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INFLUENCE OF A MYCORRHIZAL FUNGUS AND/OR RHIZOBIUM ON GROWTH AND BIOMASS PARTITIONING OF SUBTERRANEAN CLOVER EXPOSED TO OZONE*

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Abstract. The influence of soilborne symbionts such as rhizobia or mycorrhizal fungi on plant response to ozone (O_3) has not been well defined. Leguminous plants in the field are infected by both types of organisms, which influence plant nutrition and growth. We studied the effects of infection with *Rhizobium leguminosarum* biovar *trifolii* and/or *Gigaspora margarita* on response of subterranean clover (*Trifolium subterraneum* L. cv Mt. Barker) to O_3 . Exposures were conducted in greenhouse CSTR chambers using four O_3 concentrations [charcoal-filtered (CF), 50, 100, or 150 ppb; 6 h day⁻¹, 5 day wk⁻¹ for 12 weeks] as main plots (replicated). Four inoculum types were subplot treatments, i.e., inoculated with one, both, or neither microorganisms. At 2-wk intervals, plants were exposed to ¹⁴CO₂ and harvested 24 h later for determination of biomass and ¹⁴C content of shoots and roots. Ozone at 100 or 150 ppb suppressed clover growth during the experiment. Inoculation with *G. margarita* alone suppressed clover growth by the last two harvests, whereas *R. leguminosarum* alone enhanced growth during this time period. When both symbionts were present, the plants grew similarly to the noninoculated controls. Shoot/root ratios were increased by 100 or 150 ppb O_3 compared to that for CF-treated plants. Shoot/root ratios were greater for all inoculated plants compared to noninoculated controls. Under low O_3 stress (CF or 50 ppb), plants inoculated with both *R. leguminosarum* and *G. margarita* transported a greater proportion of recent photosynthate (¹⁴C) to roots than did noninoculated plants; we attribute this to metabolic requirements of the microorganisms. At the highest level of O_3 stress (150 ppb), this did not occur, probably because little photosynthate was available and the shoots retained most of it for repair of injury. Statistically significant interactions occurred between O_3 and inoculum types for shoot and total biomass. When averaged across harvests, 50 ppb O_3 suppressed biomass in the plants inoculated with *G. margarita* alone. Apparently, the mycorrhizal fungus is such a significant C drain that even a small amount of O_3 stress suppresses plant growth under these conditions.

Key words: rhizobia, VAM

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1. Introduction

The photochemical air pollutant ozone (O_3) affects physiological function and growth of plants (Alscher and Wellburn, 1994), including limitation of photosynthesis and alteration of carbon (C) allocation and biomass partitioning (Miller, 1988). Reduced allocation of C to roots by O_3 typically suppresses root growth. Interference with C assimilation and allocation to roots may cause indirect as well as direct effects on shoot growth.

Optimum growth of leguminous plants is usually dependent on symbiotic relations with mycorrhizal fungi and nitrogen (N_2)-fixing bacteria. Mycorrhizal infection of plant roots usually stimulates plant growth through effects on nutrient uptake (especially P) and/or water supply (e.g. Safir, 1994). Under some conditions, however, mycorrhizal fungi may suppress plant growth if the benefits do not offset the sink effect on root C (Bethlenfalvay *et al.*, 1982; Modjo and Hendrix, 1986; Safir, 1994). In most cases, infection by *Rhizobium* spp. provides a benefit to growth of leguminous plants unless N is abundant. Because the response of plants to these organisms is dependent in part on the health and C budget of the roots, O_3 may affect plant growth indirectly by limiting C availability to microbial symbionts.

Ozone can suppress nodulation and N_2 fixation in leguminous plants (e.g. Tingey and Blum, 1973; Montes *et al.*, 1983; Flagler *et al.*, 1987). Similarly, mycorrhizal infection can be suppressed by O_3 . For example, treatment of tomato (*Lycopersicon esculentum* Mill. 'Heinz 1350') plants with 300 ppb O_3 (3 h, once a wk for 9 wk) suppressed infection by *Glomus fasciculatus* as much as 63% (McCool and Menge, 1983). In this case, O_3 suppressed dry weight of mycorrhizal but not of non-mycorrhizal plants. Ozone (150 ppb for 6 h day⁻¹, 5 day wk⁻¹ for 8 wks) suppressed the proportion of plant N fixed from the air by a tripartite association of *Trifolium subterraneum*, *Gigaspora margarita*, and *Rhizobium leguminosarum* (Shafer and Schoeneberger, 1991b).

In most studies of O_3 effects on plant growth, the presence of *Rhizobium* or mycorrhizal fungi has not been a controlled variable, and few experiments have addressed $O_3 \times$ symbiont interactions. In greenhouse experiments, mycorrhizal tomato plants were more sensitive than non-mycorrhizal plants to O_3 (McCool and Menge, 1983 and 1984). Similarly, root dry weight of *Rhizobium*-infected subterranean clover grown and exposed to O_3 in a greenhouse was suppressed more than roots of non-infected plants (Shafer and Schoeneberger, 1991a). In a field experiment with soybean, however, mycorrhizal plants were less sensitive to O_3 than non-mycorrhizal plants (Brewer and Heagle, 1983). Thus, reliable generalizations about $O_3 \times$ symbiont interactions are unavailable.

The objective of our study was to determine the separate and combined effects of a mycorrhizal fungus and *Rhizobium* on plant response to O_3 . Growth, partitioning of biomass between shoots and roots, and allocation of recent photosynthate to shoots and roots were quantified.

2. Methods

Congaree loam soil (fine-loamy, mixed nonacid, thermic, Typic Udifluvents; 0–10 cm depth; pH 5.8, 0.11% N, 12.0 mg extractable P/kg soil) was collected from the Schenck Forest in Raleigh, North Carolina, sieved (6-mm mesh), and steamed (4 h at 80 °C on three consecutive days) to eliminate indigenous rhizobia and mycorrhizal fungi. Soil was spread on a greenhouse bench and air-dried, then placed in paper bags and dried for 48 h at 70 °C. Polyethylene pots (11.4-cm-diam) with drain holes covered by fiberglass screen were filled with soil, and each was placed inside another pot of the same size. Seven planting holes approximately 5 mm diam \times 5 mm deep were made in the surface of the soil in each pot. The soil was not fertilized.

