Interactions of Simulated Acidic Rain with Root-Knot or Cyst Nematodes on Soybean

S. R. Shafer, S. R. Koenning, and K. R. Barker

Research plant pathologist (USDA-ARS) and associate professor; research analyst; and professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

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ABSTRACT

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The influence of simulated acidic rain on interactions of root-knot (*Meloidogyne hapla, M. incognita*) or cyst (*Heterodera glycines*) nematodes with soybean plants (*Glycine max*) was investigated in greenhouse experiments. Seedlings inoculated with rhizobia were transplanted into pots of nematode egg-infested soil (one nematode species per pot) or noninfested soil. Three days later, plants and soil were exposed to simulated rain (2 cm in 1 h) adjusted to pH 5.3, 4.3, 3.3, or 2.3. After three rains per week for 8 wk, major effects on plants and nematodes (e.g., shoot dry weight and production of cyst nematode eggs suppressed by approximately 80 and 90%, respectively) occurred only after rains at pH 2.3 (relative to those exposed to rains at pH 5.3). Characteristics of polynomial dose-response relationships indicated that the effects of simulated acidic rain on plants and nematodes were nematode species-dependent; dose-response relationships for many dependent variables (plant biomass, nodulation, disease symptoms, nematode reproduction measurements) versus rain pH differed between cyst nematodes and root-knot nematodes, but most dose-response characteristics for the two *Meloidogyne* spp. were similar. Acid deposition can influence nematode-plant interactions, but the acidity of simulated rain required to cause major changes exceeded that known in the United States.

Additional keywords: acid deposition, acid precipitation, *Bradyrhizobium japonicum*, nodulation, pollutant-parasite interaction.

Characteristics of the environment in which plant-pathogenic nematodes occur affect population densities, distribution, and activities of these obligate parasites. In general, soil chemical factors such as pH and mineral nutrient concentrations that are optimum for these parasites are very similar to those that optimize growth of the host (26,27). Concentrations of mineral nutrients in samples collected from geographically widespread locations were not correlated with the occurrence of important *Meloidogyne* spp. (37), but soil chemical characteristics may influence ectoparasitic nematodes more than endoparasites (25).

Air pollution can influence many types of plant diseases (10,12). The influence of air pollutants on soilborne pathogens has received far less attention than have their effects on foliar pathogens, and impacts on plant-parasitic nematodes have been studied infrequently. In greenhouse experiments (41), exposure of soybean plants to ozone (O_3) or a mixture of O_3 and sulfur dioxide (SO_2) inhibited reproduction of soybean cyst nematodes (*Heterodera glycines*) and *Paratrichodorus minor* but not *Belonolaimus longicaudatus*. The reproduction of *Pratylenchus penetrans* associated with soybeans was stimulated by SO_2. In experiments with tomato plants (33), parasitism of roots by *P. penetrans* enhanced the negative effect of O_3 and SO_2 on leaf growth but suppressed the negative effect of the gas mixture on axillary shoot growth. The effects of pollutant stress on host-parasite interactions involving nematodes cannot be generalized from currently available information.

Influences of gaseous air pollutants, especially O_3, on soilborne microorganisms associated with roots probably are plant-mediated because the gases do not penetrate the soil significantly (2,39). Effects of acid deposition on plants and soilborne microorganisms, however, could be either plant- or soil-mediated, and both types of mechanisms could affect plant-parasite interactions. Many plant responses to acid deposition, usually in the form of simulated acidic rain, have been identified in experimental situations during the past 20 yr (9,12,17). Numerous types of soilborne microorganisms associated with plant roots have been studied as well, including rhizobia (35), mycorrhizal fungi (5,16,23,28,32,36), a pathogenic fungus (31), and bacteria and fungi in the rhizosphere (29,30).

Impacts of acid deposition on nematodes are poorly described. In experiments with the pine wilt nematode (*Bursaphelenchus xylophilus*), simulated acidic rain treatments at pH 3.6 delayed wilting and suppressed nematode reproduction in white pine seedlings relative to plants treated with deionized water, and Scots pine seedlings treated with simulated acidic rain lost the normal tolerance to the pathogen and wilted (3). Changes in nematode population densities (parasites and nonparasites) following experimental acidification of soil in field plots have been inconsistent (14,38). An indigenous population of *Meloidogyne hapla* caused root knot on field-grown kidney bean plants that were exposed to repeated simulated rains at pH 6.0 or 3.2 on 3 days per week for 8 wk. Relative to plants exposed to rains at pH 6.0, rains at pH 3.2 suppressed root galling and numbers of eggs per root system by 48 and 34%, respectively (34). Acidity of simulated rain, however, did not affect indigenous population densities of five different genera of plant-parasitic nematodes, including *Meloidogyne*, in field plots of soybean (11). Both of these latter studies (11,34) were designed to study impacts of simulated acidic rain on the plants or other host-parasite interactions; few, if any, studies have specifically examined the influence of acid deposition on interactions of important soilborne parasitic nematodes with plants.

The objective of this study was to quantify the impact of simulated acidic rain on selected aspects of host-parasite interactions

involving the sedentary endoparasitic nematode species *Heterodera glycines*, *M. hapla*, or *M. incognita*.

MATERIALS AND METHODS

Plant culture and inoculation. Seeds of *Glycine max* (L.) Merr. 'Lee 68' were inoculated with a commercially available preparation of *Bradyrhizobium japonicum* and sown in moist MetroMix 220 (W. R. Grace & Co., Cambridge, MA). Two days later, nematode inocula (eggs) were prepared, soil was infested, and seedlings were transplanted into the infested soil. Eggs were extracted (15) from 4-mo-old pot cultures of *M. hapla* Chitwood or *M. incognita* (Kofoid and White) Chitwood race 3 on tomato (*Lycopersicon lycopersicum* (L. Karsten) 'Rutgers' and from 4-mo-old pot cultures of *Heterodera glycines* Ichinohe on soybean. Eggs were suspended in Terra-Sorb (Industrial Services International, Inc., Bradenton, FL), a starch polymer (0.42 g L^{-1} of water), to maintain a homogeneous suspension during infestation. Plastic pots (15 cm diameter) with fiberglass screen in the bottom were filled with soil-sand mix (equal volumes of steam-pasteurized sandy loam soil and steam-pasteurized sand, hereafter called soil; chemical characteristics summarized in Table 1). Approximately 1.5 L of soil was placed in each pot in three 400-g layers. The surface of each layer was lightly misted with tap water and infested with 10 ml of an egg suspension before the next layer of soil was added. Each pot was infested with 5,000 eggs in 30 ml delivered by a peristaltic pump (10 ml per 400-g soil layer). Each infested pot received inoculum of a single species; noninfested controls received Terra-Sorb without eggs. The topmost layer of inoculum was covered by a 200-g layer of soil, and one soybean seedling with a 2- to 3-cm-long radicle was transplanted into each pot. A 15-cm-high collar of plastic-laminated screen was stapled around the rim of each pot to provide a barrier against possible cross-contamination due to water splash. The soil surface in each pot was kept moist by occasional misting with tap water during the next 2 days.

