Effects of Long-Term Ozone Exposure and Soil Moisture Deficit on Growth of a Ladino Clover-Tall Fescue Pasture

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ABSTRACT

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Most field studies relating seasonal ozone (O_3) exposure to crop yield have been performed in the absence of plant moisture stress. Loss estimates from such studies may be too large if moisture stress, which occurs during most growing seasons, decreases plant sensitivity to O_3. Thus, we examined the response of a mixture of ladino clover (*Trifolium repens* L. 'Regal') and tall fescue (*Festuca arundinacea* Schreb. 'Kentucky 31') to chronic doses of O_3 at two soil-moisture levels over two growing seasons. The mixture was seeded on 15 September 1983 and exposed to six levels of O_3 in open-top chambers for 12-hr day^{-1} from 26 April to 18 October in 1984 and from 13 April to 22 October in 1985. The six seasonal 12-hr day^{-1} mean O_3 concentrations ranged from 0.025 to 0.092 $\mu L\ L^{-1}$. The soil-moisture treatments, obtained by differential irrigation, were well-watered or water-stressed. A soil-moisture deficit occurred intermittently in water-stressed plots during both seasons. Shoots were harvested when plants reached a height of 20–25 cm. Total forage (clover and fescue) yield in the water-stressed plots (O_3 levels combined) was 12% less than that in the well-watered plots in 1984 and 14% less in 1985. Clover was much more sensitive than fescue to O_3. Ozone-induced suppression of clover growth was accompanied by an increase in fescue growth, and these effects increased as the O_3 level increased. There was a statistically significant interaction between soil moisture and plant response to O_3 only in 1985. This effect probably occurred because clover growth exceeded fescue growth only in the charcoal-filtered air (CF) well-watered treatment. There were no significant effects of soil moisture on response to O_3 when CF was eliminated from the analysis of variance. Over the two seasons, estimated effects of ambient levels of O_3 (2-yr seasonal 12-hr day^{-1} mean of 0.046 $\mu L L^{-1}$) were a 10% decrease in total forage yield, a 19% decrease in clover yield, and a 19% increase in fescue yield (compared to 0.028 $\mu L\ L^{-1}$ of O_3). The decrease in total forage yield and decreased quality caused by decreased growth of clover suggest a need for ladino clover lines that are tolerant to O_3.

Additional keywords: air pollution, yield effects.

Ladino clover (*Trifolium repens* L.) and tall fescue (*Festuca arundinacea* Schreb.) grown together comprise an important forage crop in the southeastern United States. Clover provides soil nitrogen and enriches the protein content of the mixture (4) but typically persists for only a few years in mixture with grass (3). Factors suggested as a possible cause for clover decline include infectious diseases, insects, poor nutrition, adverse climate, and competition (3,14). Ambient levels of ozone (O_3) can suppress yields of many crop species (10,12) and also may cause clover decline (2,14,15). Two greenhouse studies (14,15) have shown that ladino clover is sensitive to acute doses of O_3. In field studies with a clover-fescue mixture, O_3 adversely affected clover and indirectly enhanced fescue growth, probably because of less competition from clover (2,17). Similar results occurred in a pot study with a mixture of annual ryegrass (*Lolium multiflorum* Lam.) and crimson clover (*Trifolium incarnatum* L.) (1). None of these studies included enough O_3 doses to adequately describe an O_3 dose-yield response relationship.

In most field studies of plant response to O_3, plants were provided enough soil moisture to minimize water stress (12). However, plant water stress caused by inadequate amounts of water (soil moisture deficit) probably decreases stomatal aperture and foliar gas exchange. If so, decreased effects from a given level of O_3 would be expected (11). Studies of water-stress effects on yield response to seasonal O_3 exposure have shown that water stress decreased plant response to O_3 in some situations but not in others (7,13,19).

Assessments of O_3 effects on crop production would be strengthened by defining dose-response relationships for important forage crops and by further investigating the effects of water stress on plant response to O_3. Our objectives were to determine the interrelationships between water stress and long-term exposure to O_3 on yield of ladino clover and tall fescue grown together in the field.

MATERIALS AND METHODS

A mixture of ladino clover, cv. Regal, and tall fescue, cv. Kentucky 31, seeds was sown in a 0.5-ha field of Appling sandy loam soil (clayey, kaolinitic, thermic, Typic Hapludults) on 15 September 1983. The field had been fertilized previously according to soil-test recommendations. On 2 April 1984, 28 3-m-diameter plots were chosen based on the depth of the soil A horizon and on stand of both species. The population density was approximately 30–35 plants of each species per square meter.

A factorial design of seven O_3 treatments and two soil-moisture levels in each of two randomized complete blocks was used. Six of the O_3 treatments were in 3-m-diameter open-top field chambers (6). The other treatment was exposure to ambient air (AA) with no chamber, which was included to identify chamber effects on plant growth. The chamber O_3 treatments were charcoal-filtered air (CF), nonfiltered air (NF), and four NF treatments to which O_3 was added to obtain different proportions of the ambient O_3 concentration. The target O_3 proportions were 1.25 (NF × 1.25), 1.50 (NF × 1.50), 1.75 (NF × 1.75), and 2.00 (NF × 2.00) times the ambient O_3 concentration. Methods for controlling chamber O_3 concentrations at set proportions of the continuously changing ambient O_3 concentration have been published (8,9). Chamber frames were installed on 3 April 1984 and clear polyvinyl-chloride-film chamber panels were installed on 25 April 1984. Chamber fans were run from 0500 to 2200 hr eastern standard time (EST) each day. Ozone was added from 26 April to 18 October 1984 and from 13 April to 22 October 1985 for 12-hr day^{-1} (0800 to 2000 hr EST) whenever ambient O_3 concentrations exceeded 0.03 $\mu L\ L^{-1}$ except during day-long harvests, irrigation, or system malfunction. Harvests, irrigation, and system malfunction prevented O_3 addition for 9.7 and 5.4% of the possible dispensing time in 1984 and 1985, respectively.

