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Response of Two Wheat Cultivars to CO_2 Enrichment under Subambient Oxygen Conditions[1]

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ABSTRACT

Two cultivars of wheat (*Triticum aestivum* L. cvs Sonoita and Yecora Rojo) were grown to maturity in a growth chamber within four subchambers under two CO_2 levels (350 or 1000 microliters per liter) at either ambient (21%) or low O_2 (5%). Growth analysis was used to characterize changes in plant carbon budgets imposed by the gas regimes. Large increases in leaf areas were seen in the low O_2 treatments, due primarily to a stimulation of tillering. Roots developed normally at 5% O_2. Seed development was inhibited by the subambient O_2 treatment, but this effect was overcome by CO_2 enrichment at 1000 microliters per liter. Dry matter accumulation and seed number responded differently to the gas treatments. The greatest dry matter production occurred in the low O_2, high CO_2 treatment, while the greatest seed production occurred in the ambient O_2, high CO_2 treatment. Growth and assimilation were stimulated more by either CO_2 enrichment or low O_2 in cv Yecora Rojo than in Sonoita. These experiments are the first to explore the effect of whole plant low O_2 treatments on growth and reproduction. The finding that CO_2 enrichment overcomes low O_2-induced sterility may help elucidate the nature of this effect.

The great stimulation of vegetative growth of C_3 plants under low O_2 conditions due to the inhibition of the oxygenase activity of Rubisco[3] was first observed by Björkman *et al.* (2, 3). *Mimulus cardinalis* and *Marchantia polymorpha* responded with a 130- to 1000-fold increase in dry matter accumulation over a 10 day period in 4% O_2 compared with 21%, while *Zea mays*, a C_4 plant, showed no significant response. Quebedeaux and Hardy (12, 13) performed numerous experiments at 5% O_2 and confirmed the findings of Björkman *et al.* (2, 3) regarding the stimulation of vegetative growth in C_3 (but not C_4) plants by low O_2 concentrations. They further noted that growth at 5% O_2 completely prevented seed development in soybean, and they concluded that an O_2-mediated process controls the balance between reproductive and vegetative growth by its effect on the transfer of photosynthate from leaves to developing seeds.

Both Björkman *et al.* (2, 3) and Quebedeaux and Hardy (12, 13) exposed only the aerial portions of the plants to subambient O_2 while root zones experienced air levels of O_2. In view of reports of gas transport between root and shoot (6), it was of interest to conduct similar experiments in which the whole plant would be exposed to the treatment gas regime. The present experiments were designed to test further the interaction of O_2 and CO_2 levels on growth and reproduction in two cultivars of wheat. An underlying goal was to evaluate whether plants can grow and produce seed in a low O_2 growth environment as might be used in space settings.

MATERIALS AND METHODS

All experiments were conducted in a controlled environment chamber in the Duke University Phytotron. The chamber operated on a 14-h photoperiod and maintained the interior of four Plexiglas subchambers at 26/20°C day/night 14-h thermoperiod and light levels at 400 μmol m^{-2} sec^{-1} PAR. The subchambers (30 cm wide × 80 cm long × 90 cm high), equipped with fans and watering ports as described by White (17), were used to maintain separate gas treatments with flowthrough rates of 1 L min^{-1} (Fig. 1).

Low O_2 (5%) treatments were achieved by diluting air with bottled N_2. CO_2 levels (350 and 1000 μl L^{-1}) were maintained by the Phytotron's computer-controlled CO_2 injection system. Sampling ports on the sides of the chambers permitted sequential harvesting without substantial loss of control over gas composition (low O_2 treatments did not exceed 8% while the ports were open). Watering (one-half strength modified Hoagland solution [4] AM, deionized water PM) was accomplished through a remote system.

Wheat seeds (*Triticum aestivum* L., cvs Yecora Rojo and Sonoita), obtained from Dr. Bruce Bugbee, Utah State University, Logan, UT, were planted in Phytotron mix (gravel: vermiculite:turface, 1:1:1) in plastic 'rocket' pots (0.6 L volume) which were held in racks inside the four subchambers. For growth analysis experiments, five plants of each cultivar were harvested from each subchamber at weekly intervals (on d 14, 21, and 28 after planting). Final harvests were made 63 and 79 d after planting (for early maturing Yecora Rojo and later maturing Sonoita, respectively). Each experiment was repeated at least twice with similar results. The data in Tables I to IV are from one representative experiment; those in Table V are the average of two experiments.

