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Effects of Simulated Acid Precipitation on Growth and Nodulation of Leguminous Plants

F.-H. Chang[1] and M. Alexander

Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, NY 14853-0144

Nitrogen is frequently the element limiting primary productivity in terrestrial ecosystems. Because chemical fertilizers are not applied to natural ecosystems, nodulated legumes in these ecosystems may be significant sources of nitrogen for the growth of nonleguminous plants. For this reason, any reduction in nitrogen fixation by legumes in natural ecosystems exposed to acid precipitation is of considerable importance.

Acid precipitation may affect legumes by its influence either on the foliage or by altering the chemical or biological properties of the soil in a manner that is detrimental to the plants. Attention has been recently given to the effect of acid rain on several leguminous species. These studies demonstrated that the means of exposing the plants to acid rain resulted in no inhibitory effect on acetylene reduction by alfalfa at acidities above pH 2.0 (PORTER and SHERIDAN 1981), inhibited nodulation of kidney beans and soybeans at pH 3.2 (SHRINER and JOHNSON 1981), but had no significant effect on soybean growth and seed yield at pH 3.2 (IRVING and MILLER 1981). These investigations thus show that plants are sometimes affected deleteriously, but the studies were conducted using nonacid, agricultural soils unlike those representative of the acid soils common in the northeast.

Although acid rain may have no direct influence on the aboveground portion of a plant, acid solutions may alter properties of the soil that result in harm to the root system or to nodule formation or activity. The adverse influence of soil acidity on legumes has long been known (BARBER 1967). MUNNS (1968) and VINCENT (1965) differentiated among factors related to soil acidity and high H^+ activity in the soil and among the effects of these factors on the rhizobia, nodulation, and the plant and its function. Among the factors that are believed to be involved are H, Ca, Al, Fe, or Mn ions. Hydrogen ions and Ca affect nodulation and nitrogen fixation of alfalfa and clovers (LONERAGAN and DOWLING 1958, MUNNS 1970), and more Ca is needed to promote adequate nodulation with increasing acidity. Although Al or Mn toxicity may be of importance (DOBEREINER

[1]Present address: Center for Environmental Studies, Bemidji State University, Bemidji, Minnesota 55601 USA.

1966, SCHMEHL et al. 1950), HOYT and NYBORG (1972) reported that nitrogen fixation by alfalfa may be restricted by soil acidity even without the presence of toxic amounts of Al and Mn.

Despite the large literature on the development of legumes in acid soils and factors involved in the often poor growth, the possible impact of acid precipitation on legumes in natural ecosystems of the northeastern United States remains uncertain. Not only are the properties of these soils different from those of land used for agricultural crops but the acid enters only at the surface, and the surfaces of unfarmed soils are not regulargly mixed by agricultural operations, in contrast with farm lands. Hence, an investigation was conducted to determine the effects of simulated acid rain on two legumes in forest soils of the northeast. One agricultural soil was also tested for comparative purposes.

MATERIALS AND METHODS

Soil samples were collected from the Panther Lake (Potsdam-Crary series, coarse-loamy, mixed, frigid Typic Fragiorthod), Sagamore Lake (Adams series, mixed, frigid Typic Haplorthod), and Woods Lake (Berkshire series, coarse-loamy, mixed, frigid Typic Fragiorthod) watersheds of the Adirondacks region of New York. The pH values of these soils were 4.1, 3.1, and 3.9 for the organic layers and 4.6, 3.9, and 4.1 for the mineral layers, respectively. The first two soils were from under a coniferous canopy, and the third was taken under a deciduous canopy. The organic layer was to a depth of 15 cm, and the samples of the mineral soil were from a depth of 15-40 cm. In one study, an agricultural soil (Howard silty clay loam, glossoboric Hapludalf, loamy-skeletal, mixed, mesic, pH 6.7) was used.

The simulated rain was formulated as described by COGBILL and LIKENS (1974) and EVANS and RAYNOR (1976). The pH was adjusted with 1.0 N HCl or 1.0 N KOH. Except as stated otherwise, 500 mL of simulated rain was added every other day for 14 d to each pot, and 1500 mL was applied daily for 14 d to each root box before the seeds were sown. The solutions were carefully poured to avoid disturbing the soil and to distribute the liquid evenly over its surface. This quantity of solution was equivalent to 100 cm of precipitation.

In one study, the plants were grown in root boxes (12.5 cm wide, 60 cm long, 21 cm deep) having a glass side placed at a 45° angle to the vertical. The soils were mechanically mixed for 20 min with a commercial legume inoculant, and then each root box received the simulated rain. Surface-sterilized seeds were sown 2 days after the simulated rain application was completed. Red clover was grown in one half of the root box, birdsfoot trefoil in the second. In this instance, the data represent the means of triplicate root boxes, each containing 15 plants.