Two soil-moisture levels were obtained by differential irrigation during periods of low rainfall. No attempt was made to exclude rain from the plots. The well-watered treatment was set to minimize plant water stress. All well-watered plots were irrigated when soil matric potential in one or more plots reached −0.4 MPa. All plots assigned to the water-stressed treatment were irrigated when soil matric potential in one or more plots reached −1.2 MPa. Soil moisture in the top 30 cm of soil (adjacent to growth-measure quadrats) was measured gravimetrically in each of two cores (2.5-cm-diameter × 30 cm) collected from each of the plots in the NF × 1.25 and NF × 1.75 treatment plots. These plots were selected for soil-moisture monitoring because they represented intermediate O_3 treatments and because such disturbance of other plots would have prevented measurement of O_3 effects on shoot and root carbohydrate levels (18). Gravimetric analyses made from the CF, NF × 1.50, NF × 1.75, and NF × 2.00 plots on one date in 1984 and from all O_3 treatment plots on three dates in 1985 indicated that soil moisture was not affected by the O_3 treatment. Soil matric potential was estimated using the gravimetric values and soil-moisture release curves (determined with pressure-plate). Gravimetric measures were made 1 day after rainfall or irrigation or at least two times each week. Soil removed for gravimetric analysis was replaced from outside the plots to decrease the effect of soil sampling on soil drying. For each irrigation, 2.5 cm of water was applied over the entire plot area at a rate of approximately 1.2 cm hr^{-1} with a wide-angle, solid-cone spray nozzle (Spraying Systems Co., Wheaton, IL) suspended 2.4 m above the center of each plot.

Plant measurements. On 18 and 19 April 1984 (before initiation of exposures), plants were cut to a height of 7 cm in each of 10 36- × 51-cm quadrats per plot. Clover, fescue, and weeds were separated, dried (70 C for 2 days), and weighed. These data were used in assessing the value of pretreatment weights as covariates in subsequent analyses and to determine the optimum number of quadrats to harvest after exposures began, considering statistical advantage versus personnel limitations. Negligible improvement in standard errors was obtained when more than six quadrats were used. Therefore, six contiguous quadrats in the center of each plot (minimum distance of 0.8 m from the edge of any quadrat to the chamber wall) were marked for all subsequent harvests. Whenever mean canopy height reached an average of 20–25 cm, plants from each quadrat were harvested by cutting to a height of 7–8 cm. At each harvest, clover, fescue, and weeds were separated, dried at 70 C for 2 days, and weighed. Plants were sprayed with dimethoate (2E, 6.3 ml L^{-1}) within 2 days after each harvest to minimize insect infestations. The plastic chamber panels were removed for the winter on 22 October 1984 and replaced with new ones on 12 April 1985. In 1985, plants in all plots were harvested on 9 and 10 April, before initiation of 1985 exposures.

Statistical analyses. Analyses of variance (AOV) were performed on the total clover, total fescue, and total clover-fescue dry weights. The initial AOV were performed by year and moisture level, separately, to determine if the quadrat position (within chambers) effect was significant, to test the value of the covariate data, and to test for homogeneity of error variances. Dose-response models were fit using regression analyses. The regression approach was used because O_3 concentrations were not evenly spaced across treatments and were slightly different (within ± 0.005 $\mu L\ L^{-1}$) for replicates of the same O_3 treatment. There are no data that identify a threshold O_3 concentration required to cause growth effects or that quantify the relative impact of high ambient O_3 levels interspersed with low ambient O_3 levels for plants exposed for a complete season. Rather than assigning an arbitrary differential weighting to different O_3 levels, we used the seasonal (period of O_3 addition) 12-hr day^{-1} concentration for each plot as the independent variable in regression analyses. One-year total shoot weights per plot was the dependent variable. For analyses with years combined, the 2-yr seasonal O_3 mean per plot was used as the independent variable and the 2-yr total shoot-weight values were the dependent variables.

RESULTS

Ozone treatments. The ambient O_3 concentration was the baseline for all O_3 treatments. Thus, at all times, concentrations in all treatments were directly related to O_3 concentrations in ambient air as illustrated by seasonal mean diurnal curves (Fig. 1A and B). Daily curves were similar to the seasonal mean curves but their amplitude changed in response to the daily ambient levels of O_3 (Fig. 2A and B). In 1984 and 1985, there were 49 and 87 days, respectively, with daily 12-hr mean ambient O_3 concentrations greater than 0.06 $\mu L\ L^{-1}$. The distribution of these days was fairly uniform in 1984 (Fig. 2A) but in 1985, most high-O_3 days occurred from 15 April to 22 July (Fig. 2B). The 1984 and 1985 seasonal 12-hr day^{-1} mean O_3 concentrations for each treatment (Table 1) show that, for the target proportional additions of 1.25, 1.50, 1.75, and 2.00 times the concentrations in NF chambers, we achieved proportions of 1.41, 1.61, 1.80, and 2.00, respectively, in 1984, and 1.27, 1.53, 1.69, and 1.88, respectively, in 1985.

Soil-moisture levels. Seasonal temperature, photosynthetically active radiation, rainfall, and irrigation are shown in Table 2. In 1984, differences in soil matric potential between the well-watered and water-stressed plots occurred mainly near the end of the season (Fig. 3A). The 1985 season was drier than in 1984 and differences in soil matric potential between moisture treatments occurred at fairly regular intervals throughout the season (Fig. 3B).

Foliar injury. Injury caused by O_3 was first observed on clover in the two highest O_3 treatments on 30 April 1984 (4 days after exposures began). Symptoms included small (0.1–1.0 mm), light tan, irregular necrotic areas on the upper leaf surface and chlorosis. With increased concentration and duration of exposure, foliar injury increased, which was followed gradually by general foliar senescense and abscission. For a given O_3 treatment, symptoms were more severe on old than on younger leaves, probably because at any given time old leaves had been exposed longer than young ones. Symptoms on clover leaves in the NF and AA treatments were visible after several ambient O_3 episodes with 12-hr means greater than 0.07 ppm.

Ozone caused much less foliar injury on fescue than clover. The most noticeable symptom on fescue was chlorosis on leaf margins or general chlorosis. The first obvious symptoms on fescue occurred near the end of the 1984 season at the two highest O_3 levels.

Shoot dry weight. The pretreatment shoot weights used as covariate data did not account for a significant part of the field variation and were not used in subsequent analyses. The quadrat effect was not significant for either species in 1984. In 1985, the quadrat effect was significant except for the clover in the water-stressed plots. It was caused by more (approximately 10%) growth of fescue in the north than in the south quadrats for both moisture levels and by more growth of clover (approximately 8%) in the south than north quadrats of the well-watered plots. The O_3 by quadrat interaction was significant only for fescue in the 1985 water-stressed plots. However, there was no systematic pattern to account for this interaction. Therefore, plot means were used in all subsequent analyses. Error variances were homogeneous across moisture treatments. Thus, subsequent analyses were performed for moisture levels combined for each year separately and for the total 2-yr yield data. Chamber effects on plant growth can be seen by comparing the NF and AA data in Table 3. The comparative growth of each species in NF or AA varied with the year and moisture treatment. Because our main objective was to estimate effects of O_3, rather than effects of chambers per se on yield, AA data were excluded from further analyses. The results of the AOV with moisture treatments combined are shown in Table 4. The O_3 effect was significant in all analyses except for fescue in 1985. Moisture stress decreased clover shoot weight by 10% in 1984 and by 33% in 1985 (Table 3) and the effect was significant (Table 4). However, there was no significant moisture effect on fescue growth in either year (Table 4).

