EFFECTS OF SIMULATED RAIN ACIDIFIED WITH SULFURIC ACID ON HOST-PARASITE INTERACTIONS[1,2]

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ABSTRACT

Wind-blown rain, rain splash, and films of free moisture play important roles in the epidemiology of many plant diseases. The effects of simulated rain acidified with sulfuric acid were studied on several host-parasite systems. Plants were exposed, in greenhouse or field, to simulated rain of pH 3.2 ± 0.1 or pH 6.0 ± 0.2. Simulated "rain" of pH 3.2 resulted in: 1) an 86% inhibition in telia production of *Cronartium fusiforme* on *Quercus phellos*; 2) a 66% inhibition of reproduction of *Meloidogyne hapla* on field-grown *Phaseolus vulgaris*; 3) a 10% decrease in the severity of *Uromyces phaseoli* on field-grown *Phaseolus vulgaris*; and 4) an inhibition of *Rhizobium* nodulation of *Phaseolus vulgaris* and *Glycine max* by an average of 73%. Effects on halo blight of kidney bean (caused by *Pseudomonas phaseolicola*) depended upon the segment of the disease cycle in which the "rain" occured: a) simulated rain of pH 3.2 applied to plants before inoculation stimulated disease development; b) suspension of inoculum in "rain" of pH 3.2 decreased inoculum potential; and c) "rain" of pH 3.2 applied to plants after infection inhibited disease development. Scanning electron microscopy of epicuticular waxes on leaves of *Quercus phellos* and *Phaseolus vulgaris* showed marked erosion of those surfaces by "rain" of pH 3.2, indicating possible influences on the structure and function of plant cuticles.

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These results suggest that the acidity of rain is a new parameter of environmental concern, and underline the need for study of the consequences of prolonged exposure of both agronomic and natural ecosystems to this stress factor.

INTRODUCTION

Preliminary studies of effects of acidic precipitation on vegetation have suggested that significant direct effects on plant productivity, as measured by growth and yield, are unlikely to be of major short-term importance under conditions of precipitation acidity currently occurring over widespread areas of N.A. and Europe (Wood and Bormann, 1974). Of equal interest, however, are the possible indirect effects, chronic and subtle in nature, which may result in long-term effects on ecosystems as a result of man's increased burdening of such systems with acidic precipitation. Typical of such indirect effects are the interactions of plants as hosts with the large variety of organisms capable of parasitizing those plants.

Many plant-parasitic microorganisms require wind-blown rain, rain splash, or films of free moisture for dispersal, movement on leaf surfaces, and establishment, growth and reproduction of these organisms once dispersed (Walker, 1957).

For these reasons, the effects of a simulated rain solution acidified with sulfuric acid were studied with a range of host-parasite systems.

MATERIALS AND METHODS

Seeds of Red Kidney beans *(Phaseolus vulgaris* 'Red Kidney') were either planted in field plots or germinated in vermiculite, and transplanted after one week to 10 cm clay pots in a greenhouse. Plants were inoculated with commercial *Rhizobium* inoculum (Nitragin Co., Milwaukee, Wisc.) at planting or transplanting. Detailed methodology has been discussed (Shriner, 1974). Plants were subsequently exposed to sulfuric acid solutions as simulated rain. Chemical constituents of the base water solution were chosen to approximate concentrations of major ions known to be present in natural precipitation. Concentrations of sodium, calcium, magnesium, potassium, ammonium, nitrate, chloride, and sulfate in the simulant are given in Table I, as compared to average concentrations occuring at four research watersheds in the United States. Two conditions of acidity were compared, pH 6.0 ± 0.2 and pH 3.2 ± 0.1, with sulfuric acid being the primary source of added

Table I. Comparison of chemical composition of the rainfall simulant with natural rainfall collected at four watersheds in the U.S.

	Na^+	Ca^{++}	Mg^{++}	K^+	NH_4^+	NO_3^-	Cl^-	$SO_4^=$
	Concentration, mg/ℓ							
pH 6.0 ± 0.2 Simulant	0.09	0.14	0.04	0.06	0.22	0.12	0.19	< 0.20
pH 3.2 ± 0.1 Simulant	0.09	0.14	0.04	0.06	0.22	0.03	0.19	50-70
Walker Branch-[1] Watershed, TN	0.26	0.93	0.14	0.22	0.13	0.26	--	--
H. J. Andrews-[1] Watershed, OR	0.08	0.21	0.04	0.01	0.02	0.01	--	--
Coweeta-[1] Watershed, NC	0.22	0.25	0.05	0.11	0.02	0.14	0.35	--
Hubbard Brook-[2] Watershed, NH	0.12	0.21	0.06	0.09	0.22	1.31	0.42	3.1

[1]Henderson, 1975.