TABLE 1. Properties of sand-soil mix before and after exposure to simulated rains in experiment 1[a]

Soil property	Before exposure[b]	After exposure to rains at pH[c]			
		5.3	4.3	3.3	2.3
pH	4.93 (0.15)	5.06 (0.06)	5.04 (0.06)	4.84 (0.05)	3.87 (0.04)
Ac[d] (meq/100 cm^3)	0.87 (0.06)	1.11 (0.04)	1.06 (0.10)	1.17 (0.05)	1.50 (0.17)
Na (meq/100 cm^3)	0.11 (0.03)	0.04 (0.01)	0.05 (0.01)	0.04 (0.02)	0.04 (0.01)
K (meq/100 cm^3)	0.09 (0.02)	0.01 (0.00)	0.02 (0.01)	0.01 (0.01)	0.02 (0.01)
Mg (meq/100 cm^3)	0.24 (0.02)	0.07 (0.01)	0.08 (0.02)	0.06 (0.04)	0.06 (0.02)
Ca (meq/100 cm^3)	0.92 (0.10)	0.46 (0.05)	0.51 (0.06)	0.43 (0.14)	0.26 (0.09)
CEC[e] (meq/100 cm^3)	2.23 (0.16)	1.68 (0.09)	1.71 (0.07)	1.72 (0.22)	1.87 (0.18)
BS[f] (% of CEC)	61 (4)	34 (2)	38 (5)	31 (7)	20 (6)
Mn (mg/1,000 cm^3)	19.0 (1.7)	14.1 (0.5)	14.1 (0.5)	13.5 (0.7)	7.1 (0.7)
Zn (mg/1,000 cm^3)	2.4 (0.1)	1.5 (0.1)	1.6 (0.2)	1.5 (0.2)	0.8 (0.2)
Cu (mg/1,000 cm^3)	0.8 (0.1)	0.7 (0.1)	0.7 (0.1)	0.7 (0.1)	0.8 (0.1)
P (mg/1,000 of cm^3)	94 (10)	79 (5)	84 (2)	91 (3)	97 (13)
Humic matter (%)	0.34 (0.02)	0.33 (0.02)	0.32 (0.02)	0.35 (0.02)	0.30 (0.03)

[a] Mean (standard deviation).
[b] $n = 4$.
[c] $n = 9$ (one sample per noninfested pot) except for pH 4.3 (one sample lost).
[d] Exchangeable acidity.
[e] Cation exchange capacity.
[f] Base saturation.

Between exposures to simulated rain, plants were maintained on benches in a nonshaded greenhouse that was cooled by charcoal-filtered air passed through evaporative pads. Simulated rain supplied all water once exposures to rain began, except one warm day on which rain was not scheduled and on the day before harvest; on those days, 150 ml of deionized water was applied directly to the soil surface, which moistened the soil without complete saturation or leaching.

Rain simulation. Plants were exposed to simulated rain solutions in a charcoal-filtered greenhouse room adjacent to the plant growth room. The rain simulation room was covered by 30% shade cloth so that simulated rain was never applied under full sunlight. The first exposure (on 10 April) was applied 3 days after seedlings were inoculated with rhizobia and transplanted into nematode-infested soil. Immediately before each exposure, plants were placed on rotating turntables beneath nozzles as described previously (29). Solutions for simulated rain (pH 5.3, 4.3, 3.3, or 2.3) were prepared from deionized water, a mixture of H_2SO_4 and HNO_3 (70 meq SO_4^{-2}: 30 meq NO_3^-) and a standardized solution of background ions (32). Plants were exposed to simulated rain on 3 days per week for 8 wk. Each plant usually received a 2.0-cm deposition of simulated rain solution in 1 h at each exposure; the second and third exposures were only half the normal (1.0 cm of simulated rain in 0.5 h) because the water demand by the seedlings was small. Foliage was permitted to dry for 1 h after each exposure, and plants were returned to the plant growth room.

Mean deposition per exposure (for the 2-cm exposures) was within 6% of the intended 2.0 cm on each table. Mean pH values for the 24 exposures were 5.27, 4.27, 3.29, and 2.34, with standard deviations of 0.05–0.07. Estimated total deposition of major anions in simulated rain adjusted to pH 5.3, 4.3, 3.3, or 2.3 was equivalent to 5.7, 12.6, 82.4, or 779.9 kg of SO_4^{-2} ha^{-1} and 3.8, 7.7, 46.3, or 432.4 kg of $NO_3^-ha^{-1}$, respectively. In comparison, wet deposition of SO_4^{-2} and NO_3^- approached 50 and 35 kg ha^{-1}, respectively, at certain locations in eastern North America in 1980 (19).

Plant harvest and nematode assays. Plants were harvested on the third and fourth days after the last exposure to simulated rain. Soil or plants from different pots were not bulked in any way. The shoot of each plant was severed just above the soil and combined with any leaves that had previously senesced and abscised; abscised leaves had been collected, dried, and stored in envelopes during the experiment. Shoot tissues were dried for 72 h at 60 C and weighed.

The root-soil mass from noninfested controls and pots with soil infested with either *Meloidogyne* species was tapped from the pot and shaken to dislodge loose soil into a plastic bag. The soil was mixed in the plastic bag, and a 500-cm^3 portion was collected for nematode assays, bagged, and stored at 4 C. A 300-cm^3 portion from each noninfested pot was collected for chemical analyses by the North Carolina Department of Agriculture (20–22). Roots were washed on a 0.5-mm screen under running tap water, blotted with paper towels, weighed, and stored at 4 C with a moist paper towel in a plastic bag. All nematode extraction procedures for controls and plants grown in *Meloidogyne*-infested soil were completed within 2 days after harvest. The number of *Bradyrhizobium* nodules on the root system (nearest five nodules) and the percentage of the root system that was galled (nearest 5%, except trace = 1%) were estimated visually. Proportion of the root system that appeared necrotic (darkened) also was estimated in 5% increments. Fresh weight of the root system was recorded. The root system was gently flattened between clean paper towels, removed from the towels, and cut into 1-cm-wide strips perpendicular to the tap root. Strips were selected at random until 5 g fresh weight had been collected for extraction of eggs

for quantification (7); eggs were extracted from the entire root system if the fresh weight was less than 5 g. Males and second-stage juveniles of *Meloidogyne* spp. were extracted from soil by elutriation (6,13) and centrifugation (15) and counted.

The entire soil-root mass from each pot of *Heterodera*-infested soil was stored intact in a plastic bag at 4 C, and all extraction procedures were completed within 6 days after harvest. Loose soil was collected by shaking the roots over a plastic bag, and a 500-cm^3 portion was processed to extract second-stage juveniles, males, cysts, and free eggs by elutriation and centrifugation. Roots were dipped in a bucket of tap water, washed gently to remove the adhering soil, and weighed. Roots then were spread on a 0.5-mm screen and sprayed vigorously with tap water to dislodge cysts, which were collected in a bucket beneath the screen. Cysts were crushed with a Ten-Broek tissue homogenizer to free eggs for enumeration. Root necrosis and nodulation by *Bradyrhizobium* were evaluated as described previously.