Ozone suppressed clover growth over the two seasons in both moisture treatments (Figs. 4 and 5). The suppression of clover growth was accompanied by a stimulation of fescue growth, especially during 1984. For example, in the high O_3 treatments, clover growth was the least while fescue growth was the greatest.

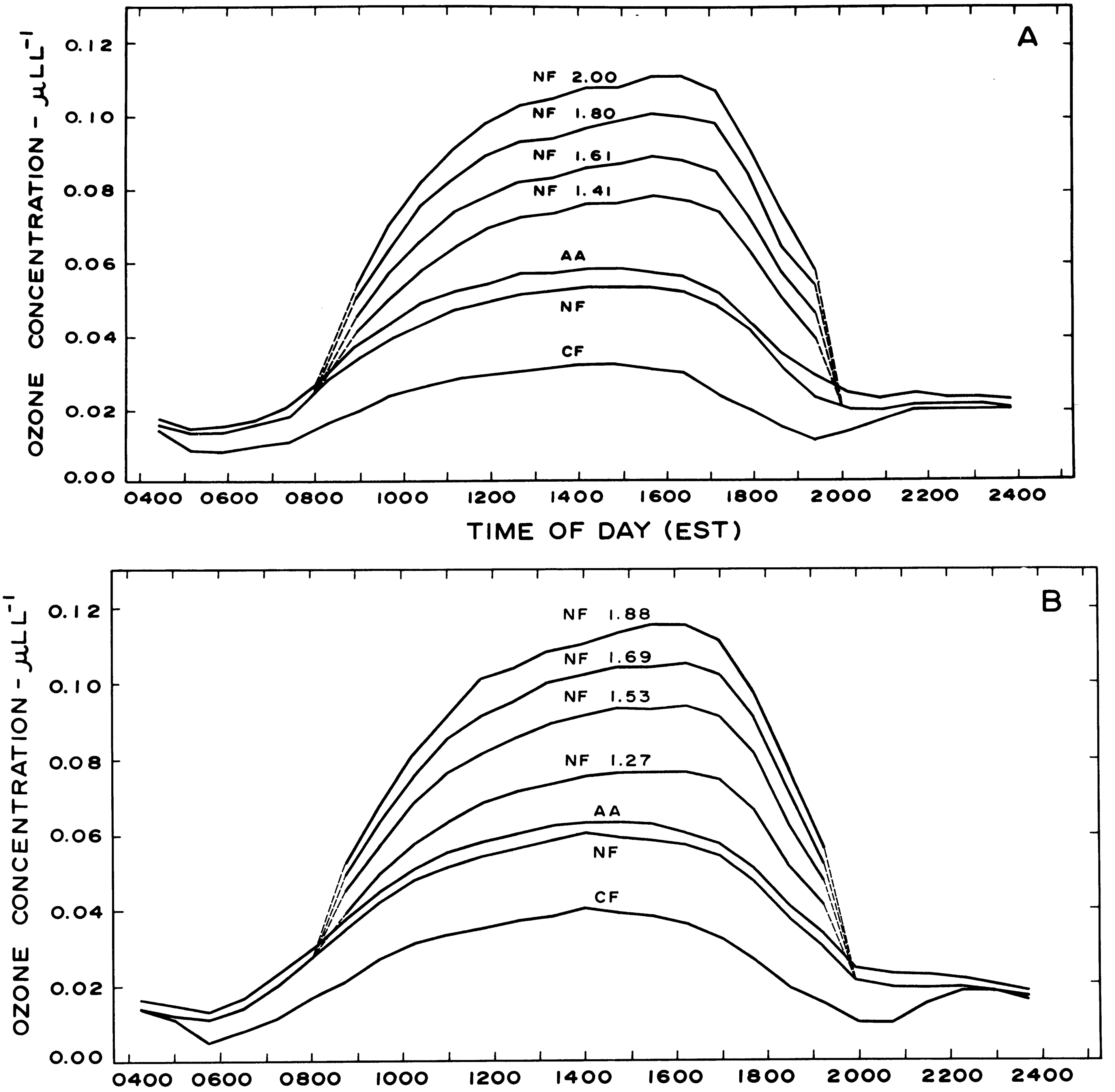

Fig. 1. Seasonal mean diurnal fluctuations in O_3 concentrations for the different O_3 treatments: **A,** from 21 April to 18 October 1984; **B,** from 13 April to 22 October 1985. CF = charcoal-filtered air; NF = nonfiltered air; NF numbered treatments = nonfiltered air plus the proportions of O_3 in NF air added. The ambient air (AA) curves resulted from measurements at a height of 5 m. The diurnal concentration curves for the AA plots were almost identical to those for NF plots. EST = eastern standard time.