Seeds of subterranean clover (*Trifolium subterraneum* L 'Mt. Barker') were surface-disinfested in 80% ethanol for 10 min, rinsed five times in sterile deionized water, soaked in sterile water for 30 min, rinsed, and placed on sterile moist filter paper in foil-covered Petri dishes for 24 h. One seedling with a 1–2 mm-long radicle was transplanted into each of the seven holes in the soil in each pot.

Rhizobium leguminosarum biovar *trifolii* isolate R039 (obtained from A. G. Wollum, Dept. of Soil Science, North Carolina State University, Raleigh, NC USA) was grown at room temperature in yeast-extract-mannitol broth (YEM; Vincent, 1970) cultures on a wrist-action shaker for 4 days. Half the cultures were bulked for live inoculum, and the others were autoclaved (20 min at 121 °C) and bulked for *Rhizobium* controls. Inoculum (1.5 ml per pot) was pipetted onto the seedling in each planting hole. Spores of the mycorrhizal fungus *Gigaspora margarita* Becker and Hall recovered from roots and soil from pot cultures with Bahaia grass (obtained from R. Roncadori, University of Georgia, Athens, GA) were surface-disinfested in 10% chlorine bleach for 2 min, rinsed on a sieve under running tap water for 5 min, and diluted to 85 spores mL⁻¹ in sterile dilute water agar (1 g agar L⁻¹ water). Control inoculum for mycorrhizal fungi was sterile water agar. Inoculum (2 mL per pot) was pipetted onto the seedling in each planting hole. The soil was then closed over the seedlings.

Plants were maintained on a bench in a nonshaded greenhouse cooled by passing charcoal-filtered air through evaporative pads. Soil was moistened several times daily with a fine mist of deionized water. Fourteen days after planting, plants were thinned to three per pot. Eighteen days after planting, the soil surface in each pot was covered by a 1.5-cm-deep layer of autoclaved perlite to insulate the soil from heat and provide some protection against soil splash.

The treatment design was a factorial combination of O₃ concentrations, rhizobial inocula, and fungal inocula. Four O₃ concentrations [charcoal-filtered air (CF, <10 ppb O₃), and CF air to which O₃ was added to obtain 50, 100, or 150 ppb] were main-plot treatments in a replicated split-plot experimental design. Main plots were continuous-stirred tank reactor (CSTR) chambers (Heck *et al.*, 1978) in a nonshaded greenhouse (one CSTR per O₃ concentration in each of two randomized

complete blocks). The subplot treatments were four inoculum types, i.e. inoculated with *R. leguminosarum*, *G. margarita*, both microorganisms, or neither (controls). Initially, 12 pots of each inoculum type were placed in each chamber. Plants were exposed to O₃ beginning 24 days after planting and during the subsequent 12 wk for 6 h day⁻¹ (0900–1500 h EST), 5 day wk⁻¹ (Monday through Friday). Ozone was produced from oxygen with a corona discharge ozonator (Griffin Technics Corp., Lodi, NJ USA) and monitored by ultraviolet absorbance (Thermoelectron model 49, Thermal Electron Instruments, Hopkinton, MA). Temperatures within the CSTRs were monitored with thermocouples and averaged 21 to 22 °C for the 12-wk exposure period (average daily high 24 to 26 °C and average daily low 19 to 20 °C).

Plants were labeled with ¹⁴C and harvested at 2-wk intervals (six harvests) starting at 16 days after initiating O₃ treatments. Plants were labeled with ¹⁴C by placing two pots of each inoculum type (selected randomly) from each chamber in a plexiglass cuvette and introducing 0.01 mCi of ¹⁴CO₂ (generated from Na₂CO₃) for 15 min. Plants were labeled on two successive days (one block per day) between 1000 and 1200 h EST. After ¹⁴C labeling, each pot was returned to its respective CSTR. After 24 h, the plants were harvested in the order in which they were labeled. Plants were divided into roots and shoots. The roots were washed thoroughly, and all tissues were dried rapidly at 80 °C. Plant tissues were ground to a fine powder, and 50-mg samples in Harvey Carbon-14 Cocktail (Ox-161) were combusted to CO₂ with an R.J. Harvey Biological Material Oxidizer (Model OX-200). Samples were counted with a Packard Tri-Carb Scintillation Counter.

Data were tested for homogeneity of variance, and transformations were made as directed by results of the Box-Cox test [shoot dry wt. (inverse square root); root dry wt., total dry weight, shoot/root dry wt. ratio (natural log)] (Box and Cox, 1964). Analysis was performed by GLM (SAS, 1988) with O₃ as the main-plot treatment. Sums-of-squares for O₃ and harvest were partitioned into single-degree-of-freedom (1df) components by orthogonal polynomial equations. Replicates (R, =blocks) and O₃ were tested against the R × O₃ term (error a). All other effects and interactions were tested against the error b term. Three pre-planned 1-df contrasts (non-inoculated vs. dual-inoculated, *G. margarita* only-inoculated vs. dual-inoculated, and *R. leguminosarum* only-inoculated vs. dual-inoculated) were used to test inoculum effects.

The statistical model was built sequentially, starting with the full model, which included all possible polynomials. The sequence of entry of the polynomial interaction terms in the model was based on the degree (order) of the polynomial, which is defined as the sum of the powers of the components in the interaction terms (Rawlings, 1988). The lower-degree terms were entered first. The inoculum (INOC) effect was not partitioned into 1-df contrasts during model building because the three 1-df contrasts of interest had been identified in the experimental design. When all possible polynomial contrasts were included in the model, the error b term was the sum of the sums of squares associated with the effects R ×

INOC, $R \times O_3 \times \text{INOC}$, $R \times \text{HARV}$, $R \times O_3 \times \text{HARV}$, $R \times \text{INOC} \times \text{HARV}$, and $R \times O_3 \times \text{INOC} \times \text{HARV}$, with 92 df, for which HARV=number of weeks elapsed from the onset of exposures to O_3 until harvest.

