Interactions Between *Glomus geosporum* and Exposure of Soybeans to Ozone or Simulated Acid Rain in the Field

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ABSTRACT

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The effects of chronic exposure of soybean plants to ozone (O_3) or to simulated acid rain on interactions between *Glomus geosporum* and soybean plants were studied at a field site near Raleigh, NC. In one experiment, plants grown in open-top field chambers were exposed to seasonal 7-hr (0930–1630 hours EDT) per day mean O_3 concentrations of 0.025, 0.049, or 0.079 ppm O_3 for 139 days (from 12 days after emergence until plant harvest). In a concurrent experiment, plants were exposed twice per week for 13 weeks to simulated rain of pH 5.5, 4.0, 3.2, or 2.8. Ozone at 0.049 and 0.079 ppm caused foliar injury and growth reduction; O_3 at 0.079 ppm also decreased pod yield. Soybeans infected by *G. geosporum* were less sensitive to adverse effects caused by 0.079 ppm O_3; pod yield of mycorrhizal plants was reduced by 25%, whereas pod yield of nonmycorrhizal plants was reduced by 48%. Seasonal changes in soil P content indicated that only at 0.079 ppm O_3 were mycorrhizal plants more efficient in P uptake than were nonmycorrhizal plants. *G. geosporum* produced 40% fewer chlamydospores per gram of root at 0.079 than at 0.025 ppm O_3. In the concurrent experiment, neither rain acidity nor *G. geosporum* significantly affected foliar nutrient ion content, vegetative growth, or yield. However, *G. geosporum* produced 39% fewer chlamydospores per gram of root when plants received simulated rain of pH 2.8 than of pH 5.5.

Vesicular-arbuscular (VA) mycorrhizae can enhance the growth or yield of soybeans (23,25) and several other agricultural crops (3,6,9). This effect has been attributed to enhanced phosphorus (P) uptake by mycorrhizal roots (3,6,9); it may be increased by low soil fertility (3,8,9,23), decreased by competition with other soil microorganisms (24,26), or altered by environmental conditions (6,17). McCool et al (17) exposed citrange orange seedlings for 19 wk to 0.45 ppm ozone (O_3) (two 3-hr events per week), 0.90 ppm O_3 (one 6-hr event per week), or charcoal-filtered air. Growth enhancement of citrange by *Glomus fasiculatum* (Thaxter) Gerd. & Trappe was reduced by 0.90 ppm O_3. Growth of nonmycorrhizal plants was not significantly affected by O_3. No other reports on the effects of mycorrhizal infection on host sensitivity to air pollutants have been published.

Effects of ambient oxidants or controlled O_3 exposures on several agricultural crops were recently reviewed (14). Soybeans grown in field chambers were injured by seasonal, 6-hr daily exposures to 0.05 ppm O_3, and growth and yield were inhibited by exposures to 0.10 ppm O_3 (11). Open-top field chambers have been used to demonstrate relationships between O_3 doses and yields of soybeans, winter wheat, spinach, and field corn (12). However, more data relating O_3 doses to crop yields are needed.

Ambient rain in the eastern United States is acidic; annual means range between pH 4.1 and 4.6 (22). Hydrogen ions from sulfuric and nitric acids account for the majority of the acidity in wet deposition (21); acidity from dry deposition has not been well quantified. There are no reports of crop injury attributed to ambient acidic deposition. Several plant species, including soybeans (29), have been injured by greenhouse exposures to simulated sulfuric acid rain. In field studies, treatments simulating the chemistry and deposition of natural rain have caused foliar injury to soybean at a pH as high as 3.5 (16); soybean seed yield has been stimulated, reduced, or not affected in the range of pH 4.0–2.7 (4,15).

Most studies concerning interactions between air pollutants and host-parasite relationships have been conducted in greenhouse or controlled-environment chambers. Acute O_3 exposures have inhibited nodulation by *Rhizobium* species (1,18), enhanced colonization by saprophytic *Fusarium* species (18), and altered the feeding behavior of some nematode species (30). Numbers of externally borne chlamydospores of *G. fasiculatum* per gram of root of citrange orange seedlings were not affected by 0.45 or 0.90 ppm O_3 (17). Effects of field O_3 exposures on interactions between crop plants and soilborne organisms have not been reported. Simulated sulfuric acid rain at pH 3.2 has reduced Rhizobium nodulation of soybeans and kidney beans (27,29) and telia formation by *Cronartium fusiforme* Hedgc. and Long (27).