Harvest data were analyzed using one-way ANOVA and the significance values were determined using Fisher's LSD multiple comparison test at the 0.05 level. O_2 and CO_2 effects on harvest data were compared at the 0.05 significance level using two-way ANOVA. All analyses were performed using Number Cruncher Statistical System (7) statistical software.

RESULTS

Table I shows that at 350 μl L^{-1} CO_2, no seeds were formed by any of the plants in the 5% O_2 treatment. CO_2 enrichment

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[3] Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase-oxygenase; NAR, net assimilation rate; RGR, relative growth rate; ΔW, dry matter gain.

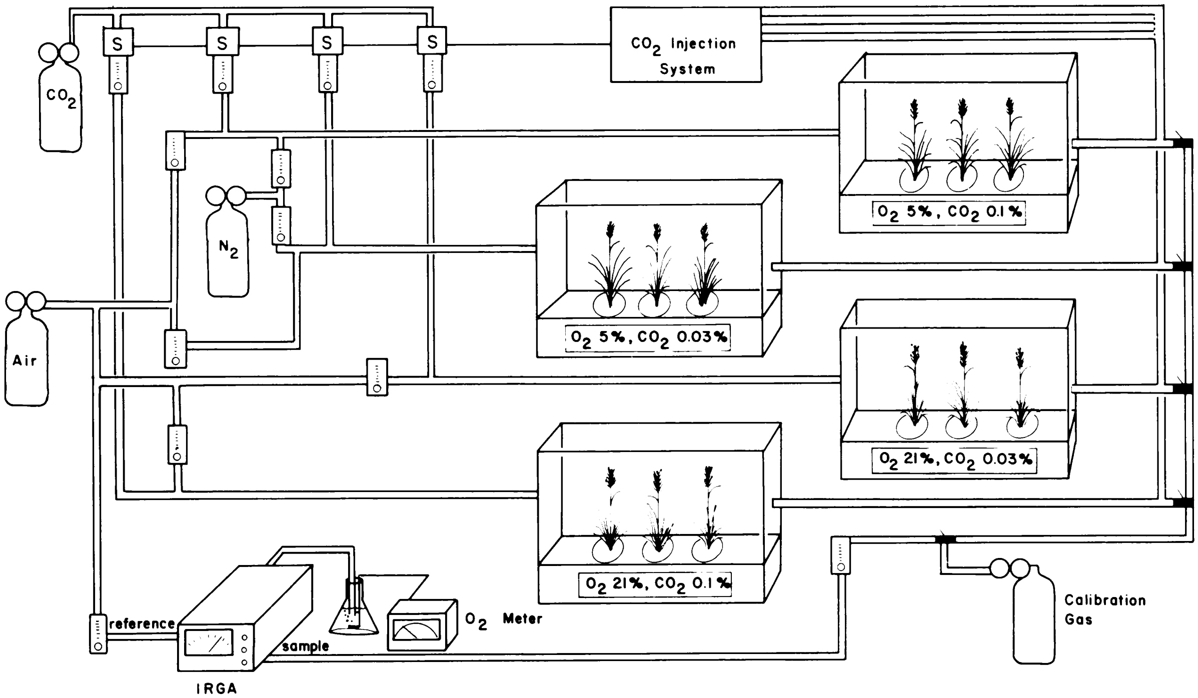

FIG. 1. A four subchamber system for imposing two levels of O_2 and two levels of CO_2 on dwarf wheat plants as they grow from seed to maturity. Low O_2 levels were maintained by diluting air with bottled N_2. CO_2 levels were maintained by the computer-controlled CO_2 injection system in the Phytotron.

Table I. *Number of Seeds Produced per Plant by Each Wheat Cultivar in One Representative Experiment*

Letters denote statistically different values within the four gas treatments at the 0.05 level.