The model was reduced by eliminating nonsignificant higher-order terms from the model one at a time, starting with the highest-order interaction. Sums of squares and associated degrees of freedom for nonsignificant higher-order effects were pooled with those for the error b term. The regression analysis was re-run each time a term was eliminated, and the sums of squares associated with the next highest-order term were tested against the new error term. If nonsignificant, that effect was also pooled with the error term and the process was repeated. All lower-order terms that were components of significant higher-order terms were retained in the model. The simplest model that fit all biomass variables was selected (Table I). The error term used to test the biomass variables (error b) is the sum of the sums of squares for: $R \times \text{INOC}$, $R \times O_3 \times \text{INOC}$, $O_Q \times \text{INOC}$, $O_C \times \text{INOC}$, $R \times \text{HARV}$, $R \times O_3 \times \text{HARV}$, $O_L \times H_W$, $O_Q \times H_F$, $O_C \times H_C$, $O_Q \times H_W$, $O_C \times H_F$, $O_C \times H_W$, $R \times \text{HARV} \times \text{INOC}$, $H_C \times \text{INOC}$, $H_F \times \text{INOC}$, $H_W \times \text{INOC}$, $R \times O_3 \times \text{HARV} \times \text{INOC}$, $O_Q \times H_L \times \text{INOC}$, $O_L \times H_C \times \text{INOC}$, $O_Q \times H_Q \times \text{INOC}$, $O_C \times H_L \times \text{INOC}$, $O_L \times H_F \times \text{INOC}$, $O_Q \times H_C \times \text{INOC}$, $O_C \times H_Q \times \text{INOC}$, $O_L \times H_W \times \text{INOC}$, $O_Q \times H_F \times \text{INOC}$, $O_C \times H_C \times \text{INOC}$, $O_Q \times H_W \times \text{INOC}$, $O_C \times H_F \times \text{INOC}$, $O_C \times H_W \times \text{INOC}$, with 152 df, for which subscripts L, Q, C, F, and W denote linear, quadratic, cubic, quartic, and lack-of-fit polynomial components, respectively, of sum of squares for O_3 or HARV.

The model-building procedure was repeated for the proportion of ^{14}C in shoots, which was measured at harvests 2–6, and a slightly different model was obtained. Error b for the final model for the proportion of ^{14}C in shoots contained the sums of squares for: $R \times \text{INOC}$, $R \times O_3 \times \text{INOC}$, $O_Q \times \text{INOC}$, $O_C \times \text{INOC}$, $R \times \text{HARV}$, $R \times O_3 \times \text{HARV}$, $O_C \times H_C$, $O_C \times H_Q$, $O_Q \times H_F$, $O_C \times H_C$, $O_C \times H_F$, $R \times \text{HARV} \times \text{INOC}$, $H_Q \times \text{INOC}$, $H_C \times \text{INOC}$, $H_F \times \text{INOC}$, $R \times O_3 \times \text{HARV} \times \text{INOC}$, $O_L \times H_L \times \text{INOC}$, $O_L \times H_Q \times \text{INOC}$, $O_Q \times H_L \times \text{INOC}$, $O_L \times H_C \times \text{INOC}$, $O_Q \times H_Q \times \text{INOC}$, $O_C \times H_L \times \text{INOC}$, $O_L \times H_F \times \text{INOC}$, $O_Q \times H_C \times \text{INOC}$, $O_C \times H_Q \times \text{INOC}$, $O_Q \times H_F \times \text{INOC}$, $O_C \times H_C \times \text{INOC}$, $O_C \times H_F \times \text{INOC}$, with 132 df (Table I).

3. Results and Discussion

Biomass. Statistical analyses (Table I) demonstrated that main effects of O_3 , inoculum, and harvest were significant for shoot, root, and total biomass. Two-way and three-way interactions of O_3 with harvest and/or inoculum occurred, however; thus, generalizations about main effects must be interpreted with respect to these interactions.

Ozone at 100 or 150 ppb suppressed shoot, root, and total clover biomass over the course of the experiment, especially by the later harvests ($O_l \times H_l$, $p < 0.01$;

Table I

Mean squares for treatment effects on biomass, shoot/root ratio, and C-14 allocation in subterranean clover^a