Seeds of Arlington red clover (Trifolium pratense L.) and Viking birdsfoot trefoil (Lotus corniculatus L.) were selected for uniformity of size and were surface-sterilized by immersion for 1 min in 95% ethanol and for 5 min in 2.6% sodium hypochlorite followed by eight rinses in sterile distilled water. In all but one experiment, 250-g portions of mineral soil were placed in the bottom of 12.5-cm (inner diam.) pots, and 200 g of the organic layer was placed on top of the mineral soil. The seeds were sown at a depth of 0.5 cm.

Twenty seeds were sown in each pot, and 40 seeds were planted in one half of a root box. When the seedlings emerged, they were thinned to 10 plants per pot and 15 plants per half root box. The commercial inoculants for red clover and birdsfoot trefoil were obtained from Stanford Seed Co., Buffalo, N.Y. Unless otherwise stated, the plants were grown at 23 ± 2°C under 12 h of artificial light (250 µEinstein $sec^{-1}m^{-2}$) each day. Before the plants were harvested, the final soil pH was measured in triplicate or quadruplicate by inserting the electrode (Orion model 601) to a depth of 3 cm in the organic layer and at 10 cm for the mineral layer while the soils were saturated with deionized distilled water. The plants were harvested at 14 weeks, and the aboveground portions and roots were dried at 45°C for 7 days. The dried tissues were ground to pass through a 40-mesh screen and analyzed for nitrogen content (BREMNER 1965). The values presented are the means of four replicate pots, each containing 10 plants.

RESULTS

After sowing uninoculated seeds, the soils were treated with simulated rain at pH 3.5, 4.1, and 5.6. Nodules did not appear on the roots of either plant species. The nitrogen content and the yield of the tops and roots are given in Table 1. These data indicate that, with but a single exception, the growth of both crops was significantly reduced by simulated rain at pH 3.5, 4.1, or both. Moreover, the nitrogen content of birdsfoot trefoil and red clover was often reduced by the treatments. Neither red clover nor birdsfoot trefoil grew in Sagamore soil, probably a result of the low pH of the organic layer. In this study, the plants were grown in summer under sunlight in the greenhouse, and the temperature reached 35°C in the day and fell to about 25°C at night.

To determine the effect of acid rain on nodule formation, the soils were treated with simulated rain at the same pH values. In this instance, surface-sterilized seeds that were coated with a commercial inoculant were sown 2 d after the 100 cm rain application was completed. The data show that acid rain had no detectable effect on the number of plants nodulated in the agricultural soil (Table 2), and the depth of nodulation in the Howard soil was usually greater than that in the forest soils. The percentage of plants bearing nodules in the forest soils was signifi-

Table 1. Influence of simulated rain on yield and nitrogen content of tops and roots of red clover and birdsfoot trefoil derived from uninoculated seeds

Soil	pH of rain	Final soil pH		Plant	Dry wt (mg/plant)		Nitrogen content (mg/plant)	
		Organic layer	Mineral layer		Roots	Tops	Roots	Tops
Panther	3.5	3.95	4.43	Trefoil	135 A*	216 A	7.40 A	9.20 A
	4.1	4.12	4.50	Trefoil	164 AB	219 A	8.70 B	9.75 A
	5.6	4.48	4.91	Trefoil	181 B	244 A	7.55 A	11.6 B
	3.5	3.90	4.41	Clover	100 A	162 A	2.46 A	5.56 A
	4.1	4.10	4.46	Clover	142 B	198 B	4.65 B	7.88 B
	5.6	4.53	5.04	Clover	161 B	240 C	6.10 C	7.75 B
Woods	3.5	3.78	3.97	Trefoil	73 A	125 A	3.74 A	8.81 A
	4.1	3.95	4.11	Trefoil	107 B	153 AB	6.02 B	9.06 A
	5.6	4.37	4.75	Trefoil	163 C	168 B	6.62 C	10.1 B
	3.5	3.73	3.93	Clover	54 A	81 A	3.59 A	5.99 A
	4.1	3.90	4.07	Clover	75 B	100 AB	3.71 A	7.38 B
	5.6	4.32	4.70	Clover	115 C	180 B	3.73 A	8.95 C

*Values followed by different letters for each plant species grown in a single soil are significantly different at the 0.05 probability level by Duncan's multiple range test.