Our objectives were to determine whether *G. geosporum* (Nichol. and Gerd.) Walker could affect host sensitivity to O_3 or simulated acid rain, and whether O_3 or simulated acid rain could affect interactions between a VA mycorrhizal fungus (*G. geosporum*) and soybean plants.

MATERIALS AND METHODS

The experiments were conducted at a field site 8 km south of Raleigh, NC, during the summer of 1979, using soybeans grown in 15-L pots. Sandy-loam soil (Appling - clayey, kaolinitic, thermic, Typic Hapludults) and quartz sand in a 2:1 ratio by volume were fumigated with methyl bromide (1.6 kg/m^3). This growth medium contained 122 kg P/ha and had an initial pH of 6.3.

Chlamydospores of *G. geosporum* were obtained from cultures established from isolated chlamydospores (25) and maintained on roots of soybeans grown in a greenhouse. Roots were rinsed free of soil and macerated in a blender, and chlamydospores were

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separated from the blend by wet-sieving and sucrose centrifugation (25). An electric cement mixer was used to thoroughly mix chlamydospores with steam-sterilized sand. Sand (400 ml) containing approximately 1,400 chlamydospores was mixed into the growth medium for each of one hundred 15-L plastic pots. Noninfested steam-sterilized sand (400 ml) was combined with the medium for each of 100 additional 15-L pots.

Seeds of *Glycine max* (L.) Merr. 'Davis' were inoculated with *Rhizobium japonicum* (Nitragin Co., Milwaukee, WI 53209) and planted 10 per pot in 200 pots on 8 June. Seedlings were uniformly thinned to five per pot 14 days after planting and to two per pot 31 days after planting. Plants were mulched with wheat straw and fertilized with 4 g of 5-10-10 (N-P-K) fertilizer per pot. Insects were controlled by foliar application of Carbaryl® (9 g/L of water) on 27 June. Plants were watered as necessary to prevent wilting.

Ozone exposure. Ozone exposures were conducted in six open-top field chambers (10). Three treatments were produced by modifying the air passing into the chambers: charcoal filtration (CF), which reduced ambient O_3 to residual levels (control treatment); nonfiltered air, to which 0.01–0.02 ppm O_3 was added daily from 0930 to 1630 hours (NF-1); and nonfiltered air, to which 0.05–0.06 ppm O_3 was added daily from 0930 to 1630 hours (NF-2). There was one treatment per chamber and three treatments (chambers) per replicate. All treatments were replicated twice. Within each chamber, six pots contained inoculum of *G. geosporum* and six pots contained no inoculum, providing a split-plot design. Plants were exposed to O_3 for 139 days (from 12 days after plant emergence until harvest).

Ozone was produced, dispensed, and monitored as described previously (13). Ozone concentrations in nonfiltered-air chambers were directly related to ambient O_3 concentrations (Figs. 1 and 2). Seasonal 7-hr (0930–1630 hours) per day mean O_3 concentrations in the CF, NF-1, and NF-2 chamber treatments and in ambient air were 0.025, 0.049, 0.079, and 0.049 ppm, respectively.

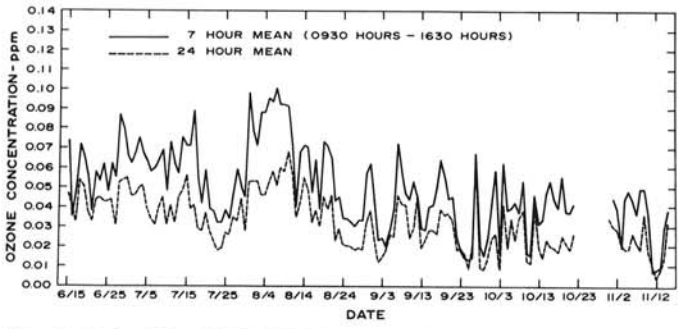

Fig. 1. Daily 7-hr (0930–1630 hours EDT) and 24-hr mean ozone concentrations for ambient air 8 km south of Raleigh, NC, during 1979.

