Effects of Simulated Acidic Rain on Host-Parasite Interactions in Plant Diseases

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ABSTRACT


The effects of simulated rain acidified with sulfuric acid were studied on five host-parasite systems. Plants were exposed in greenhouse or field to simulated rain of pH 3.2 or pH 6.0 in amounts and intervals common to weather patterns of North Carolina. Simulated acid rain resulted in: (i) an 86% inhibition of the number of telia produced by Cronartium fusiforme on willow oak (Quercus phellos); (ii) a 66% inhibition in the reproduction of root-knot nematode (Meloidogyne hapla) on field-grown kidney beans (Phaseolus vulgaris 'Red Kidney'); (iii) a 29% decrease in the percentage of leaf area of field-grown kidney beans affected by Uromyces phaseoli; and (iv) either stimulated or inhibited development of halo blight on kidney bean (caused by Pseudomonas phaseolicola), depending upon the stage of the disease cycle in which the treatments were applied. The effect varied as follows: (i) simulated acid rain applied to plants before inoculation increased disease severity by 42%; (ii) suspension of bacteria in acidic rain resulted in no infection; and (iii) acid rain applied to plants after infection inhibited disease development by 22%. Results suggest that the acidity of rain is an environmental parameter which should be of concern to plant pathologists and agricultural and forest ecologists.

Additional key words: pollution effects.

During the past decade the average acidity of rain and snow has increased from $\geq$ pH 5 to $\leq$ pH 4 in many areas of northern Europe and the eastern United States (8, 12). The change has been attributed to increased combustion of fossil fuels and the resulting wide dispersal of sulfur and nitrogen oxides which are converted into the corresponding strong acids in the atmosphere (9).

Acidic rain has been implicated in the increased leaching of nutrients from soils (13), decreased pH value of lakes and streams (15), and changes in fish populations in Sweden (15). Little is known, however, of the effects of acidic precipitation on vegetation. Several investigators have reported development of necrotic leaf spots on various plant species exposed to acid mists and simulated acidic rains (6, 17, 21). Increased nutrient leaching from vegetation by sulfuric acid mist, and decreased total plant weight and height of sugar maple and yellow birch seedlings exposed to artificial rain of pH 2.3 have been noted (5, 21, 22).

Growth and reproduction of many bacteria and certain fungi are inhibited under acidic conditions (7, 19). Bacteria generally are less tolerant of low pH than other types of microorganisms. The relative resistance of bacteria, yeasts, and fungi to acid conditions are in the approximate ratio of 1:45, respectively (14). Growth and infection by the plant pathogens Diplodacron rosae and Phytophthora infestans were inhibited when spores of these fungi were exposed to water through which SO$_2$ had been bubbled (14). The fungicidal action of SO$_2$ was the limiting factor, rather than the acidity (14). Many environmental factors affect host susceptibility to pathogens and subsequent disease development (20). Acidic buffer sprays were investigated by Leben (7) for their effectiveness as protectant sprays, and gave 53-79% disease control of Alternaria solani on tomato. The above evidence suggests that parasitism by some plant pathogens might be affected by acidic precipitation.

The objective of this investigation was to determine whether simulated rain acidified with sulfuric acid influences disease development in plants and, if so, to suggest possible mechanisms that might account for the interaction.

MATERIALS AND METHODS

Acidity levels and ionic concentrations selected for these experiments. The high acidic level, pH 3.2 $\pm$ 0.1, was chosen to approximate the most acidic condition observed in natural precipitation in Europe and North America in the early 1970's; and the lower acidic level, pH 6.0 $\pm$ 0.2, was chosen to approximate the acidity that would be expected in precipitation formed in an atmosphere relatively free of contamination with oxides of sulfur and nitrogen. The only exception was with southern corn leaf blight, for which pH 3.5 and pH 7.0 were used. Sulfuric acid was selected because it is a major source of acidity in precipitation (9). Based on the amounts of and presumed stoichiometric balance among ions in natural rain, sulfuric acid probably accounts for
about two-thirds of the total acidity (3) currently observed in precipitation in eastern North America.