Source of variation	Biomass				Shoot/root Ratio	Proportion of C-14 in shoot	
	df	Shoot dry Wt. (g)	Root dry Wt. (g)	Total dry Wt. (g)		df	C-14
Rep	1	0.782**	1.837*	1.936**	0.005	1	0.033*
Ozone (O)							
O linear (OL)	1	1.883**	11.442**	7.357**	1.227*	1	0.097*
O quadratic (OQ)	1	0.094*	1.172*	0.509*	0.377	1	0.033*
O cubic (OC)	1	0.061*	0.152	0.169*	0.001	1	0.001
Rep × O (error a) ^b	3	0.005	0.064	0.015	0.051	3	0.003
Inoculum (INOC) ^c	3	0.131**	0.457**	0.430**	0.139**	3	0.002
C vs D	1	0.024*	0.545*	0.164*	0.329*	1	0.003
D vs R	1	0.097*	0.448*	0.379*	0.008	1	0.005
D vs G	1	0.083*	0.047	0.189*	0.145*	1	0.001
OL × INOC	3	0.026**	0.039	0.033*	0.028	3	0.005*
OL × C vs D	1	0.008	0.011	0.004	0.080*	1	0.012**
OL × D vs R	1	0.001	0.006	0.001	0.037	1	0.005
OL × D vs G	1	0.064*	0.041	0.079*	0.019	1	0.001
Harvest Linear (HL)	1	20.922**	67.70**	55.88**	1.805**	1	0.021**
Harvest Quadratic (HQ)	1	2.584**	2.75**	2.827**	0.000	1	0.001
Harvest Cubic (HC)	1	0.008	0.20**	0.096**	0.040	1	0.001
Harvest Quartic (HF)	1	0.000	0.030	0.005	0.030	1	0.001
Harvest Lack-of-Fit (HW)	1	0.036**	0.11*	0.003	0.440**		
OL × HL	1	0.040**	0.220**	0.429**	0.077*	1	0.000
OL × HQ	1	0.004	0.030	0.025	0.001	1	0.002
OQ × HL	1	0.004	0.040	0.034	0.007	1	0.000
OL × HC	1	0.005	0.020	0.000	0.032	1	0.000
OQ × HQ	1	0.002	0.004	0.010	0.003	1	0.005
OC × HL	1	0.022*	0.002	0.004	0.023	1	0.005
OL × HF	1	0.002	0.001	0.003	0.004	1	0.010*
OQ × HC	1	0.004	0.009	0.017	0.003		
OC × HQ	1	0.023*	0.040	0.067**	0.017		
HL × INOC	3	0.181**	0.546**	0.567**	0.005	3	0.013**
HL × C vs D	1	0.049*	0.033	0.053*	0.008	1	0.036**
HL × D vs R	1	0.008	0.166	0.131*	0.003	1	0.015**
HL × D vs G	1	0.341*	0.716	0.816*	0.012	1	0.003

Table I
Continued.

Source of variation	Biomass				Shoot/root Ratio	Proportion of C-14 in shoot	
	df	Shoot dry Wt. (g)	Root dry Wt. (g)	Total dry Wt. (g)		df	C-14
HQ × INOC	3	0.017**	0.018	0.044**	0.038		
HQ × C vs D	1	0.001	0.021	0.002	0.015		
HQ × D vs R	1	0.005	0.000	0.014	0.023		
HQ × D vs G	1	0.020*	0.029	0.057*	0.023		
OL × HL × INOC	3	0.008	0.050	0.012	0.068**		
OL × HL × C vs D	1	0.005	0.108*	0.002	0.201**		
OL × HL × D vs R	1	0.001	0.021	0.000	0.057		
OL × HL × D vs G	1	0.022*	0.000	0.018	0.033		
OL × HQ × INOC	3	0.014*	0.066*	0.034*	0.018		
OL × HQ × C vs D	1	0.005	0.121*	0.042*	0.042		
OL × HQ × D vs R	1	0.000	0.052	0.011	0.032		
OL × HQ × D vs G	1	0.033**	0.170**	0.092**	0.032		
Error b ^d	152	0.005	0.019	0.011	0.020	132 ^e	0.002

^a Data were transformed as needed [shoot dry wt. (inverse square root); root dry wt., total dry wt., and shoot/root dry wt. ratio (natural log)]. *, ** indicate significance at 0.05 and 0.01, respectively.

^b Error a used to test Rep, O₃, O_L, O_Q and O_C.

^c Pre-planned, 1-df contrasts to compare inoculum effects. C vs D = noninoculated vs. dual-inoculated; D vs R = dual-inoculated vs *R. leguminosarum* only-inoculated; D vs G = dual-inoculated vs *G. margarita* only-inoculated.

^d Error b was used to test all effects in the model not listed in ^b above. Error b contains sums of squares and associated df from all higher-order polynomial terms that did not significantly affect any of the biomass response variables and those from Rep × treatment interactions. The model-building procedure is outlined in Rawlings, 1988, p. 384.

^e The procedure described in ^d was used to produce the response model for proportion of ¹⁴C in shoots. A slightly different model was obtained. No O₃ × HARV × INOC interaction effects were significant, so all sums of squares and df for those effects were pooled with the error b term. All other interaction terms for which no df are shown in the table are also included in the error b term.

Table I). Effects of O₃ on root and shoot biomass were similar to those shown for total biomass (Figure 1), except for slightly greater suppression of roots than shoots.

Differences attributable to effects of inoculum type on shoot, root, and total biomass increased over time as shown for total biomass (Figure 2) and were especially noticeable at harvests 5 and 6 (H_t × INOC, *p* < 0.01; Table I). Biomass was usually greatest for plants infected by *R. leguminosarum* only and least for plants infected by *G. margarita* only. When both symbionts were present, the effects on plant-growth by the mycorrhizal fungus and *Rhizobium* effectively cancelled, and the plants grew similarly to the noninoculated controls.

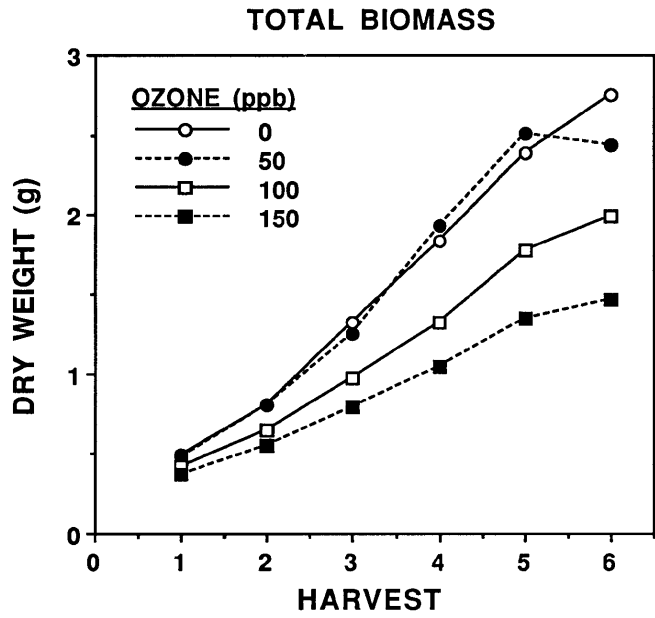


Figure 1. Main effects of O₃ on total plant biomass at 2-wk harvest intervals (data averaged across inoculum types).