Table 2. Effect of simulated rain on nodulation, yield, and nitrogen content of red clover and birdsfoot trefoil derived from inoculated seeds

Soil	pH of rain	Final soil pH		Plant	% of plants with nodules	Depth of nodulation (cm)	Dry wt (mg/plant)		N content (mg/plant)	
		Organic layer	Mineral layer				Roots	Tops	Roots	Tops
Panther	3.5	3.75	4.34	Trefoil	33 A*	4.3	305 A	342 A	15.8 A	20.1 A
	4.1	4.10	4.37	Trefoil	58 B	8.1	421 B	461 B	19.9 B	25.2 B
	5.6	4.54	5.10	Trefoil	92 C	9.3	628 C	724 C	22.3 C	28.6 C
	3.5	3.84	4.37	Clover	17 A	1.7	323 A	332 A	13.3 A	15.8 A
	4.1	4.12	4.41	Clover	33 B	2.9	354 A	371 A	16.9 B	20.6 B
	5.6	4.60	5.12	Clover	75 C	5.5	503 B	632 B	18.2 C	25.7 C
Woods	3.5	3.65	3.88	Trefoil	42 A	3.8	429 A	528 A	17.0 A	22.1 A
	4.1	3.90	4.09	Trefoil	67 B	7.5	512 AB	597 AB	21.9 B	28.6 B
	5.6	4.45	4.80	Trefoil	92 C	13.5	683 B	767 B	23.8 C	31.4 C
	3.5	3.68	3.87	Clover	17 A	1.2	297 A	365 A	14.4 A	18.2 A
	4.1	3.93	4.12	Clover	50 B	2.3	409 B	453 B	18.2 B	23.0 B
	5.6	4.49	4.78	Clover	83 C	3.5	530 C	576 C	19.8 C	26.4 C
Howard	3.5	-	6.07	Trefoil	100 A	13.2	876 B	1170 C	29.6 C	44.7 C
	4.1	-	6.24	Trefoil	100 A	18.3	831 B	907 B	26.6 B	36.3 B
	5.6	-	6.57	Trefoil	100 A	11.8	688 A	762 A	24.3 A	32.9 A
	3.5	-	6.03	Clover	100 A	11.8	847 C	958 C	28.2 C	40.8 C
	4.1	-	6.18	Clover	100 A	11.3	756 B	835 B	25.7 B	34.8 B
	5.6	-	6.53	Clover	100 A	10.0	645 A	774 A	22.3 A	30.5 A

*Values followed by different letters for each plant species grown in a single soil are significantly different at the 0.05 probability level by Duncan's multiple range test.

cantly reduced with decreased pH of the simulated rain. Based on nodule numbers and depth, the nodulation of birdsfoot trefoil in the forest soils was more tolerant to acidity than that of red clover. The depth to which nodulation occurred in two of the forest soils was reduced with decreasing pH of the simulated rain. The root depths were 5.3-13.5 and 3.7-11.8 cm for birdsfoot trefoil and red clover, respectively. No nodules appeared on plants grown in the Sagamore soil.

The dry weight and nitrogen content of plant tops and roots are also given in Table 2. Because of poor growth of the legumes in the Sagamore soil, these pots were not harvested. The simulated acid rain reduced plant yield and nitrogen levels in Panther and Woods soils but increased the yield and nitrogen content in the Howard soil. The greatest yield and nitrogen uptake of plants in Howard soil were observed in pots receiving pH 3.5 simulated rain.

The effect of simulated rain on nodulation and growth of the legumes in the root boxes is shown in Table 3. In these soils, the rhizobia were mixed throughout the soil, and hence, more of the plants bore nodules and nodulation was deeper than in the soils in which only the seeds were inoculated. Even when rhizobia were throughout the soil, acid rain reduced the number of plants bearing nodules and the depth of nodulation. The yield and nitrogen content of both plant species were significantly reduced (α, 0.05) by simulated acid rain. The inhibition even at pH 4.1 was often statistically significant. These data also confirm that the nodulation of birdsfoot trefoil was more acid tolerant than that of red clover as measured by number of plants bearing nodules and depth of nodulation. Under the test conditions, the legumes were stunted and neither species nodulated in Sagamore soil.

The simulated rain caused changes in the pH values of the soil (Tables 1, 2, and 3). The extent of the changed varied with the soil and the acidity of the rain.

DISCUSSION

Although the two species used are of economic importance in agriculture, they are present in natural environments as well. Thus, KUDISH (1975) reports that red clover is "everywhere" and birdsfoot trefoil is "locally abundant" in the Saranac Lake, N.Y. area of the Adirondacks. The organic layers in many of these soils were reported to have pH values in the range of 4.0 to 5.4. DUKE (1981) states that these two species will grow in nature at pH values down to 4.5. A 16-year stand of *Robinia pseudoacacia*, a leguminous tree planted in forests of the United States, has been noted to bring about a net nitrogen increase of 609 lb/acre in a New York soil having a pH in the surface 18 cm of 4.6 (IKE and STONE 1958). Hence, it is not unreasonable to study effects of acid precipitation on these two species.