Artificial solutions of deionized water were used in these experiments rather than natural rain water. The ion concentrations in the solutions were as follows (expressed in mg/liter): calcium, 0.14; magnesium, 0.04; potassium, 0.06; sodium, 0.09; ammonium, 0.22; nitrate, 0.12; chloride, 0.19; and sulfate, < 0.02, or 27.0 to 50.0, depending on treatment. Calcium, Mg, K, and Na were analyzed by atomic adsorption; NH₄, NO₃, Cl, and SO₄, by means of a Technicon Auto Analyzer (Technicon Instruments Corp., Tarrytown, NY 10591).

A portable apparatus, described previously (16), was used to apply controlled amounts of artificial rain. This apparatus distributed "rain" over an area 2.4 m x 2.4 m in the field or greenhouse. The mean droplet diameter was 0.9 mm and the rate of precipitation was approximately 3 cm per hr. Thus, the apparatus reasonably approximated the droplet size and intensity of natural rain (11). The term "rain" is used throughout this paper to refer to deionized water solutions applied to plants with the above apparatus.

**Greenhouse Studies.**—Bean seeds, *Phaseolus vulgaris* L. (Red Kidney), and willow oak acorns, *Quercus phellos* L., were planted in vermiculite. Kidney bean seedlings were transplanted to 10-cm-diameter clay pots in a loam:peat:sand (3:1:1, v/v/v) potting mix when unifoliate leaves were half-expanded. Willow oak seedlings were transplanted to 15-cm-diameter clay pots in the potting mix when the first leaves were fully expanded.

Kidney beans were inoculated with commercial *Rhizobium* inoculum (Nitrification Co., Milwaukee, WI 53209) by pouring 10 ml of a water suspension (20 mg/ml) onto the soil of each pot. Plants were arranged at random beneath the rain simulation apparatus. The photoperiod was extended to 16 hr by supplementing natural sunlight with 12,000-lux fluorescent light.

**Oak-pine rust.**—Beginning at transplanting, 6-wk-old willow oak seedlings were exposed to pH 3.2 or pH 6.0 "rain" (0.63 cm/10 min/day) for 14 days prior to inoculation. *Aeciosporium* (Collection No. TNC-73 from E. G. Kubel) of *Cronartium fusiforme* Hedge, and Long in vacuum-sealed ampules were rehydrated for 24 hr at 20 C. Approximately 180 mg of spores and five drops of Tween-20 (Fisher Scientific Co., Pittsburgh, PA 15219) were added to 100 ml of simulated rain water of pH 3.2 or pH 6.0, and allowed to soak for 1 hr. The spore suspensions then were applied to the adaxial surface of newly expanded oak leaves with a chromatography sprayer (VWR Scientific, Atlanta, GA 30324) until the leaf surface was wetted. The inoculated seedlings then were covered with clear polyethylene bags to maintain relative humidity near 100% for 24 hr at 20-22 C. Plants were exposed to "rain" of pH 3.2 or pH 6.0 for 14 additional days. The total amount of "rain" applied during the 29-day period was 17.6 cm. Twenty-nine days after transplanting, the numbers of infected leaves per plant and numbers of telia per infected leaf were counted on six plants per treatment.

**Southern corn blight.**—Three-wk-old corn (Zea mays L.) seedlings of one Texas male-sterile (CMS-T) and one normal (N) cytoplasm of the B-37 line were inoculated with *Helminthosporium maydis* Nisakako (Race T isolate Ch 270, from A. J. Julis). Conidia of the fungus were suspended (5,000 spores/ml of water) in water of pH 3.5 or pH 7.0 for 1 hr, and the suspensions of conidia were sprayed on corn seedlings until the leaves were uniformly wet. The inoculated plants were incubated in a mist chamber for 18 hr, and then exposed to "rain" of pH 3.5 or pH 7.0 for 10 min daily (approximately 0.25 cm/day) for 21 consecutive days. The number of lesions on the second, third, and fourth leaves, and the number of dead leaves per plant were counted 22 days after inoculation (35 days after planting).

