2. Analysis of variance for infection efficiency (%) of *Alternaria solani* on potato, 4 days after inoculation, total lesion area (square centimeter) per plant, and the natural logarithm of numbers of conidia per square centimeter of infected area, 10 days after inoculation

Source	df[a]	Infection efficiency SS[b]	Lesion area SS	No. of conidia/cm^2 SS
Block	2	43.55	95.53	46.21**
pH[c]	1	97.90**[d]	122.41*	8.92**
pH^2	1	0.03	8.23	3.34*
pH^3	1	5.23	0.03	0.13
Block*pH (error a)	6	30.47	102.92	2.23
Cultivar[e]	2	47.47**	289.62**	3.39
pH*cultivar	6	26.01**	55.97	5.05
Residual (error b)	12	7.81	57.57	6.73
R^2 (%)		7.0	92.1	91.1
C.V. (%)		25.7	37.0	11.8

[a] Degrees of freedom.
[b] Sequential sum of squares.
[c] pH of simulated mist: 2.8, 3.4, 4.0 and 4.6.
[d] Significance level: ** significant at $\alpha = 0.01$, * significant at $\alpha = 0.05$.
[e] Potato cultivars: Norchip, Monona, and Katahdin.

TABLE 3. Analysis of variance for infection efficiency (%) of *Phytophthora infestans* on potato, 4 days after inoculation, total lesion area (square centimeter) per plant, and numbers of sporangia produced per square centimeter of infected area, 6 days after inoculation of potato leaflets with *P. infestans*

Source	df[a]	Infection efficiency SS[b]	Lesion area SS	Sporangia/cm^2 SS
Block	3	0.063	2,554.1**	7,403**[c]
pH[d]	1	0.058*[e]	33.7	243
pH^2	1	0.094**	168.7(*)	1,673**
pH^3	1	0.041(*)	3.6	151
Block × pH (error a)	9	0.092	351.0	717
Cultivar[f]	2	0.126*	666.8**	5,835**
pH × cultivar	6	0.087	128.7	748
Residual (error b)	8	0.088	225.0	1,353
R^2 (%)		86.4	94.6	92.5
C.V. (%)		81.7	38.4	45.7

[a] Degrees of freedom.
[b] Sequential sums of squares.
[c] Sums of squares divided by 10^6.
[d] pH of simulated mist: 2.8, 3.4, 4.0, and 4.6.
[e] Significance level: ** significant at $\alpha = 0.01$, * significant at $\alpha = 0.05$, (*) significant at $\alpha = 0.10$.
[f] Potato cultivars: Norchip, Monona, and Katahdin.