Experimental design and data analysis. Plants assigned to different treatments were arranged on a greenhouse bench in three randomized complete blocks of split-plots. Within each block, acidity of simulated rain represented the main plots (four main plots per block), and nematode treatments represented the subplots (three species and control = four subplots per main plot). Twelve turntables arranged in three randomized complete blocks in the exposure room enabled this experimental design to be maintained regardless of the location of the plants. Each block × pH × nematode treatment combination had three plants (total $n = 144$); the three measurements for any particular plant or nematode variable were averaged for data analyses (n for analyses = 48). Data were tested for heterogeneity of variances, transformed appropriately when necessary (4), and tested by analysis of variance (ANOVA) for effects of rain acidity, nematode treatments, and interactions. Rain pH was used as the quantitative independent variable in analyses. Main effect and interaction sums of squares were partitioned further into planned orthogonal contrasts. Contrasts for the interaction compared dose-response characteristics for different nematode treatments, but dose-response relationships were not fitted to specific equations because such models would have little relevance outside the artificial system studied. This type of analysis (e.g., Table 2) was applied to all plant variables except the proportion of root systems with root-knot galls (controls and cyst-nematode-infected plants deleted from the ANOVA) (Table 3). To determine the effects of acidity on nematode reproduction (eggs, juveniles, males, and cysts), controls were deleted from the ANOVA (Table 3). Statistical analyses were conducted with SYSTAT software (SYSTAT, Inc., Wheaton, IL).

The experiment was repeated (first exposure on 2 October). Experiment 2 was conducted according to the same design as experiment 1 but with some minor changes in procedures. *Bradyrhizobium* inoculum was supplied when soil was infested with nematodes. A commercial preparation of *B. japonicum* mixed in ground peat (20 cm^3) was mixed in 9 L of tap water and 2 ml of liquid detergent, and 10 ml were dispensed onto each soil layer in each pot. Supplemental lighting (PAR photon flux ~250 $\mu mol \cdot sec^{-1}$ m^{-2} at plant height) from metal-halide lamps was provided over the benches in the plant growth room to extend

TABLE 2. Sums of squares[a] from analyses of variance for effects of rain acidity, nematode treatment, and interactions on shoot dry weight (grams) of soybean plants

Source	df	Experiment 1	Experiment 2
Block (B)	2	0.0003	0.092
Acidity (A)	3	14.814**	14.101**
A linear (AL)	1	9.360**	8.175**
A quadratic (AQ)	1	4.948**	5.188**
A lack-of-fit (AF)	1	0.507**	0.737**
Error a (B × A)	6	0.096	0.029
Nematode treatment (N)[b]	3	2.001**	1.121**
C vs others	1	0.480**	0.141**
Hg vs Mh, Mi	1	1.297**	0.954**
Mh vs Mi	1	0.224**	0.026
A × N	9	0.874**	0.237**
Con1[c]: AL × (C vs others)	1	0.066*	0.014
Con2: AQ × (C vs others)	1	0.033	0.020
Con3: AF × (C vs others)	1	0.032	0.000
Con4: AL × (Hg vs Mh, Mi)	1	0.534**	0.135**
Con5: AQ × (Hg vs Mh, Mi)	1	0.173**	0.048*
Con6: AF × (Hg vs Mh, Mi)	1	0.008	0.004
Con7: AL × (Mh vs Mi)	1	0.000	0.000
Con8: AQ × (Mh vs Mi)	1	0.000	0.014
Con9: AF × (Mh vs Mi)	1	0.028	0.001
Error b (B × N) + (B × A × N)	24	0.288	0.217

[a]Data from each experiment required the square-root transformation before analysis. Symbol * or ** indicates that the probability of obtaining a larger F value is less than 0.05 or 0.01, respectively.
[b]Nematode treatments: C = noninfested control, Hg = *Heterodera glycines*, Mh = *Meloidogyne hapla*, Mi = *Meloidogyne incognita*.
[c]The single-degree-of-freedom contrasts partitioned from the sum of squares for the acidity × nematode treatment interaction have been numbered for convenient reference in the text.

TABLE 3. Probability of obtaining a larger F value than that obtained for each variable in analyses of variance for effects of rain acidity, nematode treatment, and the interaction on plants and nematodes in two independent experiments (1 and 2)

	Transformation[a] in each experiment		Effect in each experiment					
			Acidity		Nematode treatment		Acid × Nema	
Variable	1	2	1	2	1	2	1	2
Shoot dry wt.	SQRT	SQRT	0.01	0.01	0.01	0.01	0.01	0.02
Root fresh wt.	SQRT	LOG	0.01	0.01	0.01	0.01	0.01	0.59
Nodules/root sys.	—	LOG	0.01	0.01	0.01	0.01	0.01	0.01
% roots necrotic	LOG	—	0.01	0.01	0.01	0.01	0.01	0.01
J[b,c]/pot[d]	LOG	LOG	0.01	0.13	0.01	0.01	0.01	0.86
M[c,e]/pot[d]	LOG	LOG	0.12	0.17	0.01	0.01	0.01	0.01
Eggs/g root[c]	LOG	LOG	0.01	0.01	0.01	0.01	0.03	0.55
Eggs/root system[c]	LOG	LOG	0.01	0.01	0.01	0.01	0.06	0.65
% roots galled[f]	—	SQRT $(1/y)$	0.01	0.01	0.01	0.01	0.20	0.05
Cysts/pot[d,g]	LOG	LOG	0.01	0.01				

[a]Data transformation required before analysis: SQRT = square-root, LOG = logarithmic (base e), SQRT $(1/y)$ = square root of inverse, — = transformation not required.
[b]Second-stage juveniles.
[c]Noninfested pots deleted from analysis of variance (ANOVA).
[d]1,400 cm^3 soil per pot.
[e]Males.
[f]Only plants infected with *M. hapla* or *M. incognita* included in ANOVA.
[g]Only plants infected with *H. glycines* included in ANOVA.

day length to 14 h throughout the experiment. Supplemental watering (125 ml of deionized water) was provided when needed but no more than once each week. Ion deposition in deionized water was considered negligible, and the water volume was never sufficient to cause leaching from the bottom of the pots. Data from experiment 2 were analyzed independently because environmental conditions related to time of year were unavoidably different from those in experiment 1.

RESULTS

Experiment 1. Only rains at pH 2.3 caused major changes in soil chemical properties (Table 1). The most acidic rains decreased soil pH by approximately 1 unit. All rain treatments added exchangeable acidity to the soil and leached cations (primarily calcium) from the soil, and these two changes (in addition to plant uptake of cations) caused decreases in base saturation relative to soil before exposures. Values for most properties of soil exposed to rains at pH 5.3, 4.3, or 3.3 tended to be similar and greater than those for soil exposed to rains at pH 2.3. Soil phosphorus apparently was depleted from soil by plants in an inverse relationship with rain acidity; the very stunted plants exposed to rains at pH 2.3 did not remove a measurable amount of phosphorus from the soil.