**Halo blight of kidney bean.**—Beginning 1 day after transplanting, kidney beans were treated with simulated rain of pH 3.2 or pH 6.0 for 10 min (0.63 cm/day) for 10 days prior to inoculation. *Pseudomonas phaseolicola* (Burk.) Dows. (isolate 507-1, received from L. T. Lucas) cells were washed from 5-day-old cultures on nutrient agar plates and suspended in simulated rain water of pH 3.2 or pH 6.0 for 2 hr prior to inoculation. The concentration of the suspension was adjusted to approximately 10⁷ cells/ml (OD = 0.25 at 530 nm).

Abaxial surfaces of the first and second trifoliate leaves were sprayed with the suspension from a distance of about 5 cm until the leaves were visibly watersoaked. Inoculated leaves were covered with polyethylene bags and incubated for 48 hr. To provide additional opportunity for establishment of the pathogen after the plastic bags were removed, simulated rain (10 min, 0.63 cm/day) of the same acidity as the inoculum was applied for three days. Then the plants received 11 consecutive days of post-inoculation treatment of simulated rainfall of pH 3.2 or pH 6.0. The combinations of pre-, during-, and postinoculation treatments are outlined in Table 2. Plants were exposed to a total of 13.9 cm of "rain" over the 26-day period.

Beginning 4 days after inoculation, each inoculated leaf was observed daily for 14 days for possible symptoms of halo blight. Sixteen days after inoculation, the number of dead inoculated leaflets was counted. Only leaflets that had shown symptoms of halo blight were considered to have died from the disease. Plants also were measured for root and shoot fresh weights, number of bean pods greater than 2.5 cm long, and root nodule number.

**Field studies.**—Field plots were planted at the North Carolina Agricultural Experiment Station Research Farm Unit 2 in rural Wake County to validate the effects of acidic "rain" on kidney bean nodulation observed in the greenhouse tests. In two experiments, the treatments were replicated six times in a complete, randomized block design, and were applied to the same plots throughout both experiments. Soil of the field site is characterized as a Typic Hapudult: Clayey, Kaolinitic, Thermic. Each plot consisted of five rows 3 m in length, 0.6 m apart, and 5 cm between seeds within the rows. The seeds were coated with a dry powder of commercial *Rhizobium* inoculum before planting. Treatment effects were measured on plants in the center-1.8 m of the three center rows of each plot.

"Rain" was applied in addition to natural rainfall to simulate the amounts and intervals common to weather patterns of North Carolina. Starting one day after planting, 0.25 - 0.63 cm of simulated rain of pH 3.2 or pH 6.0 was applied three alternate days per week for 9 wk (crop I) and 8 wk (crop II). The amounts of "rain" applied in the field, approximately 7.6 cm/month, were in
<table>
<thead>
<tr>
<th>Host-pathogen system</th>
<th>Acidity and duration of simulated rain</th>
<th>Disease measure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preinoculation (pH) (time-days)</td>
<td>Inoculation (pH) (time-hr)</td>
</tr>
<tr>
<td></td>
<td>3.2 (14)</td>
<td>3.2 (24)</td>
</tr>
<tr>
<td></td>
<td>6.0 (14)</td>
<td>6.0 (24)</td>
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<tr>
<td>Zea mays–Helminthosporium maydis</td>
<td>...</td>
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<td>7.0 (18)</td>
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<td></td>
<td>...</td>
<td>7.0 (18)</td>
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<tr>
<td>Field studies:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaseolus vulgaris–Meloidogyne hapla</td>
<td>3.2 (nd(^d))</td>
<td>3.2 (nd)</td>
</tr>
<tr>
<td>Phaseolus vulgaris–Uromyces phaseoli</td>
<td>6.0 (nd)</td>
<td>6.0 (nd)</td>
</tr>
</tbody>
</table>