Cultivar and O_2 Treatment	CO_2 Level (μl L^{-1}) 350	1000
	no. of seeds per plant	
Yecora Rojo		
5%	0 a	120 b
21%	116 b	165 b
Sonoita		
5%	0 a	158 b
21%	78 ab	160 b

Table II. *Number of Tillers Produced per Plant by Each Wheat Cultivar at Final Harvest in One Representative Experiment*

Letters denote statistically different values within the four gas treatments at the 0.05 level.

Cultivar and O_2 Treatment	CO_2 Level (μl L^{-1}) 350	1000
	no. of tillers per plant	
Yecora Rojo		
5%	24 c	23 c
21%	7 a	15 b
Sonoita		
5%	28 c	26 c
21%	6 a	19 b

to 1000 μl L^{-1} restored fertility in the low O_2 treatment in both cultivars.

The low O_2 treatments also had a strong effect on tillering. In both cultivars, 5% O_2 stimulated tillering three- or even fourfold over ambient values (Table II). While CO_2 enrichment to 1000 μl L^{-1} stimulated tillering at 21% O_2, it had no effect at 5% O_2. Leaf area (Table III) generally was related to tiller number and showed similar responses to the gas treatments.

These differential responses by various parts of the plant to O_2 and CO_2 resulted in a pattern of dry matter distribution near maturity as shown in Figure 2. Low O_2 treatments greatly stimulated dry matter accumulation under ambient CO_2 levels. In Yecora Rojo, an additional increase in dry matter production by CO_2 enrichment in the low O_2 treatment was largely due to the restoration of seed development under these conditions as compared with the 350 μl L^{-1} CO_2 low O_2 treatment. Root development was stimulated by the low O_2 treatments, especially in cv Sonoita.

In some ways, low O_2 and high CO_2 exerted similar effects on plant growth and development. When data from the final harvest from all four treatments were analyzed using two-way ANOVA

Table III. *Leaf Area per Plant at Final Harvest in One Representative Experiment*

Letters denote statistically different values within the four gas treatments at the 0.05 level.

Cultivar and O_2 Treatment	CO_2 Level (μl L^{-1}) 350	1000
	leaf area per plant, dm^2	
Yecora Rojo		
5%	8.4 c	6.3 b
21%	3.1 a	6.5 b
Sonoita		
5%	16.7 c	20.8 c
21%	4.3 a	12.2 b