Very little foliar tissue remained unaffected after plants had been exposed to simulated rains at pH 2.3. Irregular necrotic lesions often were torn at the margin and were accompanied by chlorosis and distortion of non-necrotic portions of the leaflets. Only widely distributed pinpoint necrotic lesions, however, occurred on leaves of plants exposed to rains at pH 3.3, and no foliar injury occurred on plants exposed to rains at pH 4.3 or 5.3. Injury symptoms were not quantified.

Transformed data (as specified for each variable in Table 3) were the basis of all statistical analyses; an indication of variability in the transformed data is provided in each figure caption (Figs. 1–6) by the maximum standard deviation among the means presented. Although back-transformed means are actually presented in the figures for ease in interpretation, all conclusions are based on analyses of the transformed data. The acidity × nematode treatment interaction was significant for all variables except the percentage of root systems galled by root-knot nematodes (Table 3). Hereafter, the single-degree-of-freedom contrasts partitioned from the interaction sum-of-squares for each variable will be referenced by number (e.g., "Con1") as shown for the sample ANOVA in Table 2.

Plants infected with cyst nematodes were stunted more than those infected with root-knot nematodes after plants were exposed to rains at pH 3.3, 4.3, or 5.3, but the severe stunting caused by rains at pH 2.3 obscured differences among nematode treatments in association with that acidity (Fig. 1). Among noninfected control plants, shoot dry weights for those exposed to rains at pH 2.3 averaged less than 15% of those exposed to rains at any other acidity. Characteristics of dose-response relationships for shoot dry weight differed among nematode treatments (Table 2). The linear component of the dose-response relationship for noninfected controls was different from that for nematode-infected plants (Con1 $P < 0.03$), and the linear and quadratic aspects for the *Heterodera*-infected plants differed from those for *Meloidogyne*-infected plants (Con4 and Con 5 $P < 0.01$) because plants infected with cyst nematodes were smaller than those infected with root-knot nematodes after rains at pH 3.3 to 5.3. The dose-response characteristics relative to the two *Meloidogyne* spp., however, did not differ. Regardless of nematode treatment, rains at pH 2.3 caused the most pronounced growth suppression.

Rains at pH 2.3 severely suppressed root growth of all plants, but characteristics of the dose-response relationships for final root fresh weight varied among nematode treatments (Fig. 1). Orthogonal contrasts indicated that the dose-response relationship for controls was different from that for all infected plants combined (Con1 and Con2 $P < 0.01$); root fresh weights of plants grown in noninfested soil lessened for each increment in rain acidity, but root weights of plants in infested soil were greatest at pH 4.3. The relationship for *Heterodera*-infected plants differed from that for plants infected with either *Meloidogyne* species (Con4 $P < 0.03$, Con6 $P < 0.05$) because root systems of plants infected with cyst nematodes were smaller than those infected with root-knot nematodes after rains at pH 5.3 or 4.3. The linear aspect of the dose-response relationships for root fresh weight of plants infected with *M. hapla* or *M. incognita* differed marginally ($P < 0.07$).

Rains at pH 2.3 severely suppressed nodulation by rhizobia (Fig. 2). Among noninfected controls, the number of root nodules on plants exposed to rains at pH 2.3 averaged fewer than 10% of those for plants exposed to rains at any other acidity. Cyst nematodes suppressed nodulation more than root-knot nematodes when plants were exposed to rains at pH 3.3–5.3. The dose-response relationship for nodule numbers on control plants was significantly different from that for all infected plants combined (Con1 $P < 0.01$, Con2 $P < 0.02$), and the relationship for *Heterodera*-infected plants differed from that for plants infected with *Meloidogyne* spp. (Con4 $P < 0.02$; Con5 $P < 0.01$). The dose-

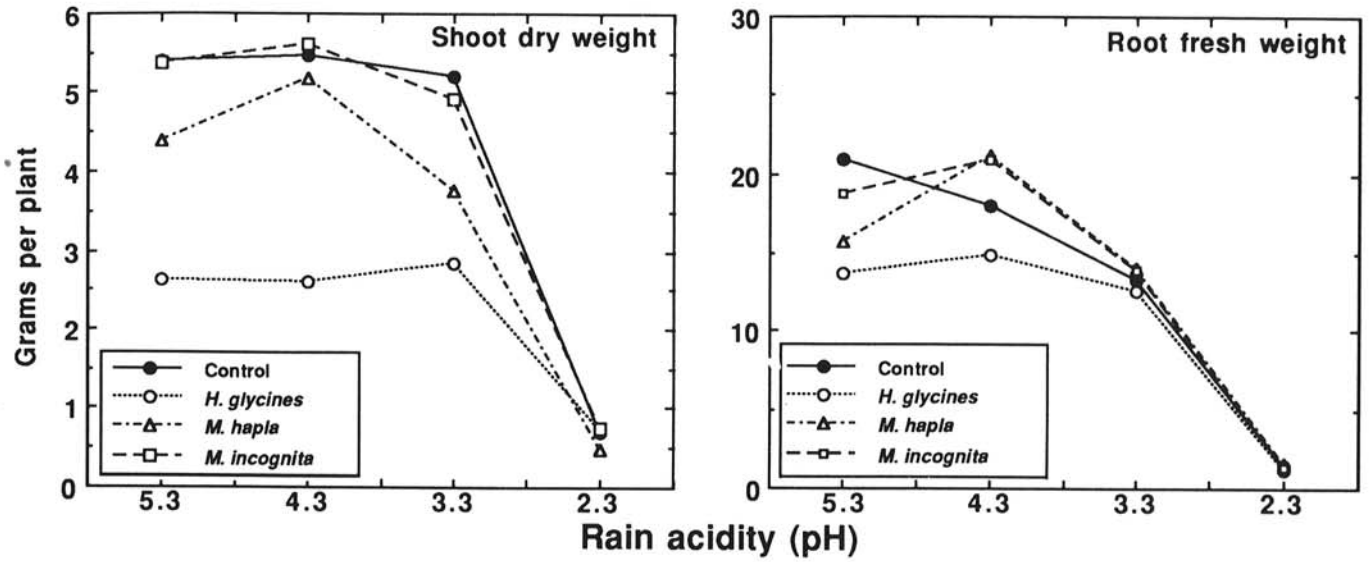

Fig. 1. Effect of acidity of simulated rain in experiment 1 on shoot dry weights and root fresh weights of soybean plants grown in noninfested soil or soil infested with 5,000 nematode eggs per pot before exposures began. Each point is the mean of three values (one value per block). Maximum standard deviation of the transformed data was 0.20 for shoot dry weights and 0.37 for root fresh weights. Back-transformed means are graphed.

response relationships for nodule numbers on plants inoculated with *M. hapla* or *M. incognita*, however, did not differ.