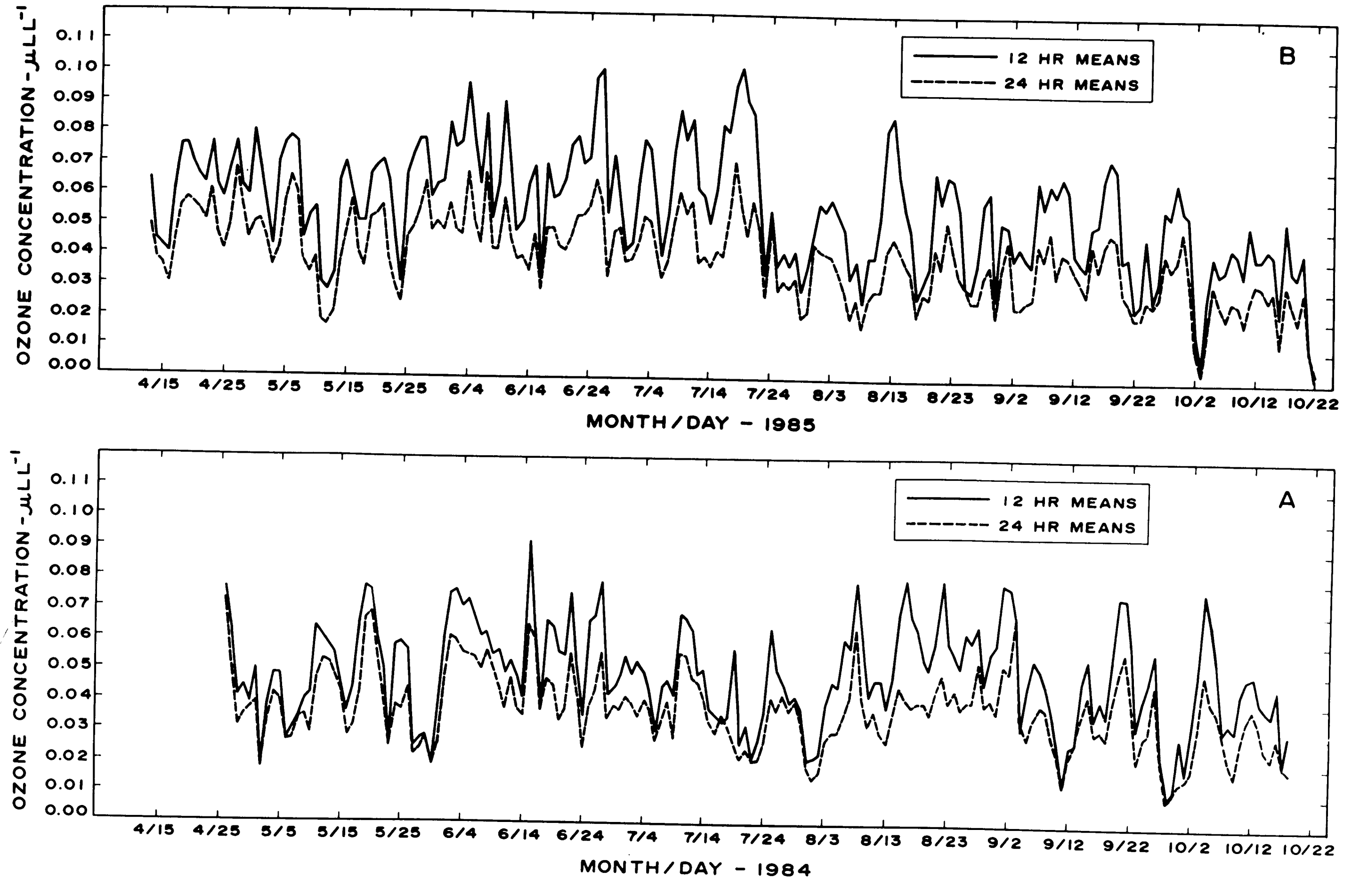

Fig. 2. Daily 12-(0800 to 2000 hr eastern standard time) and 24-hr mean O_3 concentrations in ambient air (5-m height) during the period of exposures: **A,** 1984; **B,** 1985.

TABLE 1. Mean ozone concentrations measured in studies to determine response of a clover-fescue pasture to chronic doses of ozone at different soil-moisture levels

Ozone treatment[a]	12 hr/day O_3 values (μL L^{-1})[b]				1 hr/day O_3 values (μL L^{-1})[b]			
	Seasonal means		Highest daily means		Seasonal means		Highest daily means[c]	
	1984	1985	1984	1985	1984	1985	1984	1985
CF	0.025	0.030	0.049	0.057	0.038	0.045	0.072	0.081
NF	0.044	0.049	0.095	0.091	0.059	0.064	0.123	0.118
NF×1.25[d]	0.062	0.062	0.134	0.123	0.087	0.085	0.184	0.166
NF×1.50[d]	0.071	0.075	0.157	0.151	0.100	0.106	0.202	0.217
NF×1.75[d]	0.079	0.083	0.184	0.172	0.115	0.120	0.239	0.244
NF×2.00[d]	0.088	0.092	0.205	0.193	0.129	0.138	0.260	0.274
AA(plots)	0.045	0.048	0.087	0.085	0.062	0.064	0.122	0.118

[a] CF = charcoal-filtered air; NF = nonfiltered air; AA = ambient air. Each value is the mean from two plots (one well-watered and one water-stressed plot).

[b] Values are seasonal or highest daily 12-hr or 1-hr means for the daily period from 0800 to 2000 eastern standard time for the period of dispensing (from 21 April to 18 October 1984 and from 13 April to 22 October 1985).

[c] The highest daily mean 1 hr/day value is defined as the mean of two consecutively measured values that were recorded for 3-min samples taken at 45-min intervals using time-shared monitoring.

[d] Each value is the mean from four plots (two well-watered and two water-stressed plots). Proportions (multiples of NF) are target proportions.

Conversely, in the low O_3 plots, clover growth was the greatest while fescue growth was the least. During 1985, fescue growth was not significantly affected by the O_3 treatment. Hovever, fescue growth tended to be less in the high than in the low O_3 treatments (Fig. 5B). Clover growth nearly ceased by the end of the 1985 season in all but the CF well-watered treatment (Figs. 4 and 5).

The significant O_3 × moisture interactions shown for clover and fescue in Table 4 (1985 and years combined) were probably due to the 1985 results when clover growth was greater than fescue only in the CF well-watered plots; the reverse was true for all other combinations of O_3 and moisture treatment (Table 3, Fig. 6B). When AOV were performed with the CF treatment deleted (for 1985 separately and for the years combined), there was no interaction between O_3 and moisture for either species (AOV not shown).

Polynomial dose-response models for moisture levels combined are shown in Table 5. The moisture effect (M) was included in all models where $M = 1$ for water-stressed plots and $M = 0$ for well-watered plots. The dose-response curves clearly show no interaction between O_3 and soil moisture in 1984 (Fig. 6A) and the probable reason for the O_3 × moisture interaction in 1985 (Fig. 6B). The dose-response models with 1985 CF plots omitted were linear (Table 5). The dose-response curves for the years combined (Fig. 7) show the O_3 and moisture effect on total forage, the dominance of clover over fescue at low O_3 levels, and the dominance of fescue over clover at high O_3 levels.

An increase in weeds (mainly crabgrass [*Digitaria sanguinalis* L.]) appeared to be related to increased O_3 doses in 1984 (Table 3). However, this relationship was not as clear-cut in 1985 when crabgrass increased in most plots (except CF) near the end of the season (Table 3). Probably, crabgrass incidence was in response to, rather than a cause for, decreased growth of clover, mainly near the end of each season. Therefore, statistical analyses on the influence of O_3 or moisture on crabgrass were not performed.