\(^a\) Asterisk (*) indicates analysis of variance significant difference, \(P = 0.05\).
\(^b\) Each value is the mean of six plants per treatment.
\(^c\) Each value is the mean of three plants per treatment; values in a column followed by the same letter were not significantly different, \(P = 0.05\). Duncan's multiple range test indicates means with same letter are not significantly different.
\(^d\) Texas male-sterile cytoplasm.
\(^e\) Normal cytoplasm.
\(^f\) Each value is the mean of three plants per treatment.
\(^g\) Each value is the mean of 18 plants per treatment.
\(^h\) Percent of root area galled by Meloidogyne hapla, Field Crop II only.
\(^i\) Not determined for field studies; total exposure duration 60 days.
\(^j\) Data are for Field Crop I only, each value is the mean of 18 plants per treatment.
addition to the average of 11.3 cm/month of natural rain which fell during the same period.

At harvest, three 0.3-m sections of treatment rows were randomly selected from each replicate plot. The center three plants of each 0.3-m section comprised a sample for crop I. For crop II, the center five plants of each 0.3-m section comprised a sample.

Root-knot nematode and bean rust on kidney bean.—During the growth of the first crop, root-knot nematode galls were found on plants in a portion of the plot area. Periodic sampling and analysis revealed extensive galling of bean roots and a high population of *Meloidogyne hapla* Chitwood larvae in the soil of the plot area during the second crop. Therefore, the severity of root galling was estimated and the *M. hapla* eggs were extracted from roots of three randomly chosen plants from each plot (2). The percent of root area affected by gall formation was evaluated prior to extracting the eggs.

A natural epiphytoty of bean rust, *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth., developed on both crops of kidney beans. The percent of leaf area covered with rust pustules was estimated on 4th trifoliolate leaves at 7 wk, and on 6th trifoliolate leaves 9 wk, after emergence. Three randomly selected plants per plot were sampled.

**RESULTS**

**Greenhouse Studies.—Oak-pine rust.**—Willow oak seedlings that received pH 3.2 “rain” had 42% fewer leaves infected by *C. fusiforme* than those that received pH 6.0 “rain”. The infected leaves exposed to pH 3.2 “rain” developed 86% fewer *C. fusiforme* telia per leaf than infected leaves exposed to pH 6.0 “rain” (Table 1).

Leaves exposed to pH 3.2 “rain” appeared to be lighter green, smaller, more brittle, and more irregularly shaped, than those exposed to pH 6.0 “rain.” Necrotic leaf lesions 1-3 mm in diameter were characteristic of direct tissue injury by acidic “rain.”

**Southern corn blight.**—Regardless of inoculum pH, the number of leaves (per plant) that died from *H. maydis* infection was the same for both host cytoplasm lines (CMS-T and N), when plants were exposed to postinoculation “rain” of pH 3.5 (Table 1). The response of plants exposed to pH 7.0 postinoculation “rain” varied with host cytoplasm line, but not with inoculum pH. Greatest treatment differences occurred between the pH 3.5 and pH 7.0 postinoculation treatments with the N cytoplasm line (Table 1).

The number of *H. maydis* lesions per leaf was not affected on CMS-T hosts. On N hosts, however, more lesions developed on plants which received both pH 3.5 postinoculation treatment, and pH 3.5 inoculum (Table 1).

**Bean halo blight.**—“Rain” of pH 3.2 either inhibited or stimulated development of halo blight, depending upon the part of the disease cycle during which the “rain” was applied (Table 2). Preinoculation exposure of the plants to pH 3.2 “rain” resulted in more halo blight leaflet death than plant exposures to pH 6.0 “rain” (4.2 and 4.8, compared to 2.0 and 3.2 leaflets per plant, respectively).