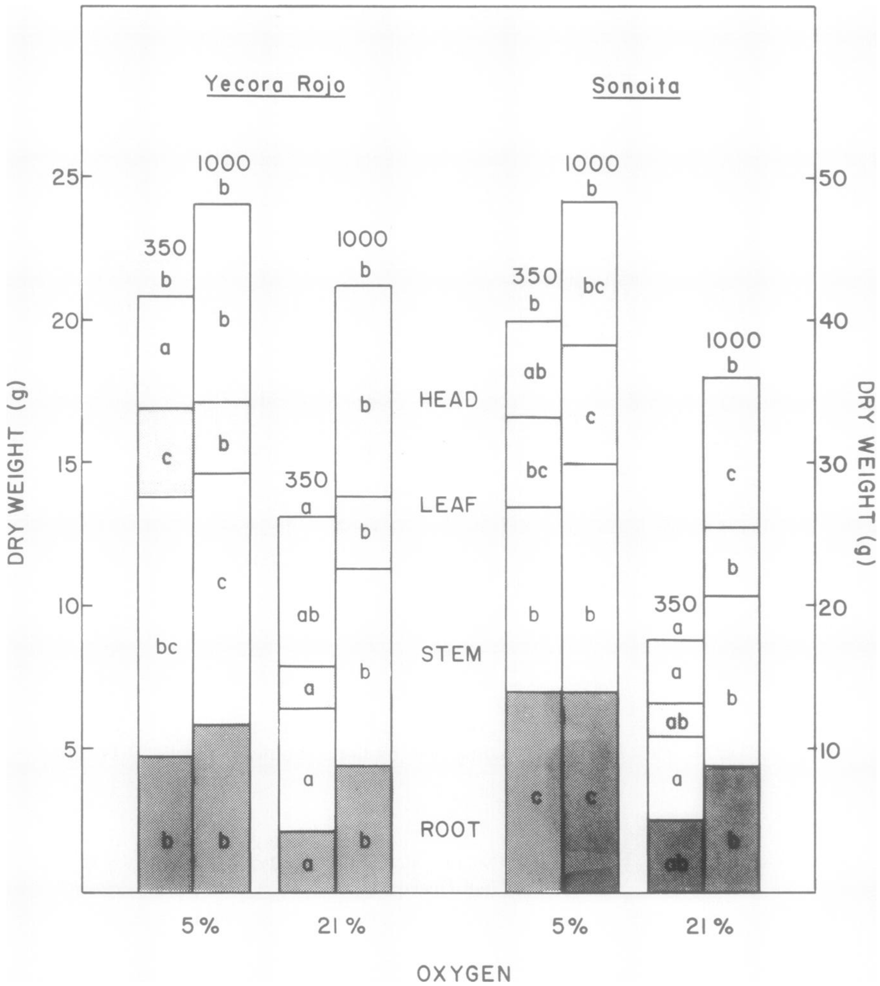

FIG. 2. Final harvest partitioning of dry matter from one representative experiment. Mean values for each tissue type are represented by the height of bars on the chart; significantly different values ($P \leq 0.05$) within tissue type and wheat cultivar are indicated by lower case letters. Bars labeled 350 are CO_2 levels of 350 $\mu l\ L^{-1}$; those labeled 1000 are CO_2 levels of 1000 $\mu l\ L^{-1}$.

to separate the O_2 and CO_2 effects, low O_2 and high CO_2 were found to have quantitatively similar effects on dry weight and number of heads. Effects on seed number were very different (Table IV), since ambient O_2 had a similar effect to high CO_2. Tillering was doubled by low O_2 treatments while it was increased by only 20% by CO_2 enrichment, indicating a stronger response of tillering to O_2 concentration than to CO_2 concentration.

To further understand the effects of the different gases on the wheat cultivars, growth analyses consisting of three weekly harvests were conducted in the prereproductive stages of growth. As shown in Figure 3, ΔW values mirrored the final harvest dry matter values (Fig. 2) for the four treatments. For cultivar Yecora Rojo, these differences also were evident in NAR and to a lesser extent in RGR. While RGR did not exhibit any differential responses to CO_2 and O_2, for ΔW and NAR, CO_2 enrichment had less of an effect at low O_2 than it did at 21% O_2; similarly O_2 depletion had more of an effect at ambient CO_2 than at high CO_2.

This saturation of response is summarized in the average of two sets of experiments in Table V where stimulation by CO_2 enrichment is expressed as a ratio of the value obtained at 1000 $\mu l\ L^{-1}$ CO_2 to the value at 350 $\mu l\ L^{-1}$ CO_2 and stimulation by O_2 depletion is expressed as the value at 5% O_2 divided by the value at 21% O_2. For cultivar Yecora Rojo, the CO_2 growth ratio was substantially larger at 21% O_2 than at 5% O_2, while cultivar Sonoita showed little difference in response. In terms of O_2 growth ratio, cultivar Yecora Rojo showed more of a response at 350 $\mu l\ L^{-1}$ CO_2 than at 1000 $\mu l\ L^{-1}$, and again cv Sonoita showed little difference in response. Also, the magnitude of stimulation of growth and assimilation by either CO_2 or O_2 was greater in cultivar Yecora Rojo than in cultivar Sonoita.

DISCUSSION

The bulk of the literature on effects of extended growth in subambient O_2 has utilized soybean as the experimental material. Quebedeaux and Hardy (12) described complete inhibition of seed set during growth at 5% O_2. Subsequent experiments were conducted to explain these observations by measuring inhibition of respiration by developing seeds (5) or of phloem unloading from the seed coat (16) in short term experiments under very low O_2 tensions. These explanations imply that it is the local O_2 environment around the developing pod which influences seed set; however, when Sinclair *et al.* (15) exposed developing soybean pods to 10% O_2, they found only a 20% decrease in seed dry weight, compared to 81% observed by Quebedeaux and Hardy (13) when the whole aerial portion of the plant was exposed to the same regimen. These results argue that seed set is indirectly influenced by subambient O_2 effects on the vegetative portion of the plant in addition to any direct inhibition of respiration and phloem unloading which may occur in the pod. (Inhibition of respiration by 4.2% O_2 observed by Gale [5] in detached soybean tissues may be an artifact due to flooding of intercellular gas spaces [1] and may not be significant in intact tissue [13]).