[2]Likens and Bormann, 1972.

acidity. The two acidity conditions were chosen because pH 6.0 reasonably approximates the equilibrium pH of CO_2 dissolved in water, and thus, a theoretical "unpolluted" rain; pH 3.2 represents an acidity of rainfall at the lower end of the range of so-called "acid" rainfall as determined from measurements in both the eastern United States and northern Europe (Likens and Bormann, 1974). Likens and Bormann (1974) found sulfate to account for 62% of the anions (milliequivalent basis) in precipitation at their New Hampshire sampling point.

The simulated rainfall was applied to the plants by means of a simulation apparatus capable of delivering droplet sizes in the range of 0.6 - 1.2 mm diameter. Droplets of mean drop size (0.9 mm) achieved approximately 80% of terminal velocity (Shriner, 1974). The simulant was applied daily for six days per week in greenhouse studies, and three days per week in field studies, where applications of the simulant were superimposed on natural rainfall incident at the site. Amounts and frequencies of simulant to be applied were based on five-year averages of precipitation data from a weather station at the field site. Simulant was applied at rates of 2.5 to 3.8 cm/hr for weekly totals between 1.9 and 5.7 cm.

RESULTS

Five host-parasite systems were investigated. In three of these five cases, significant inhibition of some parameter of disease development occurred (Table II). Production of telia of the fusiform

Table II. Effects of simulated rain acidified with sulfuric acid on host-parasite interactions.

Host-Pathogen System	Acidity of Simulated Rain: Pre-inoculation (pH)	Inoculation (pH)	Post-inoculation (pH)	Disease Measure	
Greenhouse Studies				Infected leaves/plant[1]	Telia/Infected Leaf[1]
Quercus phellos-Cronartium fusiforme Oak-Pine Rust of Oak	3.2	3.2	3.2	3.8*	15*
	6.0	6.0	6.0	6.5	115
Phaseolus vulgaris-Pseudomonas phaseolicola Holo Blight of Bean				Dead leaflets/plant[2,3] (no.)	
	3.2	3.2	3.2	0.0a[4]	
	3.2	3.2	6.0	0.0a	
	3.2	6.0	3.2	4.2d	
	3.2	6.0	3.2	4.8d	
	6.0	3.2	3.2	0.0a	
	6.0	3.2	6.0	0.0a	
	6.0	6.0	3.2	2.0b	
	6.0	6.0	6.0	3.2c	
			LSD .05	0.77	
Field Studies				Eggs/plant[5]	%root galled[5,6]
Phaseolus vulgaris-Meloidogyne hapla Root Knot Nematode on Beans	3.2	3.2	3.2	74*	26
	6.0	6.0	6.0	217	50
Phaseolus vulgaris-Uromyces phaseoli Rust of Beans				% leaf area affected at: 7 weeks	9 weeks
	3.2	3.2	3.2	22*	48
	6.0	6.0	6.0	31	45

*ANOVA significant, p=0.05

[1] Each value is the mean of 6 plants/treatment.

[2] Number of inoculated leaflets (max. 6/plant) which were dead on plants which developed symptoms characteristic of halo blight.

[3] Each value is the mean of 5 plants.

[4] Values in a column followed by the same letter were not significantly different, $LSD_{.05}$.

[5] Each value is the mean of 18 plants/treatment.

[6] Percent of root area galled by Meloidogyne hapla.

rust organism *(Cronartium fusiforme)* on the host willow oak *(Quercus phellos)* was inhibited by 86%. Natural epidemics of two pathogens were inhibited by "rain" of pH 3.2: reproduction of *Meloidogyne hapla*, a root-knot nematode was inhibited by 66% on field-grown kidney beans; there was a delay in the development of a bean rust *(Uromyces phaseoli)* epidemic on field-grown kidney beans -- this was evident after seven weeks, but not after nine weeks.