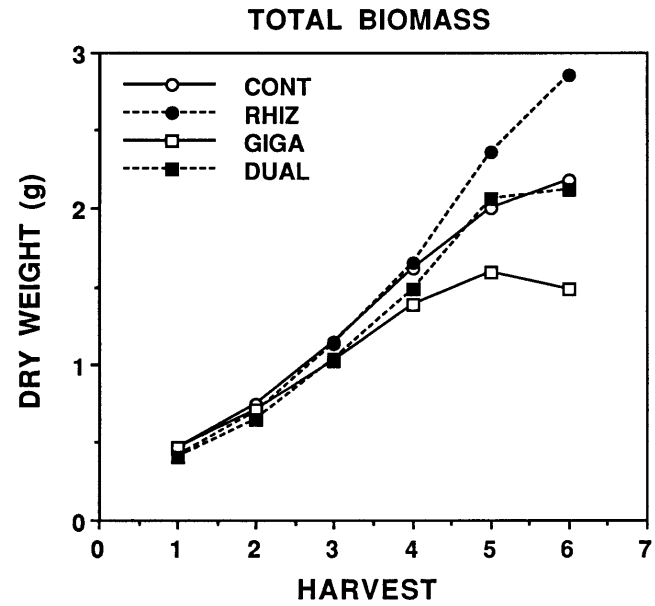


Figure 2. Effects of inocula on total plant biomass at 2-wk harvest intervals. Data averaged across O₃ concentrations. Inoculum treatments: noninoculated control (CONT), *Rhizobium leguminosarum* only (RHIZ), *Gigaspora margarita* only (GIGA), and both microorganisms (DUAL).

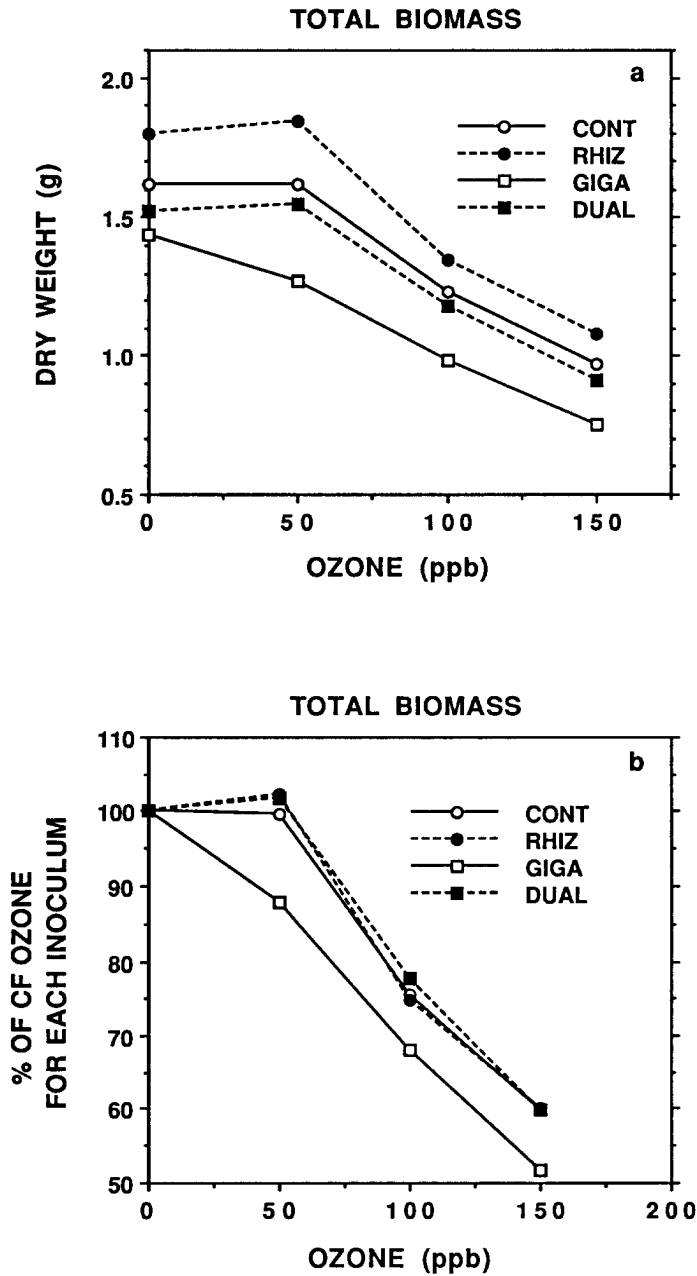


Figure 3. Effects of inocula on response of total biomass to O_3 . Data averaged across harvests. Data expressed as: (a) dry weight, (b) percentage of CF dry weight for each inoculum type.

The response of shoot and total biomass to O_3 were affected by inoculum type (Figure 3a) ($O_l \times \text{INOC}$; shoot dry wt. $p < 0.01$, total dry wt. $p < 0.05$; Table I). Plants inoculated with *R. leguminosarum* had greater biomass, and plants inoculated with *G. margarita* had less biomass across all O_3 concentrations than the noninoculated control or dual-inoculated plants. To compare relative response to O_3 , the total dry weights for plants with different inoculum types were expressed as percentages of those for plants with corresponding inoculum type exposed to CF (Figure 3b). The relative response of the plants to O_3 was similar except for plants inoculated with *G. margarita*, which were most responsive to O_3 . The $O_3 \times$ inoculum interaction was the primary interest in this experiment. The poor growth of the plants inoculated with *G. margarita* alone, even in the absence of O_3 stress, probably reflected the marginal energy status of these plants. Under the conditions of this experiment, the C sink imposed by the fungus apparently outweighed any beneficial effects, and even the ordinarily mild stress of 50 ppb O_3 suppressed growth.