The idea that a vegetative effect is a causative factor in low O_2-induced sterility is supported by the finding reported here

Table IV. *Comparison of the Main Effects of O_2 and CO_2 on Growth and Reproduction in Two Wheat Cultivars*

Underlined mean values are significantly different at the 0.05 level as assessed by two-way ANOVA. For example, in determining O_2 effects, data from the one O_2 level at both CO_2 levels are compared with data from the second O_2 level at both CO_2 levels.

	O_2	O_2 Effects		CO_2	CO_2 Effects	
		Yecora Rojo	Sonoita		Yecora Rojo	Sonoita
	%			*ppm*		
Total weight (g)	5	22.4	44.02	350	17.0	30.0
	21	17.2	27.9	1000	22.6	42.1
Root weight (g)	5	5.27	13.83	350	3.39	9.89
	21	3.23	7.08	1000	5.10	11.30
Stem weight (g)	5	8.93	14.64	350	6.69	9.90
	21	5.64	9.19	1000	7.87	14.00
Leaf weight (g)	5	2.82	7.32	350	2.30	4.70
	21	1.97	3.93	1000	2.49	6.63
Leaf area (dm^2)	5	7.36	18.71	350	5.77	11.17
	21	4.81	8.66	1000	6.40	16.45
Tillers (n)	5	23.4	27.4	350	15.6	18.7
	21	11.1	13.2	1000	18.9	22.5
Heads (n)	5	14.0	19.6	350	8.8	12.7
	21	8.4	10.9	1000	13.6	18.0
Seeds (n)	5	59.9	79.2	350	58.2	34.7
	21	140.9	123.7	1000	142.6	159.3

that seed development is restored in low O_2 under CO_2 enrichment. Quebedeaux and Hardy (13) reported no effect of CO_2 concentration on reproductive development in soybean, and it will be interesting to determine whether this is a species-dependent effect or whether soybeans would show the same effect if both roots and shoots were exposed to the experimental gas regimes. The consequence of small-scale O_2 transport within the plant (6) in these split systems remains to be determined.

Growth analysis results (Tables IV and V; Fig. 3) demonstrate that low O_2 effects on assimilation diminish as the CO_2 levels increase. Björkman *et al.* (3) proposed two causes for the lower enhancement of dry matter production by low O_2 at high as compared with ambient CO_2. As a first possibility, the effects of O_2 concentration on the rate of CO_2 fixation may decrease as the CO_2 level is increased. Sharkey (14) has postulated that under conditions of low O_2 or high CO_2, photosynthesis is insensitive to further increases in CO_2 due to limitations imposed by the rate of phosphate regeneration. As a second explanation, Björkman *et al.* suggested that under conditions when photosynthesis is already very high, the capacity of other growth processes may partially limit the rate of growth, in which case an additional increase in the rate of photosynthesis would only be partially expressed in an increased dry matter production. Either of these may explain the diminished enhancement of dry matter accumulation and net assimilation rate by CO_2 enrichment at low O_2 as compared with ambient O_2 observed in the present experiments (Table V).

The growth analysis results identified cultivar Sonoita as less responsive to increases in CO_2 and decreases in O_2 than cultivar Yecora Rojo. While other differences may account for this, it is worth noting that tissues of cv Sonoita exhibited 20 to 30% more cyanide-resistant respiration than cv Yecora Rojo (8). Musgrave *et al.* (9) demonstrated that this nonphosphorylating respiratory pathway can act as an energy overflow in metabolizing luxury carbohydrates. Pea hybrids having the pathway responded less to CO_2 enrichment in terms of increases in ΔW, NAR, and RGR than did reciprocal crosses which lack the pathway.