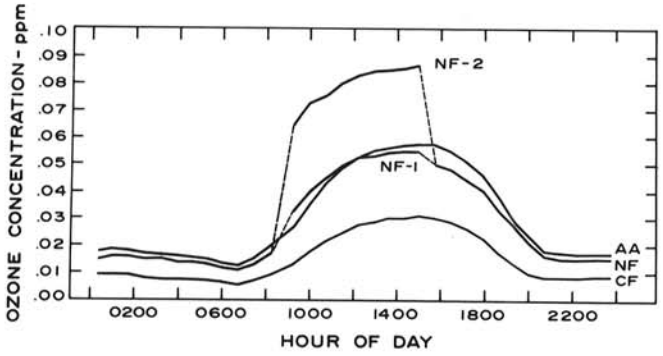

Fig. 2. Seasonal hourly mean ozone (O_3) concentrations in ambient air and in open-top field chambers. Seasonal 7-hr (0930–1630 hours EDT) per day means for ambient air (AA), carbon-filtered air (CF), nonfiltered air plus 0.01–0.02 ppm O_3 (NF-1) or nonfiltered air plus 0.05–0.06 ppm O_3 (NF-2) were 0.049, 0.025, 0.049, or 0.079 ppm O_3, respectively. Means are for the period from 27 June to 14 November.

Simulated acid rain exposure. In a separate experiment, plants in four pots containing inoculum of *G. geosporum* and four pots with no inoculum were alternated in the center row of each of sixteen 3 × 3-m field plots according to a split-plot design. Soybeans grown in the ground in a row 0.75 m to either side of the pots served as border rows (5). Simulated rains of pH 5.5, 4.0, 3.2, or 2.8 were applied to separate plots in each of four randomized complete blocks (four different treatment plots per block).

"Rain" solutions were mixed in four 760-L polypropylene reservoirs (Nalgene Corp., Rochester, NY) before each rain event. To simulate the chemical composition of ambient rain (28), microelements in the following concentrations were added to deionized water (568 L) in each reservoir: 10 μeq/L Ca^{++}, 7.8 μeq/L NH_4^+, 5.1 μeq/L Na^+, 2.3 μeq/L K^+, 4.9 μeq/L Mg^{++}, 11 μeq/L $SO_4^=$, 12 μeq/L NO_3^-, and 12 μeq/L Cl^-. A solution at pH 5.5 was obtained in one reservoir by adding a 1 *N* solution of sodium hydroxide to deionized water (initial pH 4.9–5.3). Solutions at pH 4.0 (±0.20), 3.2 (±0.20), or 2.8 (±0.15) were obtained by adding approximately 15, 100, or 800 ml, respectively, of a 1 *N* solution of sulfuric and nitric acid (70:30 ratio by volume, 33.6 g/L $SO_4^=$ 18.9 g/L NO_3^-) to three of the reservoirs. A pH analyzer (Electromark, Markson Science, Inc., Del Mar, CA) was used to measure the pH of the "rain" solutions before and after each event (5).

Each of the four reservoirs supplied solutions to one plot per block through a recirculating dispensing system (5). "Rain" with a mean droplet size of 0.9 mm (28) was dispensed to each plot through four spray nozzles (stainless steel with a polyvinyl chloride orifice plate) (28) pointed upward. The nozzles were mounted on horizontal arms that were raised to accommodate plant growth and were rotated above the plot to adjust for wind direction. The target area for the four nozzles was the entire 3 × 3-m plot area.

"Rain" (0.74 cm) was applied intermittently during a 1-hr period at a rate of approximately 1.8 cm/hr twice per week from 27 June to 4 October (26 events). "Rain" applications were usually performed between 0700 and 0900 hours to avoid winds above 8 km/hr (5). Ambient rainfall was not excluded from the plots. Plants received a total of 19.2 cm of simulated rain and 44.8 cm of ambient rain (measured and analyzed as part of the National Atmospheric Deposition Program) (21) during the experimental period (Fig. 3). The pH, amount, and chemical constituents of simulated and ambient rain are given in Table 1.