Table 3. Effect of simulated rain on nodulation, yield, and nitrogen content of red clover and birdsfoot trefoil grown in soil mixed with the homologous rhizobia

Soil	pH of rain	Final soil pH Organic layer	Final soil pH Mineral layer	Plant	% of plants with nodules	Depth of nodulation (cm)	Dry wt (mg/plant) Roots	Dry wt (mg/plant) Tops	N content (mg/plant) Roots	N content (mg/plant) Tops
Panther	3.5	3.78	4.37	Trefoil	42 A*	23.0	316 A	391 A	13.7 A	21.5 A
	4.1	4.12	4.39	Trefoil	83 B	25.0	389 B	506 B	16.0 B	26.8 B
	5.6	4.63	5.08	Trefoil	100 C	25.3	531 C	583 C	19.4 C	31.2 C
	3.5	3.89	4.41	Clover	< 10 A	10.4	271 A	354 A	11.2 A	20.2 A
	4.1	4.10	4.44	Clover	20 B	13.4	407 B	485 B	15.0 B	25.9 B
	5.6	4.60	5.07	Clover	60 C	17.7	519 C	549 C	17.8 C	27.7 C
Woods	3.5	3.67	3.91	Trefoil	92 A	15.6	423 A	464 A	15.0 A	24.5 A
	4.1	3.94	4.07	Trefoil	100 B	21.9	536 B	642 B	19.6 B	30.6 B
	5.6	4.48	4.90	Trefoil	100 B	24.8	633 C	780 C	22.8 C	35.6 C
	3.5	3.72	3.89	Clover	50 A	11.2	339 A	475 A	13.4 A	22.0 A
	4.1	3.97	4.05	Clover	83 B	13.5	510 B	545 AB	17.4 B	27.8 B
	5.6	4.53	4.84	Clover	100 C	18.0	569 B	645 B	21.0 C	31.5 C

*Values followed by different letters for each plant species grown in a single soil are significantly different at the 0.05 probability level by Duncan's multiple range test.

Three sets of experiments were conducted. The first confirmed that the acid soils did not contain sufficient rhizobia to nodulate the test legumes. WILSON (1935) also could not find rhizobia nodulating red clover in forest soils of New York. In the second, only the seeds were inoculated so that the effects of the surface-applied precipitation on nodulation would be restricted to those sites in the soil to which the rhizobia would be translocated with the growing roots or by water movement. In the third, the rhizobia were mixed with the soil so that the developing roots could make contact with rhizobia even at some depth below the surface. In each instance, however, the weights and nitrogen content of the tops and roots were reduced by the simulated acid precipitation. Thus, although nodulation was also suppressed, the legumes were affected independent of their capacity to fix nitrogen.

The reasons for the suppressions by simulated acid precipitation in the acid soils are unknown. The greater H^+ activity may be a factor, but toxicity from the greater amounts of available Al, Fe, or Mn (DOBEREINER 1966, SCHMEHL et al. 1950) or the formation of protonated organic acids at the low pH may be implicated. A decline in the level of some nutrient needed for growth may also result in diminished plant development, and the lesser availability of Mo in soils of lower pH may lead to reduced nitrogen fixation (MULDER 1948). In agreement with the findings of ANDREW (1976), conditions associated with a higher percentage of nodulated plants were those that resulted in higher dry weights of the plants that nodulated.

It is surprising, however, that the depth of nodulation diminished as the pH of the solution applied to the soil surface declined; this may result from the downward movement of some toxicant that is generated in increasing amounts as the pH of the solution applied to the surface falls. The enhancement by simulated acid precipitation of plant growth and nitrogen uptake by clover and trefoil in Howard soil (pH 6.7), in contrast, may be a reflection of the improved nutrient availability arising from chemical action of the more acid solutions.

The present findings confirm the recent report that acidified rain inhibits nodulation (SHRINER and JOHNSON (1981); however, our study involved pH values higher than those used by SHRINER and JOHNSON (1981) and higher than that found by PORTER and SHERIDAN (1981) to be needed to suppress acetylene reduction. In designing studies of this type, morever, it is important to distinguish between injury to the foliage and a deleterious change occurring in the soil. The latter change will become aggravated with time because the precipitation will continue to reach the soil each year.

The benefits of nitrogen-fixing plants in natural communities have often been noted (ASHLEY and BAKER 1968, HAINES et al. 1978). The present findings suggest that these benefits may be reduced or eliminated in acid soils subjected to acid precipitation.

Therefore, additional studies are required to establish whether the findings of our study of two legumes are applicable to the legume flora of natural ecosystems.

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