These experiments were undertaken to assess the feasibility of growing plants seed to seed in a low O_2 chamber for plant cultivation in space settings (10). The results presented here suggest that from the standpoint of effects on plant growth and reproduction, low O_2 would not limit seed production providing

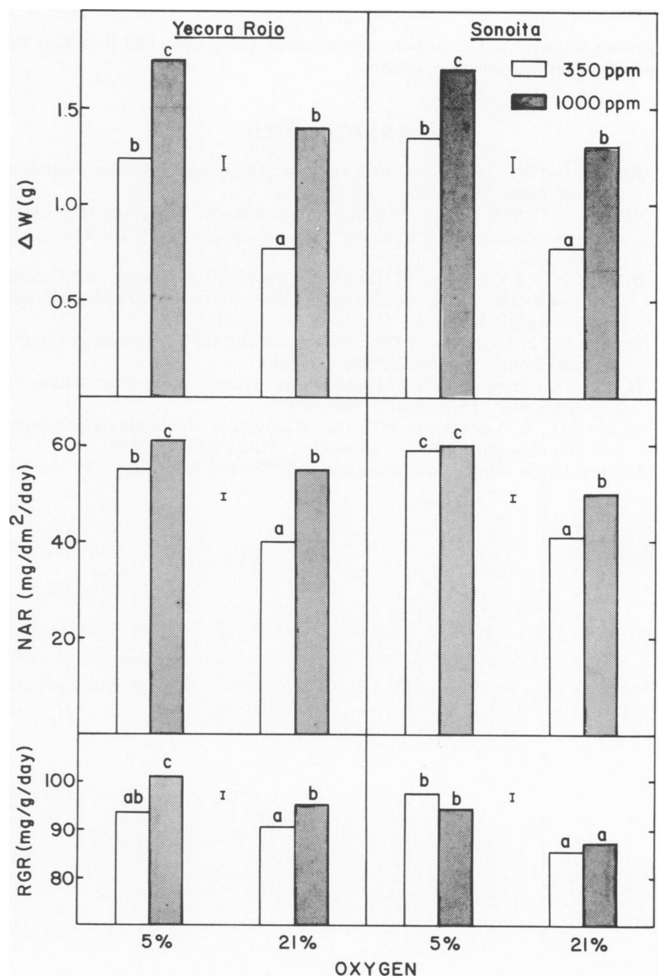

FIG. 3. Growth analysis results from one representative experiment showing ΔW, NAR, and RGR for the two wheat cultivars. Significantly different values ($P \leq 0.05$) within treatments by cultivar are indicated by lower case letters. Error bars indicate standard error of the group mean. Open bars are CO_2 levels of 350 $\mu l\ L^{-1}$; shaded bars are CO_2 levels of 1000 $\mu l\ L^{-1}$.

Table V. *Comparison of the Effects of CO_2 Enrichment and O_2 Depletion on Growth Analysis Parameters for Two Wheat Cultivars.*

CO_2 Growth Ratio = value obtained at 1000 $\mu l\ L^{-1}$/value at 350 $\mu l\ L^{-1}$; O_2 Growth Ratio = value obtained at 5% O_2/value at 21% O_2. Average of two experiments.

	O_2 Concentration (%)			
	Yecora Rojo		Sonoita	
CO_2 Growth Ratio	5	21	5	21
ΔW	1.54	1.94	1.39	1.40
NAR	1.12	1.53	1.13	1.25
RGR	1.06	1.21	1.09	0.99
	CO_2 Concentration ($\mu l\ L^{-1}$)			
O_2 Growth Ratio	350	1000	350	1000
ΔW	1.55	1.23	1.36	1.30
NAR	1.40	1.03	1.24	1.11
RGR	1.16	1.05	1.03	1.11

CO_2 enrichment is a concomitant factor. Indeed, in terms of quantum efficiency of biomass production (11), a low O_2 plant growth area would be desirable in a space setting. Future experiments will address the mechanism of the CO_2 effect on low-O_2-grown plants and will determine if it occurs in other species.

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