Plant and soil measures. Sampling methodology was the same in

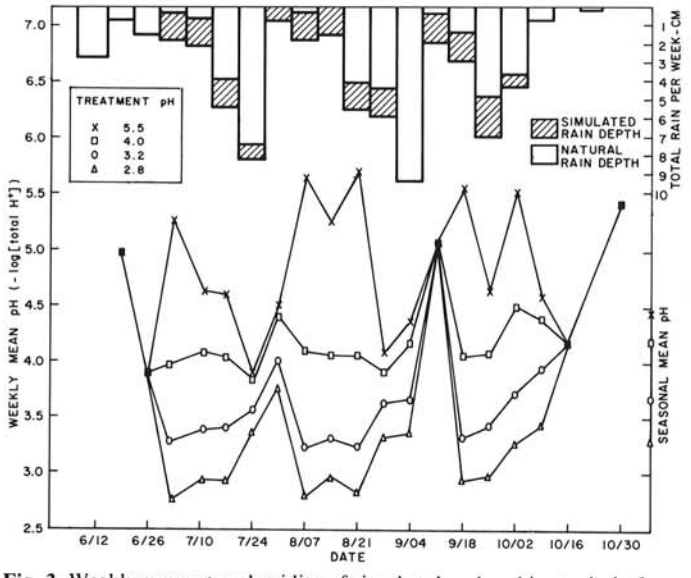

Fig. 3. Weekly amount and acidity of simulated and ambient rain in four different treatments. Weekly and seasonal mean pH values are negative logarithms of the total H^+ input from simulated and ambient rain per volume of rain received each week and over the 1979 growing season.

both studies unless otherwise stated. Equal numbers of samples of mycorrhizal and nonmycorrhizal plants were collected within each pollutant treatment.

Foliar injury was estimated as the percentage chlorosis or necrosis of main-stem trifoliolates. Ratings were made in 5% increments (0–100%); abscised leaves were rated as 100% injured. Foliar injury of 12 plants per O_3 treatment was assessed biweekly between 1 August and 11 October (six times). Foliar injury of 32 plants per "rain" treatment was estimated when plants were 6 wk old (after seven simulated rain events).

Foliar elemental contents were determined from samples of distal leaflets removed from plants on 4 October. Each sample consisted of 24 leaflets (one leaflet from each leaf at the same three node positions for each of eight plants). Eight samples per O_3 treatment and six samples per "rain" treatment were analyzed at the North Carolina Department of Agriculture (NCDA) Plant Analysis Laboratory, Raleigh, NC. Concentrations of Ca, Mg, Na, Mn, Fe, Zn, and Cu were measured by atomic adsorption spectrophotometry, K by flame emission spectrophotometry, N by Kjeldahl filtration, and P and S by colorimetric readings.

Plants were harvested on 1 November in the "rain" study and on 14 November in the O_3 study. Plant shoots were weighed at harvest. Pods were removed from each plant, air-dried for 10 wk in a greenhouse, counted, and weighed. Pods from six plants in the same treatment were pooled as one sample; eight samples of pods per O_3 or "rain" treatment were threshed. Weights of 100-seed samples were determined. Percentage protein and oil in seeds were analyzed at the USDA Regional Laboratory, Peoria, IL. Seed elemental contents were measured as described for foliage.

Soil elemental contents were determined from samples taken at the beginning (29 June) and end (29 November) of the growing season. Soil cores (2.5-cm diameter; 15-cm depth) taken one per pot were combined as four samples per O_3 treatment per date or eight samples per "rain" treatment per date. Measurements were made at the NCDA Soil Analysis Laboratory, Raleigh, NC. Soil pH in water was measured by sodium glycerolphosphate buffer reagent (20); extractable soil P, K, Ca, Mg, Mn, Cu, and Zn were measured by the Mehlich II method (19).