Interpretation of $O_3 \times$ inoculum interactive effects on biomass components (Figure 4) is complicated further by changes over time ($O_l \times H_q \times \text{INOC}$, $p < 0.05$; Table I). The 1-df contrasts for $O_l \times H_q \times$ (dual-inoculated vs *Gigaspora* only) were significant ($P < 0.05$) for shoot, root, and total dry weight (e.g. Figure 4d vs. 4b). Significance of this interaction may be due in part to the cessation of growth by the O_3 -treated, *G. margarita* inoculated plants late in the experiment, in contrast to the dual-inoculated plants.

Shoot/Root Ratios. Main effects of O_3 , inoculum, and harvest date on shoot/root dry weight ratios (S/R) were significant (Table I). Values of S/R were greatest for plants grown in the higher O_3 concentrations (O_L , $p < 0.05$), especially 150 ppb (Figure 5a). Effects of O_3 on S/R differed among harvests ($O_L \times H_L$, $p < 0.05$), although the differences were not large. Values of S/R were usually lower for noninoculated and *Gigaspora*-inoculated compared to dual-inoculated plants (C vs D and D vs G, $p < 0.05$) (Figure 5b). No differences were maintained consistently among plants grown in the other infested soils. Ratios were lower at the last three harvests than at the first three harvests regardless of inoculum type (H_L and H_W , $p < 0.01$).

The $O_3 \times$ inoculum interaction was not significant for S/R (Table I); however, the three-way interaction ($O_l \times H_l \times \text{INOC}$) was significant ($p < 0.01$; Figure 6). The interaction probably resulted in part from the relatively high S/R for the dual-inoculated/150 ppb O_3 -treated plants at harvests 2, 3, 4, and 5 (Figure 6d). High S/R resulted from slow root growth during this period, which probably occurred because of the C demands of the two microorganisms in combination with the C limitation imposed by the high O_3 stress.

^{14}C Partitioning. The partitioning of recently fixed ^{14}C between shoots and roots was measured to determine how limitation of C supply due to O_3 stress interacted with the likely increased C demand due to the presence of the symbionts. The proportion of ^{14}C in shoots was affected by O_3 (O_l and O_q , $p < 0.05$; Table I) and

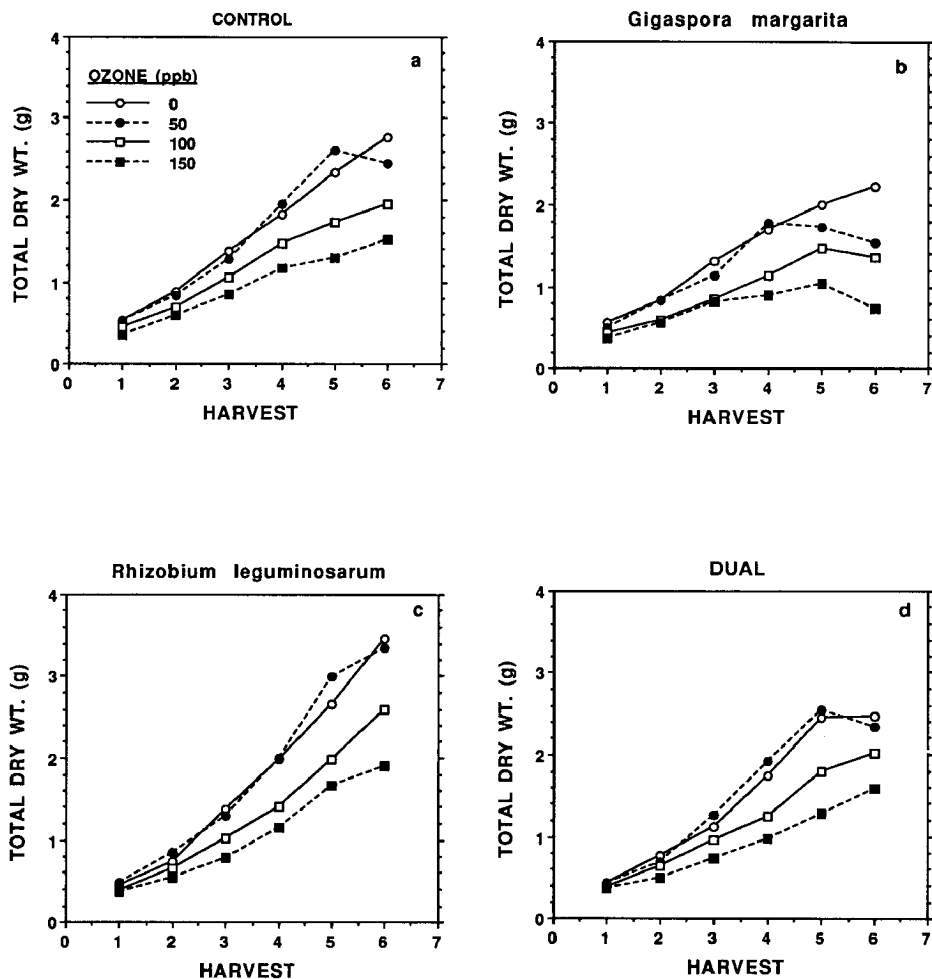


Figure 4. Effects of O₃ on total biomass at 2-wk harvest intervals for each inoculum type.

was greatest in plants treated with 150 ppb O₃ (Figure 7a and b). The allocation of ¹⁴C in response to O₃ treatment is generally consistent with observed effects on S/R ratios. For example, the greatest retention of ¹⁴C in shoots occurred in response to 150 ppb O₃, and S/R values were greatest for plants exposed to that concentration.