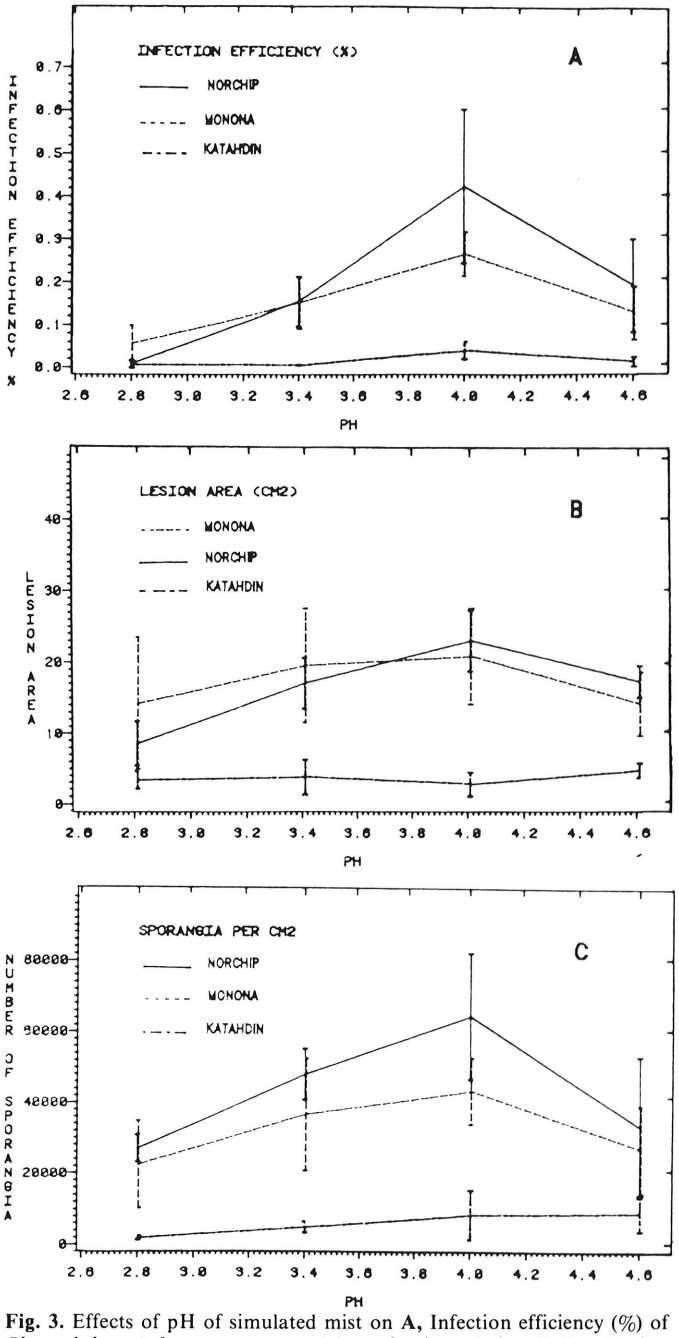

Fig. 3. Effects of pH of simulated mist on **A,** Infection efficiency (%) of *Phytophthora infestans* on potato 4 days after inoculation; **B,** Total lesion area (square centimeter) per plant; and **C,** Numbers of sporangia per square centimeter of infected area 6 days after inoculation (standard errors indicated by vertical bars).

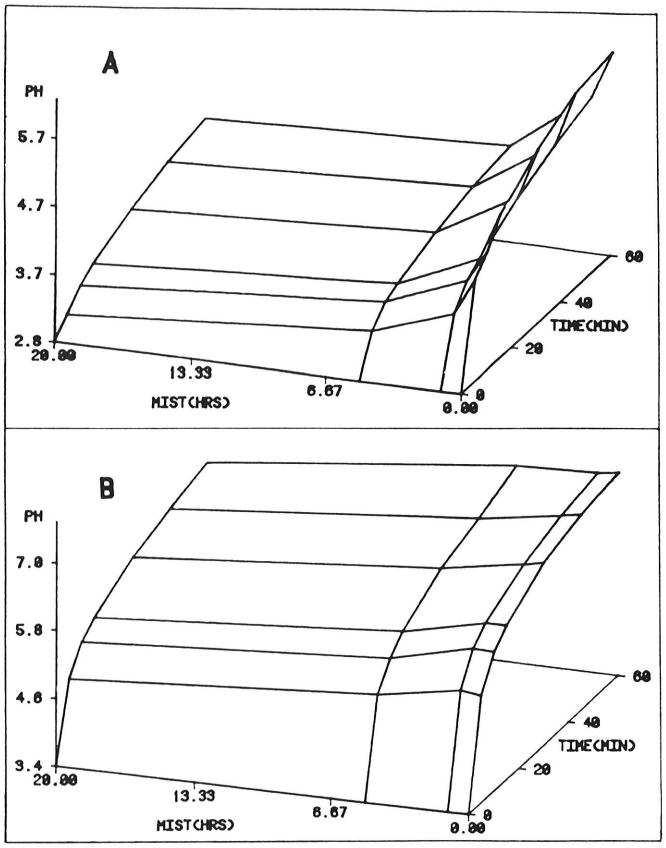

Fig. 4. Mean pH of simulated acidic mist drops on potato leaves (cultivar Norchip), measured continuously over time (minutes), after 0, 1, 5, or 20 hr of leaf exposure to simulated mist at pH 2.8 (A) or pH 3.4 (B).

between pre- and postinoculation acidity for late blight measurements. Nevertheless, when effects of acidity before inoculation (at pH 4.0 after inoculation) were analyzed separately, the number of late blight lesions appeared to be significantly increased after application of mist at pH 2.8 before inoculation ($P = 0.03$).