Mycorrhizal development. Percentage colonization of root tissue and chlamydospore production by *G. geosporum* were measured. Colonization was measured for roots of 12-wk-old plants (after 64 days of O_3 exposure or 18 "rain" events). Three soil cores (2.5 × 15 cm) from each pot were combined as one sample per pot. A semiautomatic soil elutriator (2) was used to extract roots from samples of infested soil (12 samples per O_3 treatment; eight samples per "rain" treatment). Roots were recovered on a sieve with 600-μm openings and were separated from debris by floating and decanting. Roots of uniform diameter were cut into lengths of 1–3 cm, cleared in 10% KOH, stained in 0.1% Trypan blue in lactophenol, mounted on microscope slides, and examined at ×30 (5,25). Percentage colonization was calculated as the ratio of root length (measured with a micrometer) containing hyphae, arbuscules, vesicles, and/or chlamydospores to total root length (28–42 cm per sample) observed.

Chlamydospores were counted in samples taken on 15 November and 8 December in the O_3 study, and on 15 November in the "rain" study. Four soil cores (2.5 × 15 cm) from each pot containing inoculum (12 pots per O_3 treatment or 16 pots per "rain" treatment) were combined as one sample per pot per sample date. Roots from the first sample date in the O_3 study were recovered by floating and decanting (25). All other root samples were recovered using the soil elutriator. External or internal chlamydospores (borne on hyphae outside or within the roots, respectively) per sample were recovered separately on a sieve with 53-μm openings. External chlamydospores were separated from intact roots by vigorously spraying with water. Internal chlamydospores were separated by macerating roots in a blender. External or internal chlamydospores in three replicate aliquots (10 ml) of spore suspensions (100 ml) were counted under ×30 magnification (5,25). Root debris from each sample was collected, dried, and weighed. Mean numbers of chlamydospores per gram of root were calculated.

Analyses of variance were performed on all data. Fisher's least significant differences (FLSD) between treatment means were determined where F tests indicated significant treatment effects ($P \leq 0.05$). Linear regressions relating O_3 doses to injury, growth, and yield were determined.

RESULTS

Ozone exposure. The amount of foliar injury increased as O_3 concentration and duration of exposure increased (Table 2). Mycorrhizal (M) plants exhibited consistently less injury due to 0.049 or 0.079 ppm O_3 than did nonmycorrhizal (NM) plants, but the differences were not statistically significant on any observation date.

Mycorrhizal plants were less sensitive to effects of O_3 on growth and yield than were NM plants (Table 3). Shoot weights of M plants exposed to 0.049 and 0.079 ppm O_3 were 99 and 72%, respectively, of those at 0.025 ppm O_3. Shoot weights of NM plants at 0.049 and 0.079 ppm O_3 were 73 and 48%, respectively, of those at 0.025 ppm O_3. Results for pod weights and numbers were similar to those for shoot weights. Differences between responses of M and NM plants were significant at 0.079, but not at 0.025 or 0.049 ppm O_3. Mycorrhizae did not alter the effects of O_3 in decreasing seed mass (100-seed weight).

Foliar N content was reduced by O_3 at 0.079 ppm; the mean percentage N in foliage exposed to 0.025, 0.049, and 0.079 ppm O_3 was 3.90, 3.91, and 3.44%, respectively. Seasonal changes in soil P content indicated that M and NM plants used equal amounts of soil P at 0.025 ppm (18% decrease in soil P) and at 0.049 ppm O_3 (11% decrease in soil P). However, at 0.079 ppm O_3, soil used by M plants showed a 12% decrease in P, whereas soil used by NM plants showed no seasonal change in P content. There were no differences in percentage P in foliage or seed between M and NM plants at any O_3 level. Concentrations of other nutrients in the foliage, seed, or soil were not affected by O_3 or mycorrhizae.