Approximately 90% of the root systems of control plants or those infected with root-knot nematodes were necrotic (discolored gray or brown) after rains at pH 2.3, but root necrosis averaged only ~50% on *Heterodera*-infected plants (Fig. 3). When plants were exposed to rains at pH 3.3–5.3, necrosis averaged 11% or less for control plants, ~10–20% for *Meloidogyne*-infected plants, and ~20–30% for *Heterodera*-infected plants. The dose-response relationship for root necrosis on control plants was different from that for all infected plants combined (Con1 and Con2 $P < 0.01$), and the relationship for *Heterodera*-infected plants differed from that for plants infected with *Meloidogyne* spp. (Con4 and Con5 $P < 0.01$). The shape of the dose-response relationship for plants infected with cyst nematodes appeared to have the greatest influence on these contrasts. The dose-response relationships for root necrosis on plants infected with *M. hapla* or *M. incognita* differed marginally (Con8 $P < 0.08$; Con9 $P < 0.07$).

The acidity × nematode treatment interaction for the percentage of root system galled by different species of root-knot nematodes was not significant, but the main effect of each was significant at $P < 0.01$ (Table 3). Averaged across all rain acidities, *M. hapla* caused more root galling than *M. incognita*, and galling was greatest after plants were exposed to pH 2.3 (Fig. 3). This acidity-related response can be attributed to the stunted condition of those root systems. The dose-response relationship for percent galling versus rain acidity was linear ($P < 0.01$) for both species.

A large number of juveniles (Fig. 4) were present in pots infested with *H. glycines* and exposed to rains at pH 3.3–5.3 (averages ~5,000–7,000 per pot) but averaged ~200 per pot after exposures to rains at pH 2.3. However, the number of juveniles in *Meloidogyne*-infested pots was low and apparently unrelated to rain acidity (averages ~20–150 per pot for *M. hapla* and 20–70 per pot for *M. incognita*). The dose-response relationship for total number of juveniles per pot differed for cyst nematodes versus both root-knot nematode species (Con4 and Con5 $P < 0.01$) and between species of root-knot nematodes (Con7 and Con9 $P < 0.01$).

Number of males per pot generally reflected numbers of juveniles per pot (data not shown). Mean number of males per pot ranged from ~2,300 to 2,800 in *Heterodera*-infested pots exposed to rains at pH 3.3–5.3 but averaged ~25 per pot after exposure to rains at pH 2.3. Mean number of males in *Meloidogyne*-infested pots was variable (~15–100 per pot) and unrelated to rain acidity. Thus, characteristics of the dose-response relationship for *Heterodera*-infested pots differed from that for *Meloidogyne*-infested pots (Con4, Con5, Con6 $P < 0.01$). Only the lack-of-fit component of the dose-response relationships between *Meloidogyne* spp. differed ($P < 0.01$).

M. incognita produced the largest number of eggs recovered directly from the roots of plants exposed to rains at pH 3.3–5.3 (Fig. 5). For all three species, the maximum number of eggs per root system occurred on plants exposed to rains at pH 3.3. Egg production by all species was extremely suppressed by rains at pH 2.3. The acidity × nematode treatment interaction was significant at $P < 0.06$. Characteristics of the dose-response relationship for eggs per root system differed between cyst nematodes versus root-knot nematodes (Con4 $P < 0.01$) and between *Meloidogyne* species (Con7 $P < 0.05$). Some of the suppression in egg number by rains at pH 2.3 can be attributed to the stunted root systems, but the number of eggs per gram of root tissue

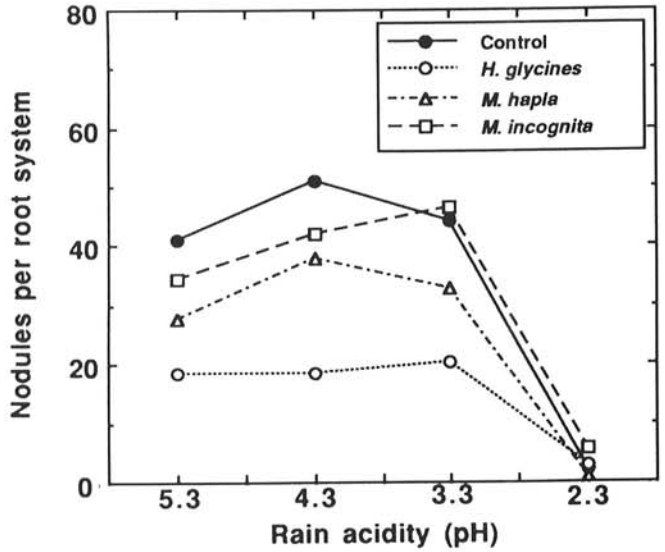

Fig. 2. Effect of acidity of simulated rain in experiment 1 on number of *Bradyrhizobium* nodules per root system on soybean plants grown in noninfested soil or soil infested with 5,000 nematode eggs per pot before exposures began. Each point is the mean of three values (one value per block). Maximum standard deviation of the data (no transformation required) was 10.0.

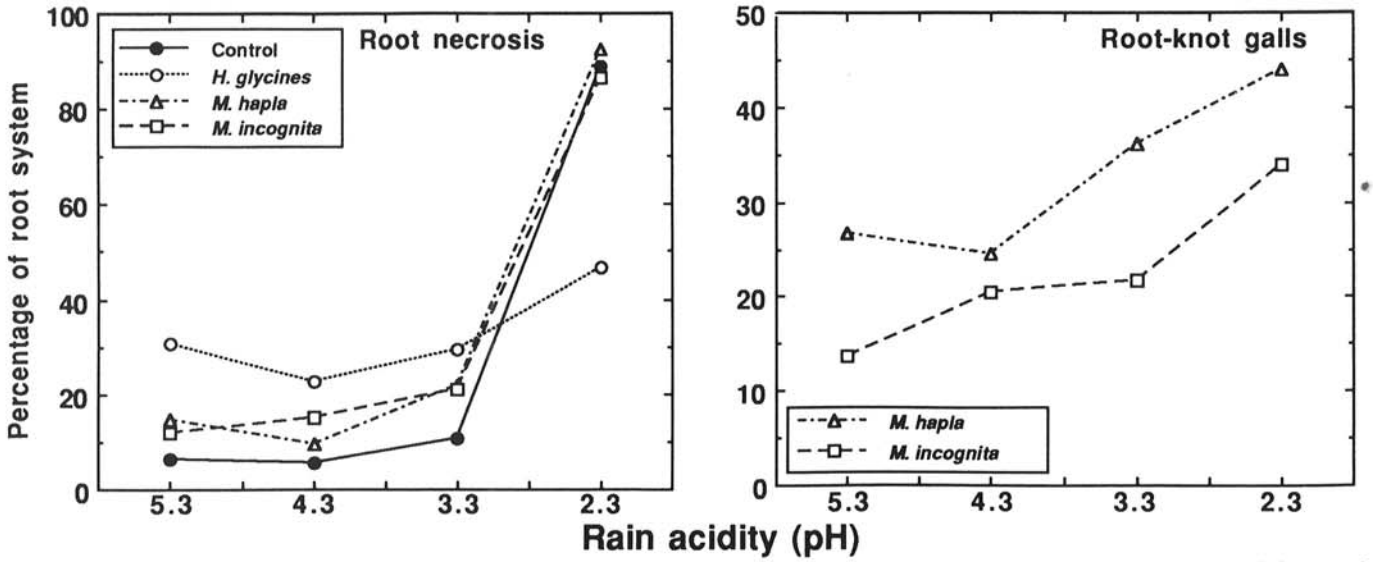

Fig. 3. Effect of acidity of simulated rain in experiment 1 on root necrosis and root-knot galling of soybean plants grown in noninfested soil or soil infested with 5,000 nematode eggs per pot before exposures began. Each point is the mean of three values (one value per block). Maximum standard deviation was 0.53 for root necrosis (transformed data; back-transformed means are graphed) and 9.2 for galls (data transformation not required).