Inoculum suspended and applied in water at pH 6.0 caused symptoms in most plants, but when the inoculum was applied in water at pH 3.2, it was apparently inactivated, because no symptoms developed. Plants exposed to pH 3.2 “rain” after inoculation and initial infection, developed less halo blight than those exposed to pH 6.0 “rain.” This difference was only significant for plants that had received preinoculation “rain” of pH 6.0. No consistent relationship was observed between the amount of halo blight and the fresh weight of shoots or roots, or the number of pods or nodules formed per plant. Shoot fresh weight and numbers of nodules per plant were generally smaller for plants exposed to pH 3.2 “rain” prior to inoculation, compared to plants exposed to “rain” of pH 6.0 (Table 2).

**Field Studies.**—Root-knot nematode and bean rust on kidney bean.—“Rain” of pH 3.2 inhibited reproduction of *M. hapla* as measured by total egg counts per root system. Kidney bean plants that had received “rain” of pH 3.2 during the second crop had 34% as many eggs per root

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**TABLE 2. Effects of simulated rain acidified with sulfuric acid on the development of halo blight, and on growth and nodulation of Red Kidney beans**

<table>
<thead>
<tr>
<th>Preinoculation (10 days)</th>
<th>Inoculation* (5 days)</th>
<th>Postinoculation (11 days)</th>
<th>Halo blight, dead leaflets (%)</th>
<th>Nodules/ plant (no.)</th>
<th>Fresh weight/plant</th>
<th>Pods 2.5 cm long/plant (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>0.0*</td>
<td>4.6*</td>
<td>4.48*</td>
<td>2.4*</td>
</tr>
<tr>
<td>3.2</td>
<td>3.2</td>
<td>6.0</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.0*</td>
<td>1.4*</td>
</tr>
<tr>
<td>3.2</td>
<td>6.0</td>
<td>3.2</td>
<td>0.0*</td>
<td>1.4*</td>
<td>1.4*</td>
<td>1.6*</td>
</tr>
<tr>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.8*</td>
</tr>
<tr>
<td>6.0</td>
<td>3.2</td>
<td>3.2</td>
<td>0.0*</td>
<td>1.4*</td>
<td>1.4*</td>
<td>1.4*</td>
</tr>
<tr>
<td>6.0</td>
<td>3.2</td>
<td>3.2</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.8*</td>
</tr>
<tr>
<td>6.0</td>
<td>6.0</td>
<td>3.2</td>
<td>0.0*</td>
<td>2.0*</td>
<td>12.2</td>
<td>2.8*</td>
</tr>
<tr>
<td>6.0</td>
<td>6.0</td>
<td>3.2</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.8*</td>
</tr>
<tr>
<td>L.S.D. (P = 0.05)</td>
<td>0.8</td>
<td>3.5</td>
<td>0.52</td>
<td>0.39</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

*Plants were covered with plastic bags at inoculation for 48 hr. After bags were removed, plants received additional “rain” treatments (0.63 cm/day) of the same pH as the inoculum, for 3 days prior to initiation of the “postinoculation” treatments.

*Number of inoculated leaflets (maximum six per plant) which were dead on plants which developed symptoms characteristic of halo blight.

*Each value is the mean of five plants.

*Asterisk (*) indicates values significantly different from the 6.0/6.0/6.0 control, P = 0.05.
system as plants that had received pH 6.0 "rain." Plants in the pH 3.2 "rain" plots had only 48% as much root surface galling as did plants in the pH 6.0 plots (Table 1).

Shoot, root, and pod fresh weights were not affected by treatment or numbers of nematodes. Acidity of the field plot soil was pH 6.3 when the experiments began, and was not changed by the treatments during the period of the experiment.

Observations on field-grown kidney beans indicated that bean rust development was slightly delayed on plants receiving pH 3.2 "rain." Differences between "rain" treatments in percentage leaf area affected by U. phaseoli occurred on the first crop after seven wk but not after 9 wk (Table 1). There were no differences between treatments in the percent leaf area affected by U. phaseoli 7 or 9 wk after the second crop was planted.