TABLE 1. The pH, amount, and chemical constituents of simulated and ambient rain received by soybeans

Rain pH	Total deposition (cm)[a]	Chemical constituents (mg/L)[b]								Seasonal mean (simulated plus ambient rain)[c]		
		SO_4	NO_3-N	NH_3-N	Ca	Mg	K	Na	Cl	pH	SO_4 (mg/L)	NO_3 (mg/L)
Simulated												
5.5	19.2	1.55	0.15	0.045	0.55	0.13	0.07	0.17	0.44	4.46	2.33	0.85
4.0	19.2	3.45	0.46	0.084	0.45	0.13	0.08	0.11	0.49	4.20	2.90	0.94
3.2	19.2	28.10	3.39	0.120	0.49	0.12	0.08	0.12	0.41	3.69	10.31	1.82
2.8	19.2	80.30	9.65	0.126	0.44	0.12	0.07	0.11	0.38	3.31	26.00	3.70
Ambient	44.8	2.67	1.14	···	0.15	0.04	0.04	0.47	0.32	···	···	···

[a] Simulated rain was applied in 26 events (0.74 cm per event) between 27 June and 4 October. Ambient rain ws not excluded from the field plots.
[b] For simulated rain values are means of samples (100 ml) collected from three field plots on 20 September. For ambient rain values are means of 20 weekly samples (12 June–30 October) collected at the site from an Aerochemetric rain collector as part of the National Atmospheric Deposition Program (21).
[c] Values are means calculated from total H^+, $SO_4^=$, or NO_3^- deposited in total (simulated plus ambient) rain (5.73×10^3 L).

Eighty-four percent of the length of roots sampled from 12-wk-old inoculated plants was colonized by hyphae, arbuscules, and/or vesicles of *G. geosporum*. Percentage colonization was not affected by O_3. However, *G. geosporum* produced 58, 32, and 40% fewer external, internal, and total chlamydospores, respectively, per gram of root at 0.079 than at 0.025 ppm O_3 (Fig. 4). Spore counts within the 0.049-ppm treatment were highly variable among replicates; therefore, O_3 treatment effects were not statistically significant when results of all three treatments were analyzed. In analyses of variance between the 0.025- and 0.079-ppm O_3 treatments, the 40% reduction of total chlamydospores per gram of root was significant. Weights of sampled roots were not affected by O_3 concentration.

Simulated rain exposure. Weekly and seasonal mean pH values were computed from the total hydrogen ion input per total volume of simulated plus ambient rain received per week and per season (Fig. 3). The pH of ambient rain ranged from 3.9 to 6.5. Seasonal mean pH values for the four treatments were 4.46, 4.20, 3.69, and 3.31.

Chlorotic lesions were observed on the newly expanded leaves of immature plants receiving simulated rain of pH 2.8 and (infrequently) pH 3.2. Less than 15% of the leaf area was injured, and new lesions were not observed on any leaves after plants were 9 wk old.

Neither "rain" acidity nor mycorrhizae significantly affected soybean growth, yield, seed protein, or oil content. Trends toward increased foliar S and N and towards decreased foliar Mn were observed as "rain" acidity increased. Soil pH changed over the season from 6.3 to 5.3 in the pH 2.8 "rain" treatment and from 6.3 to 5.4 in the other three treatments. Trends towards less extractable Ca and Mg in soil were observed as the pH of the "rain" treatment decreased. Concentrations of other elements in foliage, seeds, and soil were not affected by "rain" acidity or mycorrhizae.

Seventy-two percent of the length of roots of 12-wk-old-inoculated plants was colonized by *G. geosporum*. Percentage colonization was not affected by "rain" treatment. However, *G. geosporum* produced 39% fewer internal chlamydospores per gram of root at pH 2.8 than at pH 5.5 "rain" (Fig. 4). This result was significant in an analysis of variance for the pH 5.5 and 2.8 treatments but not in the analysis for all four treatments. External chlamydospores per gram of root were similar in all treatments. Sampled roots from plants in the pH 2.8 "rain" treatment weighed significantly less (18%) than those from the other three treatments.