also was suppressed by the most acidic rains yet were greatest on plants exposed to rains at pH 3.3 (Fig. 5). When cyst nematode eggs in the soil (quantified for that species only) were added to those from the roots, total number per pot exhibited the same acidity-related trend. Approximately 90,000–100,000 cyst nematode eggs per pot were recovered after exposures to rains at pH 3.3–5.3, but only ~2,000 per pot were recovered after exposures to rains at pH 2.3 (data not shown).

The number of *Heterodera* cysts per pot was greatest after exposure to simulated rains at pH 5.3 and were smaller with each increment in rain acidity (Fig. 4). Although average number of cysts ranged from ~1,800 to 2,300 in pots exposed to rains at pH 3.3–5.3, the number averaged less than 100 per pot exposed to rains at pH 2.3. Linear, quadratic, and lack-of-fit aspects of the dose-response relationship for cysts per pot versus acidity all were significant (all $P < 0.01$). Much of the major suppression of cyst number in association with the most acidic rains can be attributed to a similar suppression of root tissue available for colonization.

Experiment 2. General conclusions drawn from experiment 1 were verified when the experiment was repeated (Tables 2 and 3; Fig. 6) despite differences between experiments in the magnitudes of some measurements or the significance of specific interactions. Acidity-related trends in experiment 2 were very similar to those in experiment 1 and verified the conclusion that effects of rain acidity were minor except for rains at pH 2.3. Soil in experiment 2 (same source as that in experiment 1) was not chemically analyzed.

Acidity-related trends for shoot dry weights of plants grown with different nematode treatments were very similar in both experiments (Figs. 1 and 6). Main effects of inoculum and acidity, but not the interaction, were significant for root fresh weight in experiment 2 (data not shown); a slight increase in root weight associated with noninfected plants exposed to rains at pH 4.3 apparently caused the dose-response relationship of these plants to be similar to the dose-response relationships for infected plants. Averaged across nematode treatments, acidity again suppressed root fresh weights in a general pattern similar to that in experiment

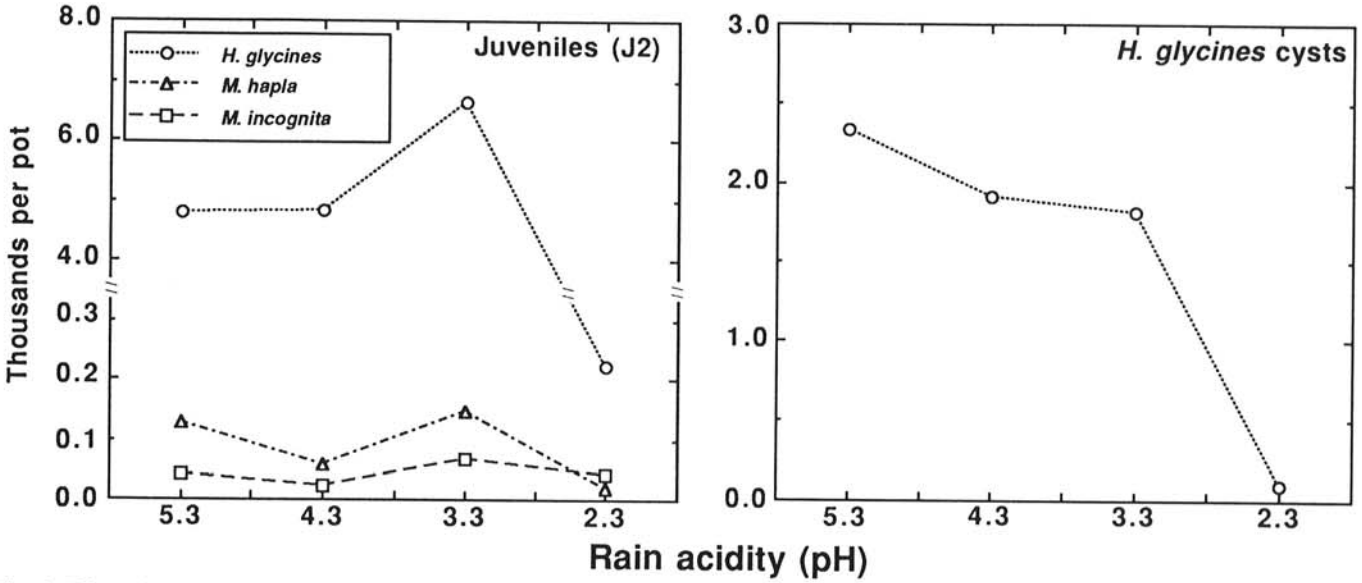

Fig. 4. Effect of acidity of simulated rain in experiment 1 on number of nematode second-stage juveniles and cysts of *Heterodera glycines* per pot of soil (1,400 cm^3) under soybean plants grown in soil infested with 5,000 nematode eggs per pot before exposures began. Each point is the mean of three values (one value per block). Maximum standard deviation of the transformed data was 0.86 for juveniles and 0.23 for cysts. Back-transformed means are graphed.