DISCUSSION

In this study, we chose to follow normal agronomic practice by harvesting plants when they reached a height of 20–25 cm. Because plants grew slower in the high O_3 treatments than in the low O_3 treatments, plants in the CF, NF, NF× 1.25, and AA plots were harvested on different dates than plants in the NF × 1.50,

TABLE 2. Monthly temperatures, photosynthetically active radiation (PAR), rainfall, and irrigation during O_3 dose-yield response studies with a clover-fescue mixture in 1984 and 1985[a]

Variable	Moisture treatment	1984							
		April	May	June	July	Aug.	Sept.	Oct.[b]	Total
Mean max. temp., C	Both	19.9	25.7	31.6	29.3	31.7	25.5	24.5	
Mean min. temp., C	Both	7.5	12.9	18.8	19.7	20.1	14.4	12.1	
Mean dew-point temp., C	Both	6.2	11.1	17.1	22.3	18.7	13.0	11.0	
Mean daily (24-hr) PAR μmol/ m^2/ sec	Both	439	542	626	493	535	414	367	
Rain, cm	Both	9.1	20.8	11.8	15.4	2.1	6.2	3.9	69.3
Irrigation, cm	Well-watered	0.0	5.0	5.0	0.0	7.5	7.5	5.0	30.0
	Water-stressed	0.0	2.5	2.5	0.0	5.0	2.5	5.0	17.5
		1985							
Mean max. temp., C	Both	25.5	27.4	31.0	31.1	30.6	28.5	26.0	
Mean min. temp., C	Both	9.7	13.6	17.6	20.4	19.2	15.7	13.5	
Mean dew-point temp., C	Both	4.7	12.0	15.2	19.0	17.8	13.3	12.5	
Mean daily (24-hr) PAR μmol/ m^2/ sec	Both	638	535	592	479	449	487	346	
Rain, cm	Both	0.9	9.3	7.6	14.5	9.9	0.9	6.8	49.9
Irrigation, cm	Well-watered	5.0	10.0	10.0	5.0	2.5	7.5	2.5	42.5
	Water-stressed	5.0	5.0	7.5	2.5	2.5	5.0	0.0	27.5

[a] Temperature, light, and rainfall measures were made 120 m south of the experimental field.

[b] For the period from 1 to 18 October 1984 and from 1 to 22 October 1985.

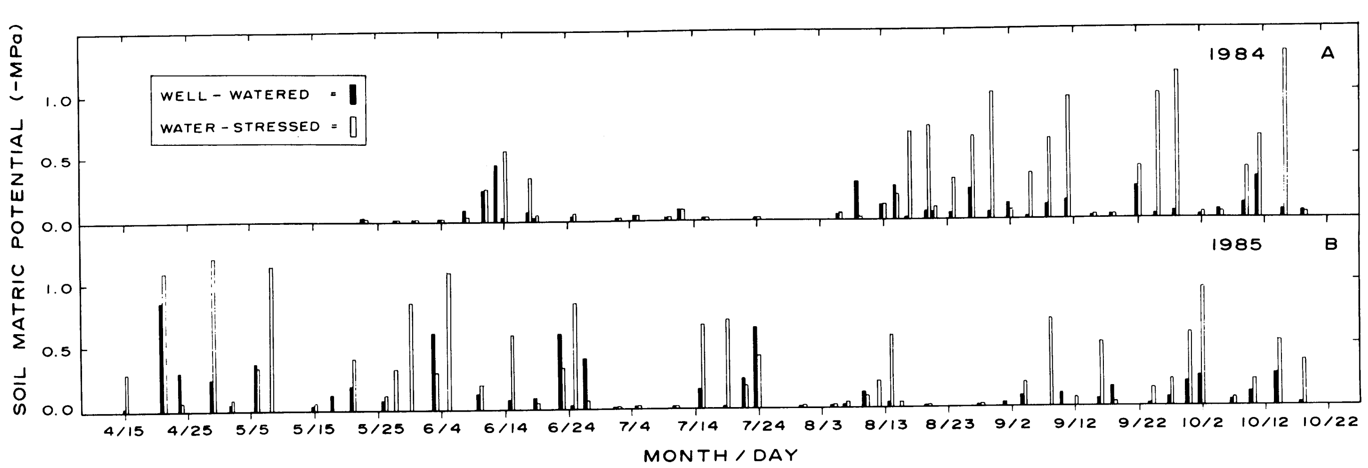

Fig. 3. Soil matric potential for two well-watered and water-stressed plots: **A,** during the 1984 season; **B,** during the 1985 season. Each bar represents the mean from eight samples (two cores, two O_3 treatments [NF × 1.25 and NF × 1.75], and two blocks).

TABLE 3. Effects of chronic doses of ozone on seasonal yield of a clover-fescue pasture grown under well-watered or water-stressed conditions

Moisture treatment	Ozone treatment	1984 conc. (μL L^{-1})[b]	Total biomass measured in 1984 (g m^{-2})[a]					1985 conc. (μL L^{-1})[b]	Total biomass measured in 1985 (g m^{-2})[a]					2-yr conc. (μL L^{-1})[c]	Total biomass measured in 1984 and 1985 (g m^{-2})[a]				
			Clov.	Fesc.	Total clover-fescue	Weeds	Ratio clover: fescue		Clov.	Fesc.	Total clover-fescue	Weeds	Ratio clover: fescue		Clov.	Fesc.	Total clover-fescue	Weeds	Ratio clover: fescue
Well-watered	CF	0.025	1,039	83	1,122	10	12.5	0.029	610	203	813	144	3.0	0.027	1,649	286	1,935	154	5.8
	NF	0.043	928	189	1,117	6	4.9	0.047	281	632	913	236	0.4	0.045	1,209	821	2,030	242	1.5
	NF×1.25	0.062	761	200	961	26	3.8	0.062	181	480	661	493	0.4	0.062	942	680	1,622	519	1.4
	NF×1.50	0.071	594	350	944	18	1.7	0.074	148	513	661	230	0.3	0.072	742	863	1,605	248	0.9
	NF×1.75	0.079	533	372	905	41	1.4	0.082	112	539	651	313	0.2	0.081	645	911	1,556	354	0.7
	NF×2.00	0.088	467	433	900	60	1.1	0.091	138	467	605	306	0.3	0.089	605	900	1,505	366	0.7
	AA	0.045	894	100	994	9	8.9	0.048	422	475	897	135	0.9	0.046	1,316	575	1,891	144	2.3
Water-stressed	CF	0.025	867	149	1,016	2	5.8	0.031	250	577	827	54	0.4	0.028	1,117	726	1,843	56	1.5
	NF	0.045	833	148	981	2	5.6	0.050	198	498	696	125	0.4	0.047	1,031	646	1,677	127	1.6
	NF×1.25	0.061	689	194	883	10	3.6	0.062	198	489	687	342	0.4	0.061	887	683	1,570	352	1.3
	NF×1.50	0.070	539	319	858	11	1.7	0.076	83	421	504	120	0.2	0.072	622	740	1,362	131	0.8
	NF×1.75	0.079	494	317	811	23	1.6	0.084	134	419	553	143	0.3	0.081	628	736	1,364	166	0.9
	NF×2.00	0.088	356	339	695	54	1.1	0.092	78	359	437	240	0.2	0.088	434	698	1,132	294	0.6
	AA	0.045	900	100	1,000	2	9.0	0.048	333	560	893	81	0.6	0.046	1,233	660	1,893	83	1.9

[a] Each value is the mean of 12 36- × 51-cm quadrats (six in each of two plots) from a total of six harvests in 1984 and seven harvests in 1985. Values shown in Figures 4 and 5 include data from the pre-exposure harvest in 1984.