TABLE 2. Percentage foliar injury of mycorrhizal (M) and nonmycorrhizal (NM) soybeans exposed to different levels of ozone

Days of exposure[a]	Foliar injury (%)					
	0.025 ppm O_3[b]		0.049 ppm O_3[b]		0.079 ppm O_3[b]	
	M	NM	M	NM	M	NM
34	1	1	1	3	6	12
48	5	3	3	20	16	29
61	9	7	25	44	46	56
74	32	34	46	62	65	74
91	38	56	58	81	79	86
105	45	53	76	88	90	98
Coefficient[c]	0.728	0.891	1.122	1.117	1.239	1.215

[a] Plants received either carbon-filtered air (0.025 ppm O_3) or nonfiltered air to which small concentrations of O_3 were added for 7 hr per day (0930–1630 hours EDT) for 139 days. Soil of mycorrhizal plants was amended prior to planting with 1,400 chlamydospores of *Glomus geosporum* per 15 L.

[b] Values are means of injury (chlorosis and necrosis) of eight trifoliolates (fifth through twelfth oldest leaves on main stem) of 6 M or 6 NM plants. Injury measured for plants at 0.025 ppm O_3 probably resulted mainly from normal senescence.

[c] Linear regression coefficient for days of exposure. All regressions were significant at $P > F \leq 0.01$.

DISCUSSION

The results show that soybeans infected with *G. geosporum* were less sensitive to adverse growth and yield effects of O_3. Reduced sensitivity may be related to the ability of M roots to obtain nutrients from a greater volume of soil than NM roots. Increased efficiency of M roots could compensate for an O_3-induced reduction in root biomass, resulting in less of an impact on growth

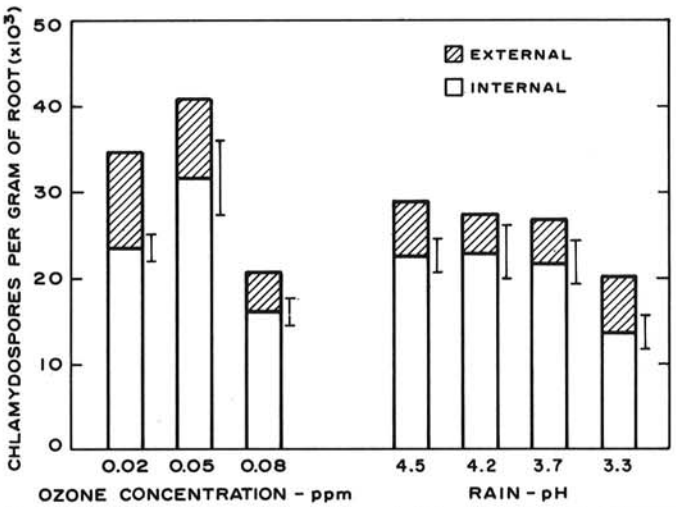

Fig. 4. Numbers of external or internal chlamydospores of *Glomus geosporum* per gram of root of soybeans exposed to different levels of O_3 or simulated rain. Intervals shown are standard errors of each mean number of internal chlamydospores per gram of root. Ozone concentrations are 7-hr (0930–1630 hours EDT) per day seasonal (27 June to 14 November) means; pH values are negative logarithms of total H^+ input from ambient and simulated rain from 12 June to 30 October. Plants received 19.2 cm of simulated rain of pH 5.5, 4.0, 3.2, or 2.8 in addition to 44.8 cm of ambient rain.

TABLE 3. Effects of ozone on growth and yield of mycorrhizal (M) and nonmycorrhizal (NM) Davis soybeans

Seasonal 7-hr per day mean ozone concentration (ppm)[a]	Shoot fresh weight at harvest[b] (g)		Dry weight of pods (with seeds) per plant[b] (g)		Pods per plant[b]		Mean weight of 100 seed[c]	
	M	NM	M	NM	M	NM	M	NM
0.025	233.3	266.8	114.2	120.7	199.9	217.8	17.0	17.3
0.049	231.3	194.4	108.3	99.9	214.1	192.8	15.5	15.3
0.079	169.2	129.1	85.2	62.8	196.7	147.9	13.5	14.5
Coefficient[d]	−1224.6*	−2535.0**	−546.6**	−1080.4*	NS[e]	−1302.7**	−64.5**	−67.6**

[a] Plants received either charcoal-filtered air (0.025 pm O_3) or nonfiltered air to which small concentrations of O_3 were added for 7 hr (0930–1630 hours) daily for 139 days. Soil of mycorrhizal plants was amended with 1,400 chlamydospores of *Glomus geosporum* per 15 L.