DISCUSSION

All five host-parasite systems were affected to some degree by simulated rain acidified with sulfuric acid to pH 3.2-3.5. With bean halo blight, the sensitivity of P. phaseolicola to low pH was probably the most critical factor. Pseudomonas phaseolicola is reported not to grow at pH values below 5.0-5.3 (1) and was unable to survive 48 hr in nutrient broth acidified below pH 4.0. Bacterial cells were viable after 2 hr in water of pH 3.2, but were noninfective to kidney bean plants when compared to inoculum treated similarly in water of pH 6.0. Preinoculation treatment with pH 3.2 "rain" predisposed the plants to greater disease development, and a postinoculation acid "rain" reduced disease development.

The predisposition of bean plants to greater disease development from a preinoculation treatment with pH 3.2 "rain" suggests a stress of the host plant. There may have been increased avenues of penetration available to P. phaseolicola because of injury to the leaf surface by pH 3.2 "rain" (6, 16, 21). In addition to this visible injury, water droplet-induced wounding of the leaf cuticle (10)—an established barrier to plant infection, may also result in predisposition of the host. Postinoculation inhibition of disease development by "rain" of pH 3.2 may have been due to stress-induced changes in the host plant making it less susceptible to disease development.

The effects of pH 3.2 "rain" on the growth of willow oaks, combined with the high susceptibility of immature willow oak leaves to infection by C. fusiforme (4, 18) suggest that pH 3.2 "rain" may have altered the susceptibility of the oak leaves. This would account for the fewer infected leaves, and telia per leaf which were observed in treated than in nontreated plants. The results of the experiment, do not, however, eliminate the possibility that acidic "rain" directly affected the pathogen.

"Rain" of pH 3.2 may have altered kidney bean physiology, thus indirectly affecting M. hapla. Direct effects of "rain" of pH 3.2 on free-living stages of M. hapla may have occurred from short-term acidification of soil water, but it is unlikely that endoparasitic stages of the nematode, protected within root tissues, would have been directly affected.

The observed inhibition of bean rust by simulated acidic rain on naturally infested field plots suggests a delay in disease development. Treatment differences detectable at 7 wk, however, were no longer evident at 9 wk. No differences in rust incidence or severity were detectable with the second crop of beans perhaps because of the large amount of inoculum from an adjacent bean field. Effects on photosynthetic rate and carbohydrate allocation mechanisms also may have had a role in the plant's decreased susceptibility to U. phaseoli.

Development of southern corn blight probably was affected only slightly by "rain" of pH 3.2 because of the high virulence of the pathogen and the relatively high susceptibility of the host lines tested. In addition, the total amount of "rain," 2.7 cm, was relatively small, compared to the amounts applied in the other studies reported.

The relevance of the results of these investigations must be viewed with an appropriate regard for the limitations of the experiments themselves: (i) the acidity ranges of pH 3.2-3.5 and pH 6.0-7.0 are extremes of the range of rainfall acidity naturally occurring in Europe and North America; (ii) sulfuric acid was the sole donor of excess hydrogen ions in the simulated rain; and (iii) the solutions applied contained very low concentrations of other ions and substances which may occur in combination with strong acid in natural rain.

From these experiments it is impossible to assert the threshold pH levels at which biological effects could be detected or become significant. These investigations were developed on the basis of data by Likens and Bormann (8) who report H+ is the dominant cation in precipitation in the northeastern United States, and accounts for 69% (miliquivalent basis) of the cations in precipitation at their New Hampshire sampling point. Of anions, 99% of the total were accounted for by SO4²-, NO3⁻, and Cl⁻ (8).

The results of the present investigation provide evidence that the acidity of rain is an environmental parameter that can have significant biological consequences, suggesting that chemistry of rainfall should be monitored with other commonly measured environmental factors.

The results of this study have demonstrated hydrogen and sulfate ions in concentrations similar to those known to occur in "worst case" events of natural acidic rains can affect the processes of infection and disease development; research is now needed to determine whether the lower levels of acidity in naturally occurring acidic rains cause similar effects in natural and man-made ecosystems. The need for greater awareness of this environmental parameter is suggested.

LITERATURE CITED