[b] Seasonal mean 12-hr day^{-1} O_3 concentration (μL L^{-1}).

[c] Two-year seasonal mean 12-hr day^{-1} O_3 concentration (μL L^{-1}), obtained by summing the 1984 and 1985 values and dividing by 2.

TABLE 4. Analyses of variance mean squares and probability levels for shoot dry weight (g m^{-2}) of clover, fescue, and clover and fescue combined exposed to different levels of ozone when grown at two levels of soil moisture over two seasons[a]

Species	Source	df	1984		1985		Years combined	
			MSE	*P*>*F*	MSE	*P*>*F*	MSE	*P*> *F*
Clover	Block	1	27,222	0.0027	247	0.7808	22,366	0.1309
	Moisture (M)	1	48,858	0.0003	45,957	0.0022	189,597	0.0006
	O_3	5	183,889	0.0001	152,284	0.0001	432,741	0.0001
	$O_3 \times M$	5	5,339	0.3883	49,321	0.0042	3,387	0.0264
	Error	11	1,846		2,937		8,397	
Fescue	Block	1	957	0.5972	1,913	0.6017	171	0.9122
	Moisture	1	4,413	0.2653	802	0.7340	9,010	0.4304
	O_3	5	49,105	0.0001	15,216	0.1180	55,942	0.0228
	$O_3 \times M$	5	2,778	0.5332	38,333	0.0075	59,647	0.0185
	Error	11	3,205		6,670		13,443	
Combined (total forage)	Block	1	18,008	0.0268	809	0.7308	26,450	0.1576
	Moisture	1	82,636	0.0002	58,992	0.0118	281,269	0.0004
	O_3	5	45,989	0.0001	60,373	0.0011	209,641	0.0001
	$O_3 \times M$	5	2,055	0.6062	10,299	0.2431	16,795	0.2785
	Error	11	2,759		6,495		11,502	

[a] Ambient air-plot data not included.

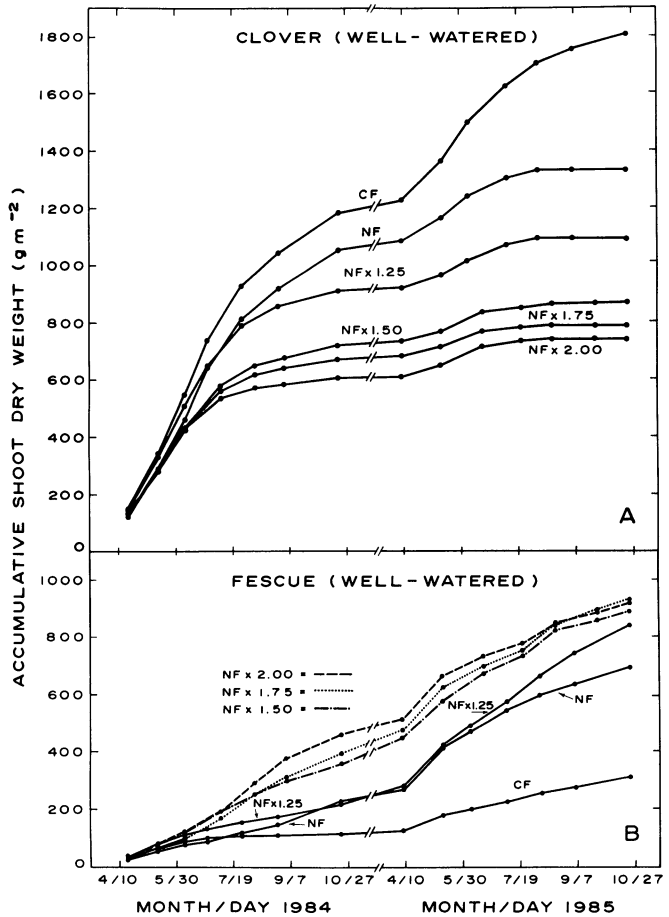

Fig. 4. Effect of O_3 on accumulative shoot-weight production during 1984 and 1985 of ladino clover (**A**) and tall fescue (**B**) in well-watered plots. CF= charcoal-filtered air; NF= nonfiltered air; NF× 1.25, NF× 1.50, NF× 1.75, and NF× 2.00= NF air with increasing amounts of O_3 added for 12-hr day^{-1} in proportion to ambient O_3 levels. Proportions shown are target proportions. Each measured value is the mean of 12 quadrats (six in each of two plots).

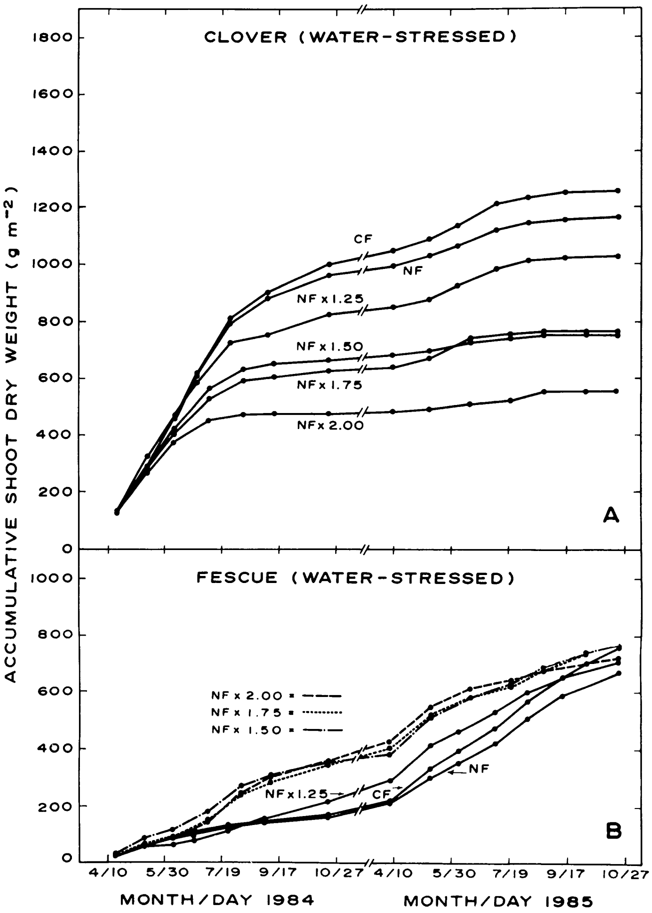

Fig. 5. Effect of O_3 on accumulative shoot-weight production during 1984 and 1985 of ladino clover (**A**) and tall fescue (**B**) in water-stressed plots. CF = charcoal-filtered air; NF= nonfiltered air; NF× 1.25, NF× 1.50, NF× 1.75, and NF× 2.00= NF air with increasing amounts of O_3 added for 12-hr day^{-1} in proportion to ambient O_3 levels. Each measured value is the mean of 12 quadrats (six in each of two plots).