Acidity of leaf surface moisture. For all initial acidity levels (pH 2.8, 3.4, and 4.0), the pH of simulated mist droplets on leaf surfaces increased over time, in the form of saturation curves (Fig. 4). Since the rise in pH was very similar for initial pH levels of 3.4 and 4.0, only the data for pH 3.4 are graphically displayed. Because the changes in acidity of the drops were similar on leaves of both cultivars, only the measurements on Norchip are given in Figure 4. When rooted potato cuttings had been exposed to simulated mist at pH 3.4 or pH 4.0, the pH in drops of 'mist' solutions with the same initial pH levels rose quickly to maximum levels of around pH 7.0–7.5, regardless of the time period of exposure to simulated acidic mist (Fig. 4 and Table 6). However, when cuttings had been treated with acidic mist at pH 2.8, the pH of mist droplets rose more slowly after 6- or 20-hr exposure to acidic mist compared with 0- or 1-hr exposure (Fig. 4 and Table 6). Regression of the increase in pH over time (expressed as the slope of the prediction equation relating measured pH to the logarithm of time) on duration of exposure to mist and pH of mist, indicated that there was a significant interaction between pH and duration of mist ($P = 0.002$). The increase in pH over time was only dependent on the duration of exposure to mist when the initial pH was 2.8, and not when the initial pH was 3.4 or 4.0. With an initial pH of 2.8, the linear effect of duration of mist was significant ($P = 0.05$) and the quadratic effect was almost significant ($P = 0.07$).

DISCUSSION

Pre- plus postinoculation exposure to acidic mist. When potato plants were exposed to simulated mist with the same acidity before

TABLE 4. Lesion numbers and areas per potato plant, and number of spores per square centimeter of infected area related to pH of simulated mist before and after inoculation with *Alternaria solani* or *Phytophthora infestans*

		Alternaria solani			*Phytophthora infestans*		
pH before inoculation	pH after inoculation	Lesions per plant (no.)	Lesion area per plant (cm^2)	Conidia per cm^2 (×100)	Lesions per plant (no.)	Lesion area per plant (cm^2)	Sporangia per cm^2 (×1,000)
2.8	2.8	8.4 ± 1.5[a]	3.5 ± 1.6	13.7 ± 4.3	28.6 ± 5.7	8.0 ± 1.1	9.6 ± 2.5
	4.0	22.0 ± 4.7	3.7 ± 1.1	13.2 ± 5.9	179.1 ± 25.1	17.1 ± 1.2	14.8 ± 2.5
4.0	2.8	12.5 ± 1.9	3.7 ± 1.5	7.7 ± 3.1	38. 2 ± 10.0	7.0 ± 0.8	8.6 ± 2.7
	4.0	17.1 ± 2.7	3.9 ± 1.4	9.9 ± 5.8	142.6 ± 27.5	16.4 ± 0.8	11.8 ± 2.7

[a] Standard error of the mean ($N = 18$).

TABLE 5. Analysis of variance for mean number of lesions and lesion area (cm^2) per potato plant and mean number of spores per square centimeter of infected area, as affected by pH of simulated mist before and after inoculation with *Alternaria solani* or *Phytophthora infestans* (analysis of means of five plants per treatment)

		Alternaria solani			*Phytophthora infestans*		
Source	df[a]	Lesions (no.) SS[b]	Lesion area SS	Conidia (no.) SS	Lesions (no.) SS	Lesion area SS	Sporangia (no.) SS
Block	3	80.9	142.3**	79×10^5	50,529*	38.9	76×10^5
pH[c] before inoculation	1	0.6	0.9	15×10^5	721	2.7	161×10^5
pH[c] after inoculation	1	334.6**[d]	4.3	0.5×10^5	64,999**	342.7**	713×10^5
Interaction[e]	1	83.3*	4.1	0.1×10^5	2,121	0.1	41×10^5
Residual	9	121.3	32.7	77×10^5	25,633	67.5	$6{,}680 \times 10^5$
R^2 (%)		80.4	82.3	55.3	82.2	85.1	12.9
C.V. (%)		24.8	48.6	78.7	55.0	22.6	76.7