[b] Values are means of 24 plants.

[c] Values are means of four seed samples (six plants per sample).

[d] Linear regression coefficients for O_3. Significance of linear regression; * = ($P > F \leq 0.05$) or ** = ($P > F \leq 0.01$).

[e] NS = not significant.

and pod weight in M plants than in NM plants. A relationship between apparent P uptake by M roots and foliar injury due to O_3 was not demonstrated.

Growth and yield enhancement by VA mycorrhizae have previously been associated with P-deficient soils (3,6,9,23). A significant mycorrhizal-induced growth response of plants at 0.025 and 0.049 ppm O_3 was not observed; this may be due to the fact that sufficient P was available in the soil for normal plant growth. Results at 0.079 ppm O_3 suggest that VA mycorrhizae may be beneficial when soil P is not limiting, if plants are otherwise stressed. Results of the simulated acid rain experiment are consistent with these conclusions. Differences between our results and those of McCool et al (17) may have been due to differences in the type of exposure or to different sensitivities of the host and fungal symbionts involved. Mycorrhizal citrange may have been affected more by O_3 than NM citrange because M plants were growing faster during the experiment than NM plants. In our study, the growth of M and NM plants in the control treatment (0.025 ppm O_3) was similar (Table 3).

Sporulation by *G. geosporum* may have been inhibited as an indirect result of O_3 effects on host metabolism. Foliar injury and reduced foliar N content of soybeans were evidence that photosynthetic capacity was suppressed by O_3. Effects of O_3 on photosynthesis and respiration (14) would likely decrease carbohydrate translocation to the roots, thus reducing the nutrient source for *G. geosporum*. This is consistent with the report that low starch content in roots of partially defoliated tobacco plants was accompanied by decreased mycorrhizal infection (6). Reduced nodulation by *Rhizobium japonicum* has also been attributed to low food reserves in roots of soybeans exposed to O_3 (1).

Sporulation by *G. geosporum* was inhibited by rain (simulated plus ambient) with a seasonal mean pH of 3.31. This inhibition may have been an indirect result of altered host metabolism. Lesser weights of soybean root samples at low pH "rain" indicated reduced carbohydrate reserves in these roots. Thus, as in the case of O_3, sporulation may have been inhibited by reduced nutrient source. Alternately, the fungus may have been affected directly by concentrations of H^+, $SO_4^=$, or NO_3^- in the soil. Previous studies have found the extent of endomycorrhizal infection to be influenced by soil pH (generally less infection in acid than in near-neutral soils) (7). The pH effect may be due to direct toxicity of H^+ or to greater solubility of pH-dependent toxic metals (Al or Mn). Hydrogen ions from simulated rain of pH 3.2 were shown to inhibit nodulation of soybean by *R. japonicum*, as were $SO_4^=$ ions (29). Since sulfates may also be fungistatic (25), their accumulation in the soil may have inhibited *G. geosporum*. Although increased N in field soils was observed to inhibit sporulation of *G. geosporum* in wheat (8), it is not clear whether N input from simulated rain was sufficient to inhibit *G. geosporum* in soybeans. These hypotheses need to be tested under controlled conditions to determine the mechanisms of inhibition.

The symbiosis between *G. geosporum* and soybean may not have been altered by either pollutant. Since root colonization and apparent P uptake of mycorrhizal roots were not inhibited by either pollutant, reduced fungal sporulation may not indicate reduced benefit to the host. Because soil fertility and competition from other microorganisms affect soybean mycorrhizae (24,26), fungal-pollutant interactions as observed here may be different in P-deficient or nonfumigated field soils.

Our results indicate that ambient rain at current pH and ion levels would not be expected to negatively affect soybean yield. In fact, at current levels of wet deposition, S and N from ambient rain may benefit plants grown in low-fertility soils (15). Agricultural crops have not been studied sufficiently to predict the effects of dry acidic deposition, discrete rain events with high ionic loadings, or continuous deposition at current loading levels. Longer term studies are needed to address these questions.

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