1.75, and 2.00 plots, except for the first two harvests each year (Figs. 4 and 5). If all plants had been harvested on the same dates, the physiological effects of cutting on plants cut at different heights would have been different. Because the growth period, exposure duration, and O_3 dose differed for most harvests, we did not attempt to relate the O_3 dose to growth for individual harvests.

The interaction between O_3 and soil-moisture treatment for yield of clover and fescue in 1985 was probably because the CF well-watered treatment was the only treatment combination where clover grew better than fescue. This result may have been caused by the relative tolerance of clover and fescue to moisture and O_3 stress. Clover is known to be more sensitive than fescue to both stresses (2,5,14) and our results agree with these findings. Clover dominated fescue in the absence of either stress (CF well-watered plots), but fescue dominated clover when either stress was present (all other plots).

Measured loss of clover yield caused by ambient levels of O_3 (CF vs. NF) in 1984 and 1985 was 11 and 54%, respectively, in the well-watered plots and 4 and 21%, respectively, in the water-stressed plots (Table 3). We don't believe that the increased effects seen in 1985 were caused by increased sensitivity of clover to O_3 or by differences in day-to-day O_3 levels over the two seasons. It seems more likely that the differences measured in 1985 began in 1984 with gradual shifts in species dominance caused by differences in species tolerance to O_3 and moisture stress. Soil-moisture deficit probably was not much of a factor until late in 1984 and for all of 1985 (Fig. 3). Early indications that an O_3 × soil moisture interaction would occur are illustrated by the rates of shoot-weight accumulation near the end of 1984 and for all of 1985. For example, clover shoot weight continued to accumulate throughout 1985 in the CF well-watered plots but ended for the most part by

TABLE 5. Regression equations with standard errors (in parentheses) for seasonal yield (shoot dry weight) of clover, fescue, and clover plus fescue exposed to chronic doses of ozone at two soil-moisture levels

1984 (all chamber plots included)[a]							
Clover	$\hat{Y}$ =	1,059.7	− 90.1 M	− 22.8 X	− 79,992.3 X^2		
		(76)	(20)	(2,902)	(25,541)		
Fescue	$\hat{Y}$ =	−5.88	− 28.0 M	+ 4,513.3 X			
		(43)	(24)	(574)			
Clover+ fescue	$\hat{Y}$ =	1,265.98	− 116.5 M	− 4,480.07 X			
		(36)	(21)	(486)			
1985 (all chamber plots included)[a]							
Clover	$\hat{Y}$ =	1,356.1	− 1,027.5 M	− 31,740.8 X	− 203,214.1 X^2	+ 29,517.9 XM	− 208,875.8 X^2M
		(134)	(196)	(4,801)	(39,741)	(6,962)	(56,843)
Fescue	$\hat{Y}$ =	−494.1	+ 1,152.5 M	+ 32,548.0 X	− 246,881.4 X^2	− 35,088.3 XM	+240,069.3 X^2M
		(242)	(355)	(8,686)	(71,918)	(12,600)	(102,869)
Clover+ fescue	$\hat{Y}$ =	1,057.8	− 91.5 M	− 5,339 X			
		(63)	(34)	(827)			
1985 (CF plots excluded)[a]							
Clover	$\hat{Y}$ =	406.8	− 28.8 M	− 3,325.8 X			
		(59)	(23)	(765)			
Fescue	$\hat{Y}$ =	746.3	− 84.7 M	− 3,107.4 X			
		(68)	(27)	(901)			
Clover+ fescue	$\hat{Y}$ =	1,152.8	− 113.5 M	− 6,433.2 X			
		(88)	(34)	(1,144)			
1984, 1985 combined[b] (all chamber plots included)							
Clover	$\hat{Y}$ =	2,647	− 1,451.8 M	− 39,160 X	+ 181,658 X^2	+ 40,560.6 XM	− 288,511.4 X^2M
		(217)	(317)	(7,753)	(64,216)	(11,251)	(91,852)
Fescue	$\hat{Y}$ =	182.8	+ 494 M	+ 8,781 X	− 8,357.8 XM		
		(129)	(178)	(1,847)	(2,632)		
Clover+ fescue	$\hat{Y}$ =	2,345	− 202 M	− 9,976 X			
		(86)	(46)	(1,128)			

[a] X = seasonal 12-hr day^{-1} mean O_3 concentration ($\mu L\ L^{-1}$); Y = grams dry weight per square meter; M = 0 for well-watered plots and 1 for water-stressed plots.

[b] X = 2-yr seasonal 12-hr day^{-1} mean O_3 concentration; Y = grams dry weight per square meter; M = 0 for well-watered plots and 1 for water-stressed plots.