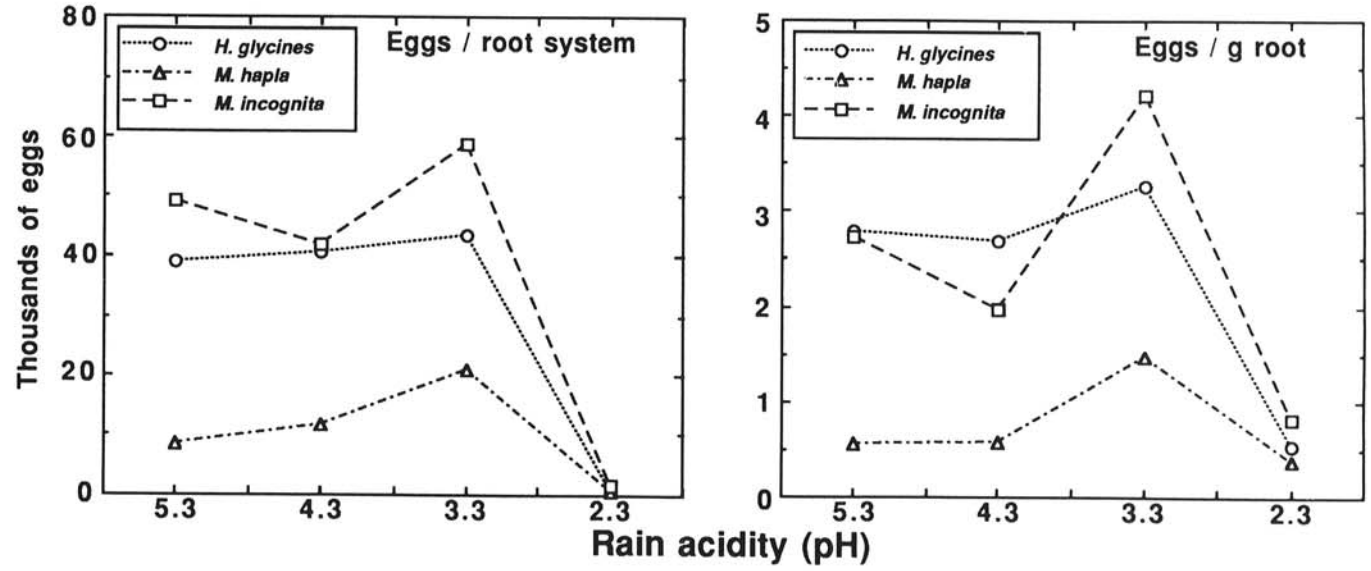

Fig. 5. Effect of acidity of simulated rain in experiment 1 on final number of nematode eggs per root system and number of nematode eggs per gram (fresh weight) of root tissue of soybean plants grown in soil infested with 5,000 nematode eggs per pot before exposures began. Each point is the mean of three values (one value per block). Maximum standard deviation of the transformed data was 0.82 for eggs/root system and 0.72 for eggs/g root. Back-transformed means are graphed.

1; root fresh weights after rains at pH 2.3 averaged <10% of those from plants exposed to rains at other acidities (linear, quadratic, and lack-of-fit components of the acidity main effect all significant at $P < 0.01$). Averaged across all acidities, the fresh weight of roots of control plants was slightly less than that of all infected plants combined (9.6 g versus 11.2 g; $P < 0.01$). Nodulation by *B. japonicum* (data not shown) was affected by the acidity × nematode treatment interaction as in experiment 1.

The influence of acidity on the amount of root galling caused by root-knot nematodes was the same for both species in experiment 1 but differed in experiment 2 ($P < 0.05$) because rain acidity had no consistent effect on galling by *M. incognita* in experiment 2 (Fig. 6). Galling by *M. hapla*, however, was increased again by rains at pH 2.3 (Con7 and Con8 $P < 0.04$) even though the overall extent of galling in experiment 2 was less than in experiment 1.

Magnitudes of most variables indicative of nematode reproduction were less in experiment 2 than in experiment 1. The significant main effect of nematode treatment on number of juveniles per pot was attibutable to a greater number of *Heterodera* juveniles (ranging from ~100 after pH 2.3 rains to ~1,000 after pH 5.3 rains) than *Meloidogyne* juveniles (less than 20 per pot regardless of rain acidity) (contrast of *H. glycines* versus both *Meloidogyne* species $P < 0.01$); thus, although the main effect of rain across all three nematode species was not significant ($P < 0.13$), the pH-related effect on cyst nematode juveniles was clear. The number of eggs per root system also was smaller in experiment 2 (Fig. 6) than in experiment 1 (Fig. 5) and was not affected by the acidity × nematode treatment interaction (Table 3), but the suppressive influence of rains at pH 2.3 relative to other acidities occurred again (linear, quadratic, and lack-of-fit components of the acidity main effect all significant at $P < 0.01$). Numbers of eggs per root system averaged across all acidities were approximately 43,000 (*H. glycines*), 1,500 (*M. hapla*), and 19,000 (*M. incognita*). In contrast to number of juveniles or eggs, number of cysts was greater in experiment 2 than in experiment 1; the acidity-related suppression of cyst number in both experiments was nearly identical (Figs. 4 and 6).

DISCUSSION

Changes in host-parasite interactions between plant roots and sedentary endoparasitic nematodes, which were suggested by results of a field experiment (34), were verified under controlled conditions. Results clearly demonstrated that pH-related trends differed for control versus nematode-infected plants. Character-