[a] Degrees of freedom.
[b] Sequential sums of squares.
[c] pH of simulated mist: pH 2.8 and 4.6.
[d] Significance level: ** significant at $\alpha = 0.01$, * significant at $\alpha = 0.05$.
[e] Interaction between pH before and after inoculation.

and after inoculation with *A. solani* or *P. infestans*, infection efficiency, lesion area, and sporulation were reduced at pH 3.4 and 2.8. A similar trend had been observed for infection by *P. infestans* under laboratory conditions (18). Reductions in several components of the infection cycle could possibly lead to a slower development of epidemics in the field, but Campbell et al (8) reported that disease progression of late blight was not significantly affected by acidity of simulated rain under field conditions. However, simulated acidic mist could have a more profound effect on disease development than simulated acidic rain because the main function of rain in the epidemiology of aerial pathogens is dispersal and loss of propagules from the canopy, whereas that of mist is to promote initial infection processes.

Biotrophic versus necrotrophic pathogens. *P. infestans* can be classified under the biotrophic pathogens. These pathogens have generally been inhibited by simulated acidic precipitation (14), i.e., several rusts (6,28) and root knot nematodes (28), but no distinction was made between pre- and postinoculation acidity stress. In this paper, we demonstrated that infection by *P. infestans* was enhanced after exposure of potato leaves to acidic mist before inoculation.

A. solani is a necrotrophic pathogen. Effects of acidic precipitation on other members of this group varied from negative (Cercospora leafspot on peanut [8], and Goss's leaf blight on corn [20]), to neutral (Scleroderris canker on pine [5], and Septoria brownspot on soybean [8,20]), to positive (Scleroderris canker on spruce [3], southern and northern corn leaf blight [20,28], and Leptosphaerulina leafspot on alfalfa [8]). The differences in response to acidic precipitation were probably due to variations in acidity treatments, buffering capacity of the hosts, and sensitivity of the pathogens to acidity on the host.

Pre- vs. postinoculation exposure. In previous studies on effects of simulated acidic precipitation and disease development, acidic rain was applied during inoculation, after inoculation, or before and after inoculation, but not exclusively before inoculation. However, in this study we demonstrated that the effect of acidic mist on early and late blight development depends on the timing of the acidity stress in relation to the infection cycle. A preinoculation stress at pH 2.8 predisposed potato plants to infection by *A. solani* or *P. infestans* when plants were subsequently exposed to pH 4.0, whereas acidic mist (pH 2.8) applied during and after inoculation reduced infection and further disease development. A similar distinction between pre- and postinoculation acidic rain effects was made for halo blight on beans (28): preinoculation exposure to acidic rain resulted in an increase in halo blight, whereas postinoculation exposure suppressed halo blight. It would be of interest to know whether the same distinction between pre- and postinoculation acidity stress holds for other pathogens. Leben (15) sprayed tomato plants with acidic buffers before inoculation with *A. solani*, resulting in a reduction in early blight development. However, in this case, the pH of the inoculum suspension might have been reduced by the residual buffer on the leaf surface, so that the acidity stress continued during infection processes.

Effects of postinoculation exposure seem to be more important than those of preinoculation exposure, because both pre- and postinoculation exposure to acidic mist at pH 2.8 and 3.4 in one treatment resulted in a reduction of early and late blight on potato. Thus far, only in the cases of southern corn leaf blight on N cytoplasm corn (28), northern corn leaf blight (20), and Leptosphaerulina leafspot of alfalfa (8) was disease development enhanced by pre- plus postinoculation acidic rain treatments. *Exserohilum turcicum* (causal agent of northern corn leaf blight) was one of the few fungi relatively insensitive to pH 3.2 in a buffered agar medium (15). If a pathogen is relatively insensitive to a low pH, the preinoculation acidity effect on the host might override the relatively minor negative effect on the pathogen itself, resulting in an increase in disease. It might be of interest to investigate whether relative sensitivity of germinating propagules to acidity of a buffered medium could give an indication of the sensitivity of a pathosystem to acidic precipitation.