The main effect of inoculum on ¹⁴C retention in shoots was nonsignificant, but the microorganisms did affect the O₃-induced retention of ¹⁴C in shoots (Figure 7a) ($O_l \times \text{INOC}$, $p < 0.05$; Table I). The most notable differences were for the CF- and 50 ppb O₃-treated plants, among which dual-inoculated plants retained less ¹⁴C in shoots than noninoculated control plants [$O_l \times (\text{noninoculated vs dual-inoculated plants})$, $p < 0.01$]. Plants inoculated with *G. margarita* had less apparent retention of ¹⁴C in shoots compared to controls in the CF O₃ treatment, but the pre-

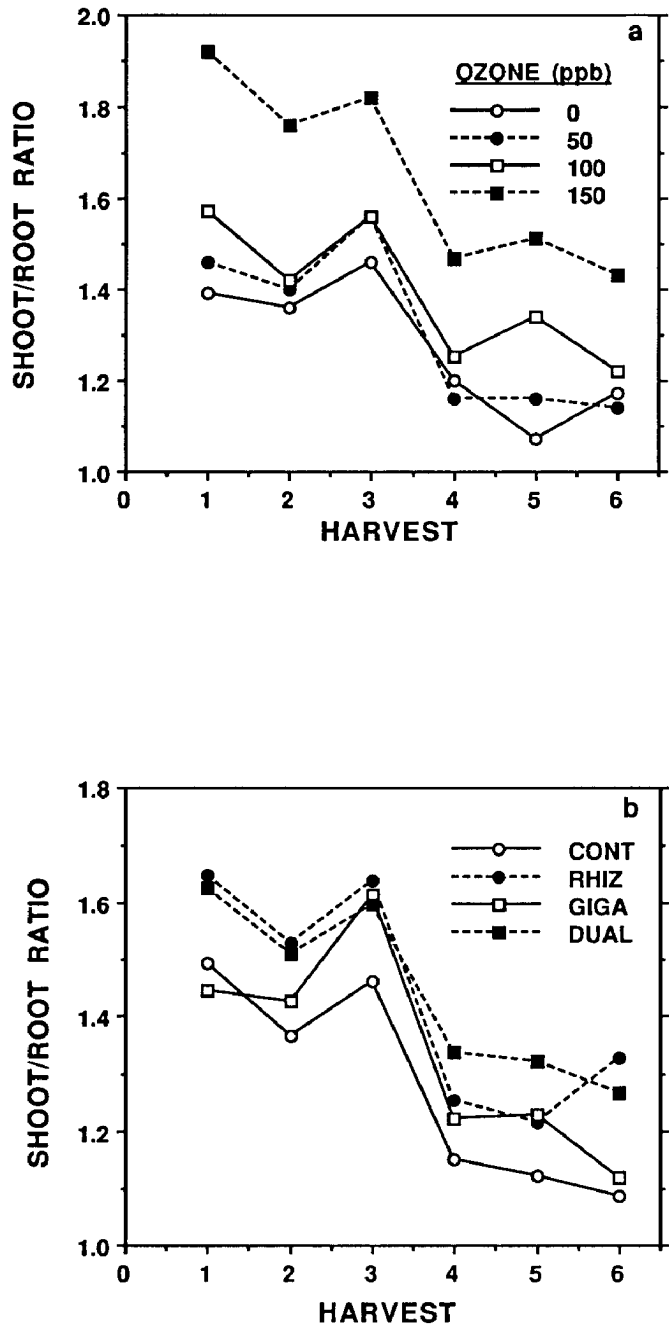


Figure 5. Shoot/root ratios at 2-wk harvest intervals. (a) Effects of O₃ for data averaged across inoculum types. (b) Effects of inoculum type for data averaged across O₃ concentrations.

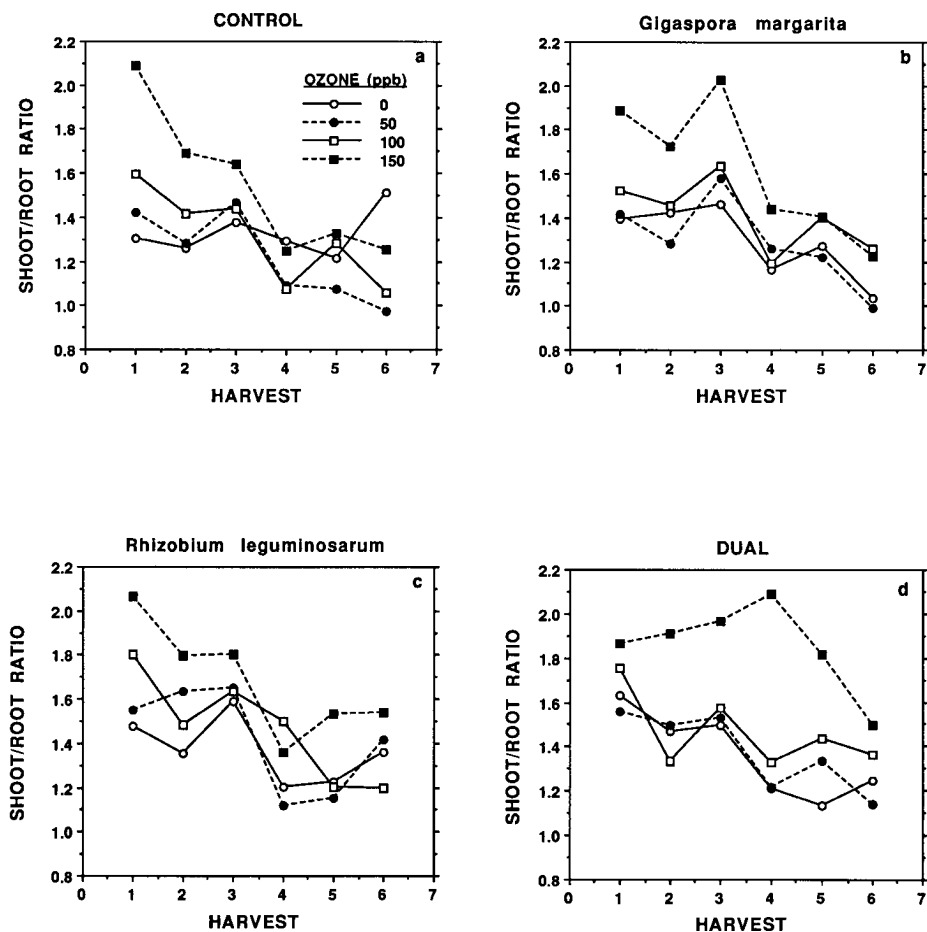


Figure 6. Effects of O₃ on shoot/root ratios at 2-wk harvest intervals for each inoculum type.