Effects on halo blight of kidney beans (caused by *Pseudomonas phaseolicola*) depended upon the segment of the disease cycle in which the acidic treatment occurred: a) simulated rain of pH 3.2 applied to plants before inoculation stimulated disease development; b) suspension of inoculum in "rain" of pH 3.2 decreased its capacity to induce disease; and c) "rain" of pH 3.2 applied to plants after infection inhibited disease development (Table II).

During routine observation of kidney beans for these host-pathogen studies, an apparent difference was observed in nodulation of the bean plants by the symbiotic nitrogen-fixing bacteria of the genus *Rhizobium*. Experiments were subsequently established to study this effect.

Greenhouse and field studies with kidney bean and greenhouse studies with soybean *(Glycine max* 'Lee'), all resulted in significant (>65%) inhibition of nodule formation on root systems of plants treated with "rain" of pH 3.2 (Table III). Assay of the plants by the acetylene-reduction technique showed a decrease in nitrogenase activity proportionate to the degree of inhibition of nodule formation. Inhibition of nodulation was accompanied by growth reductions most frequently when the plants were cultured in soils of low cation exchange capacity (< 3 me/100g whole soil). No consistant decreases in plant growth or yield were correlated with the observed effect on nodulation when the plants were grown in soils of higher cation exchange capacity (>3 me/100g whole soil), however. Studies of the relationship of time and duration of exposure suggested that the greatest inhibition occurred when the "rain" was applied during the period of greatest increase in nodule activity (Stewart, 1966). Since nodule activity requires host substrates, the removal of such materials by leaching may be important to the mechanism of nodule inhibition (Stewart, 1966). This aspect of the problem is currently being investigated by the author.

Examination of leaf surfaces of kidney beans with the scanning electron microscope following treatment with the "rain" revealed extensive erosion of the cuticular waxes of leaves exposed to solutions of pH 3.2 when compared to controls. Shriner (1974) has recently reviewed the functional role of plant cuticles in relation to these observations. Of greatest importance to the observed effects on host-parasite interactions are the role of cuticular waxes as a physical barrier to fungi and bacteria, and the efficiency of the cuticle as a barrier to loss of water from leaves. Alterations of either of these functions could help to explain the observed predisposition of host plants to greater disease development.

Table III. Effect of "rain" acidified with sulfuric acid on Rhizobium nodulation and growth of leguminous plants.

Species and Experiment No.	Simulated Rain (pH)	Nodules/ Plant (No.)	Dry Wt.[3] /nodule (mg)	Fresh Weight/Plant Shoots (g)	Roots (g)	Pods (g)
FIELD						
Kidney bean-1[4]	6.0	29	0.62	43.70	4.10	15.10
	3.2	7*	0.76	42.30	3.80	13.60
GREENHOUSE						
Kidney bean-1[5]	6.0	15	0.67	6.05	7.88	1.86
	3.2	1***	0.40	5.96	5.74	1.81
Kidney bean-2[5]	6.0	49	0.60	9.63	9.34	5.26
	3.2	11*	0.60	5.63*	8.14*	3.05*
Soybean-3[2,6]	6.0	65**	0.52	3.55	4.62	--
	3.2	24	0.51	3.02	3.53	--

[1]*Phaseolus vulgaris* 'Red Kidney'

[2]*Glycine max* 'Lee'

[3]Dried at 85 C for 24 hours

[4]Each value is the mean of 6 replications, each with 15 plants

[5]Each value is the mean of 5 replications, each with a single seedling

[6]Each value is the mean of 8 replications, each with a single seedling

*Difference significant at p=0.05; **p=0.01; ***p=0.001

SUMMARY AND CONCLUSIONS

Many environmental stress factors play important roles in the disease cycles of plant parasites. These factors may be active on the parasitic organism, on its host, or on the host-parasite complex. Results of this study demonstrate that solutions of an acidity similar to that known to occur in natural acidic rains can affect processes involved in host-parasite interactions. These results are presented with recognition and emphasis of the limitations that must be considered when interpreting their significance and implications to natural biological systems: a) the pH values selected for investigation - pH 3.2 and pH 6.0 - represent only the approximate extremes of acidity observed in natural rainfall; b) sulfuric acid was assumed to be a major component of the acidity in ambient precipitation; and c) the relative effects of cations and anions in the simulated "rain" can not be distinguished.

With those limitations in mind, however, the results underline the need for study of the consequences of prolonged exposure of both agronomic and natural ecosystems to the subtle, indirect influences of acidic precipitation on ecosystem structure and function, even in the absence of obvious direct injury to these systems.

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