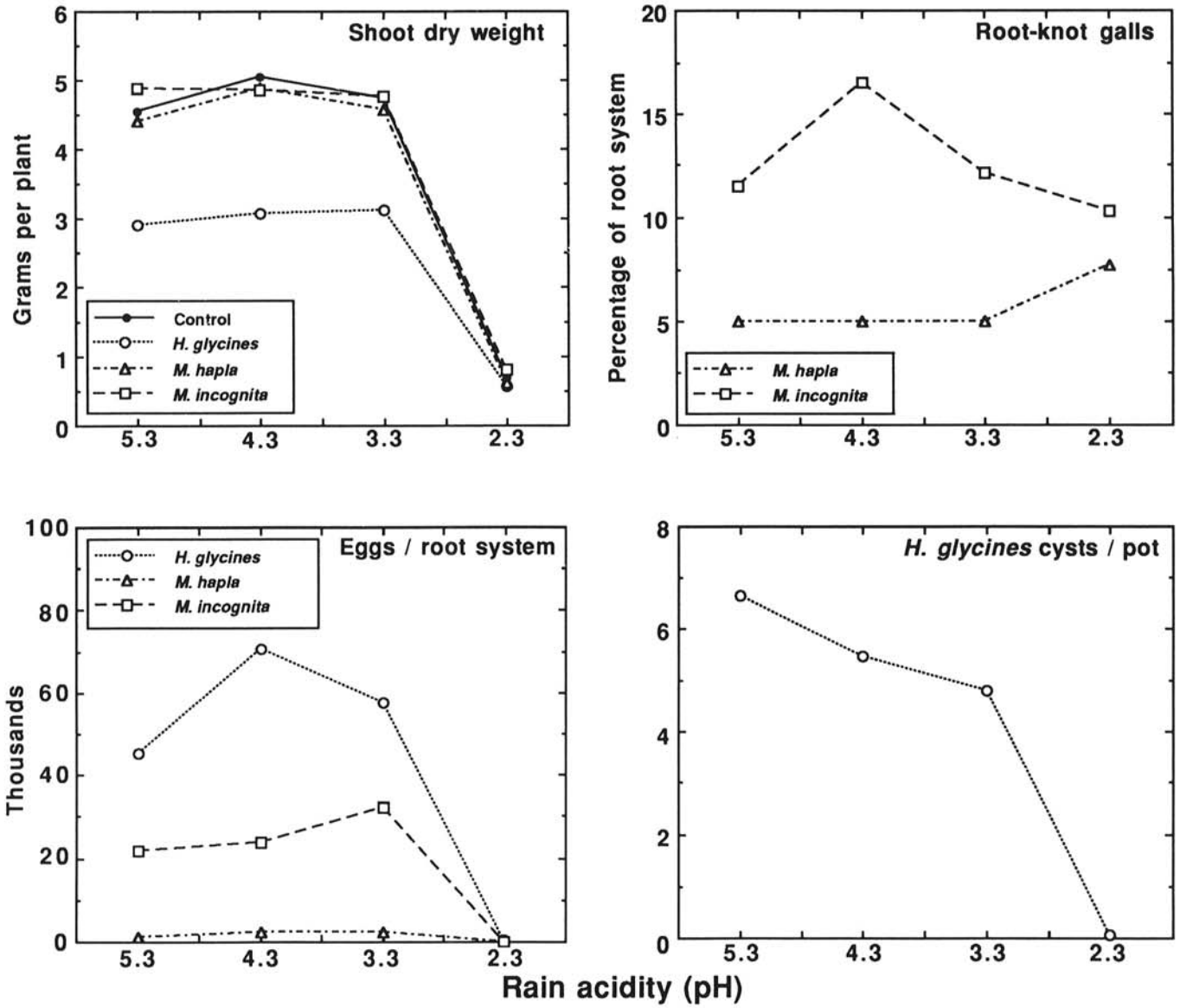

Fig. 6. Effect of acidity of simulated rain in experiment 2 on selected dependent variables. Soybean plants were grown in noninfested soil or soil infested with 5,000 nematode eggs per pot before exposures began. Each point is the mean of three values (one value per block). Maximum standard deviation of the transformed data was 0.19 for shoot dry weight, 0.06 for galls, 2.77 for eggs, and 0.81 for cysts. Back-transformed means are graphed.

istics of dose-response relationships for *H. glycines* generally were different from those for the two *Meloidogyne* species, but dose-response relationships for plants infected with *M. incognita* or *M. hapla* generally were similar. Trends were quite consistent despite differences between experiments in the magnitudes of certain measurements. Furthermore, several results of these experiments (extreme foliar injury only after exposure to simulated rains at pH < 3; plant stunting by nematodes; suppression of nodulation by acid deposition or *H. glycines*; suppression of nematode reproduction in acidified soil) were consistent with previously reported phenomena (e.g., 9,18,26,35). Current rates of acid deposition onto agronomic soils in the field, however, probably do not cause epidemiologically important changes in the types of variables measured here because major changes that occurred in our experiments were associated with acidity levels that exceed ambient deposition.

Mechanisms that control impacts of acid deposition on soilborne microorganisms associated with plant roots could be mediated by effects on the plant foliage, roots, or the soil environment. A root- or soil-mediated effect of acid deposition on soilborne microorganisms was indicated in another experiment in which population densities of rhizosphere-inhabiting bacteria, fungi, and actinomycetes were altered when simulated acidic rains were applied to sorghum foliage + soil or soil alone, but no changes were detected when rains were applied to foliage only (30). Hatching of *M. javanica* eggs can be inhibited in soils at pH < 5 (40), so one possible mechanism behind the influence of rain acidity on nematode reproduction may be acidification of the soil environment. In the present experiments, rains at pH 2.3 were required for a major increase in soil free acidity to a pH less than the pre-exposure value of 4.9 or to cause major changes in plant or nematode variables. The pH 2.3 treatments deposited more than 15× the SO_4^{2} and 12× the NO_3^{-} on a per-hectare basis in 8 wk than occurred in all of 1980 in the regions of the eastern United States that received the most acidic precipitation (19). Thus, if the primary mechanism behind the impact of acid deposition on these nematodes is soil-mediated, current ambient acid deposition rates (average pH 4.0–4.5 in most of the eastern United States) (19) probably have little impact on the host-parasite interactions in agricultural soils. This conclusion seems reasonable in the context of the buffer capacity of most agricultural soils (greater than that of the poorly buffered sand-soil mix used here) and the quantities of fertilizers and other chemicals that are applied yearly in most crop production systems. However, a soil-mediated mechanism such as an increase in acidity (and subsequent increase in aluminum), sulfate, or nitrate might have a greater impact on ectoparasitic nematodes, which remain outside the roots throughout the life cycle, than on the endoparasites we studied. This hypothesis is supported by field data, from which soil clay content and copper and sodium concentrations were useful in explaining spatial variation among population densities of *M. incognita, Tylenchorhynchus claytoni,* and *Helicotylenchus dihystera,* but variables related to the suitability of the soil as a nematode habitat, such as texture, moisture content, organic matter, and acidity, affected ectoparasites more than *M. incognita* (25). Furthermore, based on surveys of fields throughout the world, the occurrence of *Meloidogyne* spp. is poorly correlated with soil elements (37). Both soil chemical characteristics and nematode population densities are spatially quite variable, so hypothetical changes caused by ambient acid deposition on either regional soil environments or population densities would be extremely subtle and difficult to demonstrate.

Changes induced by acid deposition in plant physiological processes could have impacts on plant-parasitic nematodes that might not occur for microorganisms that do not actually inhabit or feed from the root. Plant-parasitic nematodes are obligate parasites, so changes in nematode reproduction can be mediated by the host (26). Plant stunting caused by the most acidic treatments represents suppression of carbon fixation and allocation to biomass, which may indicate restricted availability of carbon and plant metabolites for the nematodes in roots. This mechanism could account for part of the suppression of nematode reproduction associated with rains at pH 2.3. Obvious changes in the plants and nematode populations, however, were evident only at acidities that exceed current levels in precipitation, so the occurrence of measurable interactions of this type in the field seem unlikely.

The high proportion of the root system that exhibited root-knot galls after rains at pH 2.3 can be attributed to the stunted root systems. An increase in susceptibility or a decrease in tolerance to nematodes when plants are under acid-deposition stress, however, is another explanation because stress-induced changes in susceptibility to nematodes can occur. For example, population densities of *Pratylenchus penetrans* associated with eggplant were greater when plant roots were infected by *Verticillium dahliae* than when plants were not infected by the fungus (24). In studies of effects of simulated acidic rain on Phytophthora root rot of lupine, however, the pathogen apparently was more sensitive than the plant to acid deposition (31).

This research did not address possible effects of acid deposition on parasitic nematodes in unmanaged systems. The ecology of plant-parasitic nematodes in nonagricultural systems, particularly forests, has been studied very little. Forest soils are not fertilized regularly and often are poorly buffered, so forest soils in some regions are considered among the most sensitive to adverse effects of atmospheric deposition (1). Long-term atmospheric deposition could change the chemical and biological properties of such soils over many years, and plant interactions with parasitic nematodes in the system might be altered. The discovery of large numbers of a previously unknown sedentary endoparasite, *Sphaeronema sasseri* (8), on roots of declining red spruce and Fraser fir at high elevations subject to acid deposition raises questions regarding the importance of the nematodes in that area. Whether the nematodes are a primary cause or a contributing factor in the decline or whether they become important only after the trees begin to succumb to other stresses remains to be determined.

In conclusion, ambient levels of acid deposition probably have little effect on interactions of root-knot and soybean cyst nematodes with plants in agricultural soils. The impact of acid deposition on other types of nematodes, such as ectoparasites, and the species indigenous to unmanaged ecosystems remains unknown.

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