planned 1-df contrasts did not include a comparison of the *G. margarita*-treated and noninoculated control treatments. Apparently, under conditions of low O₃ stress, roots received an increased proportion of recently-fixed C when both soil microorganisms were present. As O₃ stress increased, this difference did not persist, probably because of reduced C availability due to suppression of photosynthesis by O₃ and increased demand for C by shoots for repair of injury. Under conditions of stress and limited C availability, shoots usually take priority over roots for receiving photosynthate (e.g. studies reviewed by Cooley and Manning, 1987).

Effects of O₃ on proportion of ¹⁴C in shoots (Figure 7b) changed over time (O_l × H_f, *p* < 0.05; Table I). For example, the proportion of ¹⁴C in shoots was similar among plants exposed to 0, 50, or 100 ppb O₃ by harvests 2 and 3, but the proportions diverged at the later harvests. The proportion of ¹⁴C in shoots was consistently highest across all harvest dates for plants exposed to 150 ppb O₃.

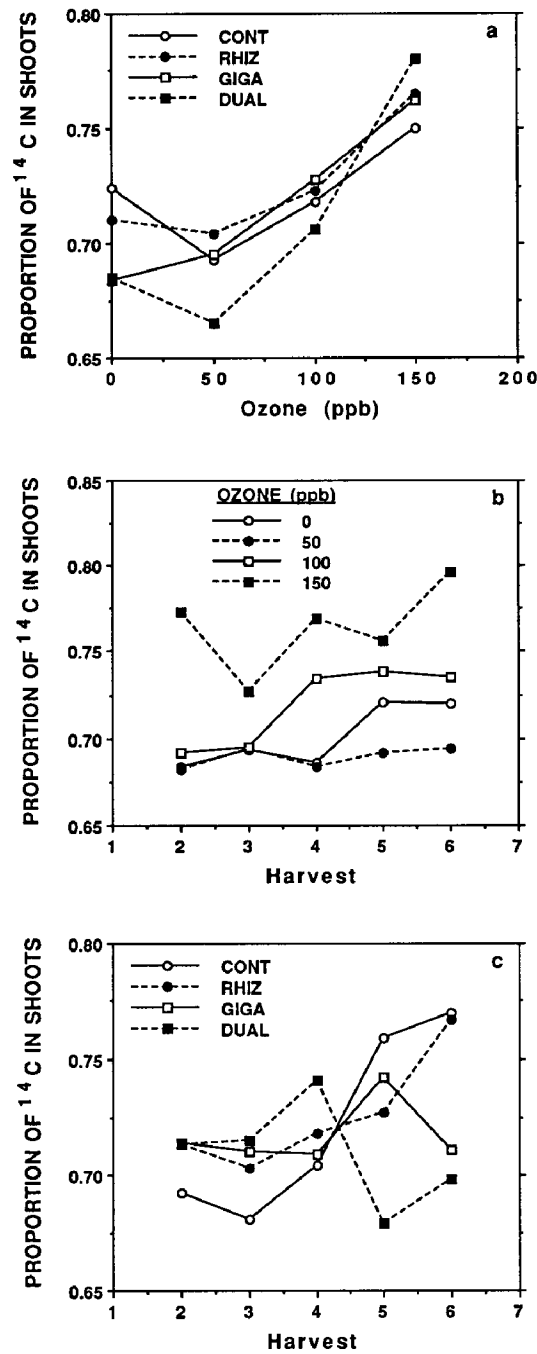


Figure 7. Proportion of C-14 in shoots. (a) Effects of O₃ for each inoculum type; data averaged across harvests. (b) Effects of O₃ concentration over time (harvest dates); data averaged across inoculum types. (c) Effects of inoculum types over time (harvest dates); data averaged across O₃ concentrations.

The proportion of ^{14}C in shoots was affected by harvest date (Figure 7c) (H_l , $p < 0.01$: Table I), but this relationship varied among plants that were inoculated differently ($H_l \times \text{INOC}$, $p < 0.01$). The proportion of ^{14}C retained in shoots was less in noninoculated control plants than in inoculated plants at harvest 2 and 3. At harvests 5 and 6 this reversed, and the dual-inoculated plants had the lowest proportion of ^{14}C in shoots [$H_l \times$ (noninoculated vs dual-inoculated plants) and $H_l \times$ (dual-inoculated vs. *Rhizobium*-only inoculated plants), $p < 0.01$]. This shift after harvest 4 probably reflects increased C demand by roots due to increased microbial activity in the dual-inoculated plants during the late stages of the experiment.

4. Conclusions

Most studies of the impact of O_3 on plant growth, development, and carbon allocation have not considered the potential role of symbiotic microorganisms as factors that can mediate plant responses. Some influences of root symbionts on plant response to O_3 have been reported previously (e.g. McCool and Menge, 1983 and 1984; Shafer and Schoeneberger, 1991a), but our study shows that these influences vary with the combination of symbionts present, among concentrations of O_3 , and over time. Furthermore, the combination of biomass, shoot/root ratio, and ^{14}C label data demonstrate the role of carbon allocation in explaining these effects. These findings have important implications with respect to the interpretation of studies of plant response to O_3 and the extrapolation of results to plants grown under different conditions. Studies of plant response to O_3 are often done in artificial or disinfested rooting media that precludes association with root symbionts. The resulting over-simplification of the plant-soil system can lead to an incomplete assessment of responses of carbon allocation and plant growth to O_3 . Our results illustrate the value of including appropriate microbial inocula in studies of plant responses to O_3 .

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