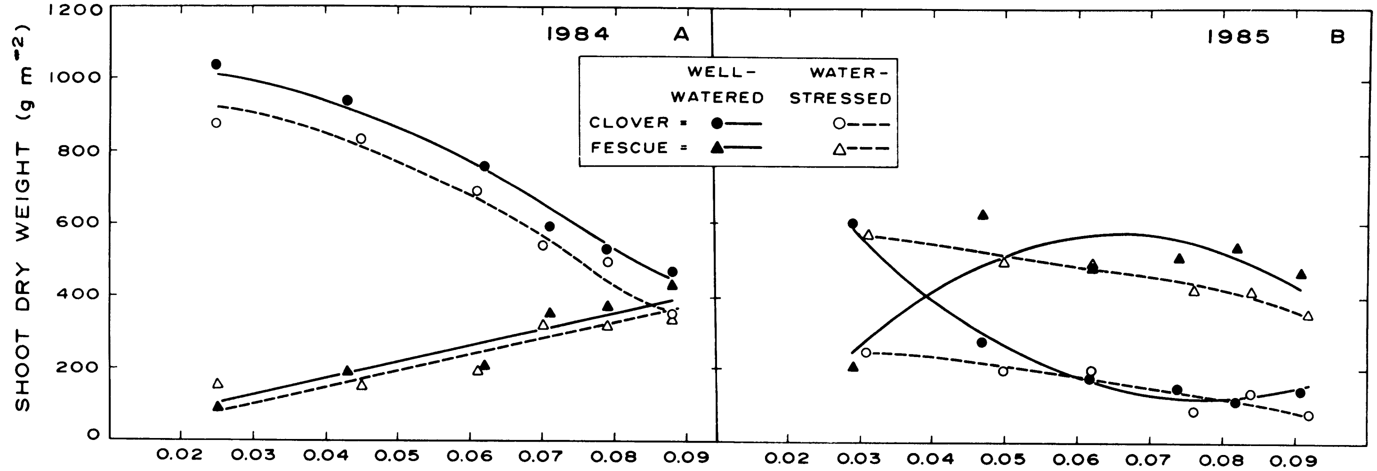

Fig. 6. Measured and estimated effects of chronic exposure to O_3 and soil-moisture deficit on yield of ladino clover and tall fescue grown in a mixture for 1984 (**A**) and 1985 (**B**). The response curves were estimated by the polynomial equations shown in Table 4.

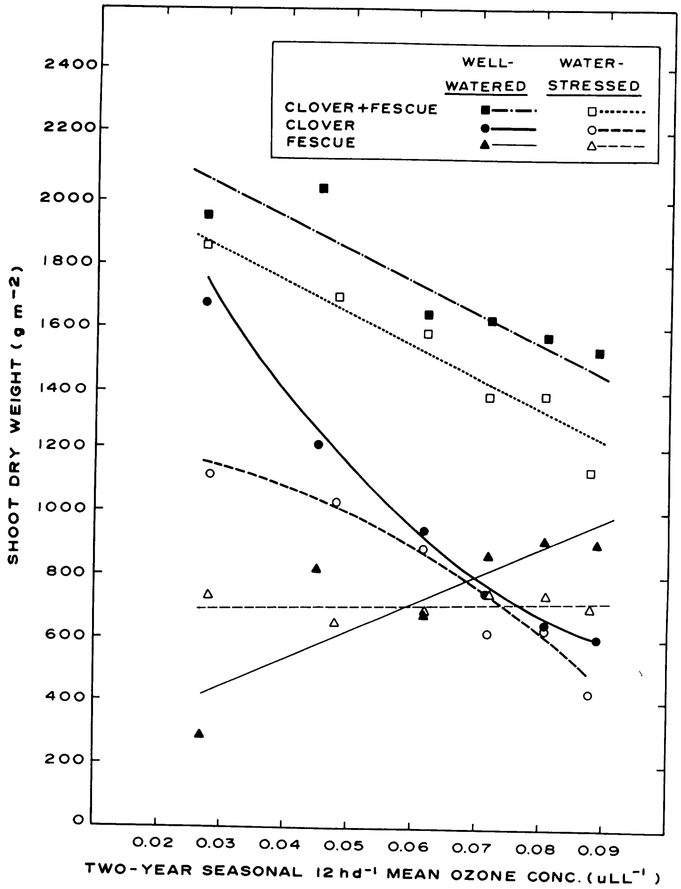

Fig. 7. Measured and estimated effects of chronic exposure to O_3 and soil-moisture deficit on total (2-yr) shoot weight of ladino clover, tall fescue, and total forage (clover and fescue). The response curves were estimated by the polynomial equations shown in Table 4. Each measured value is the mean of 13 harvests for two plots (six quadrats per plot for each of six harvests in 1984 and for each of seven harvests in 1985).

mid-July 1985 in the CF water-stressed plots (Figs. 4 and 5). Fescue biomass continued to accumulate throughout 1985 in the CF water-stressed plots, probably because fescue is more tolerant to moisture stress than clover.

Previous studies have shown that soil-moisture deficit can decrease response of cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L. Merr.) to chronic doses of O_3 under some conditions but not others (7,13,18). Apparently, a fairly high level of moisture stress is required to significantly decrease crop response to O_3, but data needed to provide an estimate of the intensity and duration of that level are extremely limited. Future studies of this type should focus on quantifying relationships between soil-moisture deficit and plant moisture status.

Our results clearly show that ambient levels of O_3 can significantly decrease yield of clover in a clover-fescue mixture under well-watered or water-stressed conditions. Although the decrease in clover yield was partly compensated by an increase in fescue yield, the overall forage quality (level of protein) decreases when the species composition shifts toward fescue (2). Our results also clearly show that clover can dominate fescue when moisture or O_3 stress is not a significant factor. Thus, increased levels of drought and O_3 tolerance in ladino clover could lead to clover-fescue mixtures that would persist for more than a few years.

Symptoms of virus infection were observed in a few plots in August 1984. A visual estimate of the percentage of clover leaves showing virus symptoms (percentage incidence) was made for the AA, CF, NF, and NF× 1.25 treatments (16 plots) on 26 July 1985. This estimate was not feasible for the higher O_3 treatments because O_3 symptoms masked virus symptoms. The four AA plots showed virus symptom incidence of 0, 1, 14, and 19%. Of the 12 chambered plots tested, three had an incidence of 0%, five had an incidence of 1–3%, three had an incidence of 8–9%, and one had an incidence of 15%. On 9 October 1985, clover samples were taken for virus assays from the 20 plots still containing an adequate amount of clover. A host assay with *Chenopodium quinoa* L. indicated that viruses were present in all but two plots. An enzyme-linked immunosorbent assay (ELISA) (16) performed on 28 November 1985 for alfalfa mosaic virus (AMV) and white clover mosaic virus (WCMV) showed that WCMV was present in all plots sampled and that AMV was present in half of these. A subsequent ELISA (16) on one selected clone from each of seven plots was performed on 7 April 1987 for eight viruses known to infect white clover. This test revealed WCMV in all plots, AMV in five plots, peanut stunt virus in six plots, red clover vein mosaic virus in two plots, and clover yellow vein virus in one plot (Mike McLaughlin, *personal communication*). We did not attempt to directly measure the influence of virus infection on clover yield or on clover response to O_3 or moisture stress. Although some virus-induced growth suppression probably occurred, there was no apparent relationship between total clover yield and the level of virus incidence. For example, clover yield was almost identical in both NF well-watered plots although estimated virus incidence was 3% in one plot and 15% in the other. Nevertheless, further work is needed to determine the relative importance of viruses and O_3 in causing clover decline and whether either of these two factors affect the impact of the other.

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