Acidity around the pathogen. The actual pH levels measured in spore suspensions and on potato leaf surfaces were higher than those used in the suspension solution or simulated mist. Germinating spores and leaves have the capacity to neutralize acidic solutions. The response of germinating propagules to acidic precipitation on leaf surfaces will probably depend on the intrinsic sensitivity of the propagules to a low pH and on the neutralizing capacity of the leaf and of the propagules themselves. Conidia of *A. solani* were added to the solution without substrate, and the pH of their suspension rose more slowly than that of a sporangial suspension of *P. infestans* which was obtained from agar plates. The agar neutralized the acidity considerably, so that maximum germination at pH 3.4–4.6 of the original solution corresponded to pH 6.0–6.6 in the suspension.

The neutralizing capacity of leaves depends on plant species (1,9,16,19) but is generally reduced at initial pH levels below 3.0 (1,16). We observed substantial neutralization of simulated mist drops on potato leaves when initial pH levels were above 3.0, even after exposure to acidic mist for 20 hr. Measurements were not made for drops on leaf surfaces exposed to acidic mist at pH 4.6. However, based on similar pH measurements on potato leaves after exposure to simulated acidic rain at pH 2.8–4.6 (*unpublished*), we expect that neutralization of drops with an initial pH of 4.6 would result in a final pH level of about 8. This pH level may have been supraoptimal for *A. solani* and *P. infestans*, so that infection and sporulation sometimes were reduced at pH 4.6 compared with pH 4.0. The pH values on the leaf surface, as measured by flat bottom electrodes, are only estimates of actual acidity on the leaf surface. In the experiments with the pathogens, mist of a lower pH was added continuously to the leaf probably resulting in acidity levels on the leaf between those applied and those measured on the surface.

Possible reasons for increased infection. Neutralization of acidic solutions takes place by cation exchange. Easily leachable cations are K^+, Ca^{++}, Mg^{++}, Na^+, and Mn^{++} (17). Adams and Hutchinson (1) found especially high concentrations of Ca in simulated acidic rain drops, and Parameswaran et al (21) observed drastic reductions in the number of calcium oxalate crystals between mesophyll cells of declining spruce and fir exposed to ambient rain in heavily polluted areas. A reduction in Ca in the cell walls or between cells may render a plant more susceptible to a pathogen (24). This may be one reason why we observed an increase in infection efficiency of both *A. solani* and *P. infestans* when the acid mist stress was applied before inoculation. In a previous study, we observed a similar increase in the number of late blight lesions on potato exposed to acidic rain (pH 2.8) before inoculation (29). Besides a reduction in Ca^{++} ions in the cell walls, erosion of wax layers (10,21), increased availability of nutrients (18), and a reduction in the microflora on the leaf surface (13) might contribute to increased susceptibility to foliar diseases after exposure to strongly acidic simulated precipitation applied before inoculation.

Effects of acidic precipitation on epidemic development. Effects of acidic precipitation on development of foliar diseases in the field would be difficult to predict, because the acidity of ambient rain varies widely from one event to another (11,23,30). Thus, the pH of precipitation events before infection may differ from those during or after infection. Field experiments under different climatological conditions, in which disease progression is monitored during

TABLE 6. Mean pH of simulated mist drops 60 min after they were placed on potato leaves that had been exposed to simulated acidic mist (pH 2.8, 3.4, 4.0) for 0, 1, 5, or 20 hr

Cultivar	pH of mist and initial pH of drops	Hours of exposure to simulated acidic mist			
		0	1	5	20
Norchip	2.8	5.7 ± 0.9[a]	5.0 ± 0.7	4.2 ± 1.6	4.0 ± 0.8
	3.4	7.0 ± 0.7	7.0 ± 0.6	6.9 ± 0.7	6.3 ± 0.9
	4.0	7.0 ± 0.7	6.9 ± 0.7	7.3 ± 0.7	6.6 ± 0.7
Monona	2.8	6.0 ± 1.6	5.0 ± 0.9	4.1 ± 0.7	4.5 ± 0.9
	3.4	7.8 ± 1.1	7.0 ± 0.1	6.6 ± 0.7	7.5 ± 0.7
	4.0	7.6 ± 0.9	7.6 ± 0.5	7.4 ± 0.7	7.7 ± 0.6

[a] Standard deviation.

several infection cycles, would be needed to evaluate the overall sensitivity of a host-pathogen system to acidic precipitation. Thus far, this approach has been followed in only a few cases (8,20).

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