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Very High CO_2 Reduces Photosynthesis, Dark Respiration and Yield in Wheat

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Although terrestrial CO_2 concentrations, [CO_2], are not expected to reach 1000 μmol mol^{-1} for many decades, CO_2 levels in closed systems such as growth chambers and glasshouses, can easily exceed this concentration. CO_2 levels in life support systems in space can exceed 10000 μmol mol^{-1} (1%). Here we studied the effect of six CO_2 concentrations, from ambient up to 10000 μmol mol^{-1}, on seed yield, growth and gas exchange of two wheat cultivars (USU-Apogee and Veery-10).

Elevating [CO_2] from 350 to 1000 μmol mol^{-1} increased seed yield (by 33%), vegetative biomass (by 25%) and number of heads m^{-2} (by 34%) of wheat plants. Elevation of [CO_2] from 1000 to 10000 μmol mol^{-1} decreased seed yield (by 37%), harvest index (by 14%), mass per seed (by 9%) and number of seeds per head (by 29%). This very high [CO_2] had a negligible, non-significant effect on vegetative biomass, number of heads m^{-2} and seed mass per head. A sharp decrease in seed yield, harvest index and seeds per head occurred by elevating [CO_2] from 1000 to 2600 μmol mol^{-1}. Further elevation of [CO_2] from 2600 to 10000 μmol mol^{-1} caused a further but smaller decrease.

The effect of CO_2 on both wheat cultivars was similar for all growth parameters. Similarly there were no differences in the response to high [CO_2] between wheat grown hydroponically in growth chambers under fluorescent lights and those grown in soilless media in a glasshouse under sunlight and high pressure sodium lamps.

There was no correlation between high [CO_2] and ethylene production by flag leaves or by wheat heads. Therefore, the reduction in seed set in wheat plants is not mediated by ethylene. The photosynthetic rate of whole wheat plants was 8% lower and dark respiration of the wheat heads 25% lower when exposed to 2600 μmol mol^{-1} CO_2 compared to ambient [CO_2].

It is concluded that the reduction in the seed set can be mainly explained by the reduction in the dark respiration in wheat heads, when most of the respiration is functional and is needed for seed development.

Key words: *Triticum aestivum*, wheat, CO_2, seed yield, harvest index, pollination, ethylene, photosynthesis, respiration.

INTRODUCTION

Predictions of impending increases in global atmospheric CO_2 concentration, [CO_2], (Keeling *et al.*, 1979) have naturally stimulated interest in the effect of high [CO_2] on photosynthesis and plant growth. Therefore, most of the studies on the biological effects of elevated CO_2 have focused on the response of plants to concentrations below 1200 μmol mol^{-1} CO_2 in air (0·12%). The majority of the cases which examined CO_2 enrichment up to 1200 μmol mol^{-1}, found a positive effect on plants in terms of increased photosynthetic rate, enhanced crop yield, and elevated vegetative dry weight biomass (Egli, Pendleton and Peters, 1970; Krenzer and Moss, 1975; Fischer and Aguilar, 1976; Sionit, Hellmers and Strain, 1980; Kimball and Idso, 1983; Mortensen, 1987; Cloux *et al.*, 1989). Although terrestrial CO_2 concentrations are not expected to reach 1200 μmol mol^{-1} for many decades, CO_2 levels in closed systems (e.g. growth chambers and glasshouses), can easily exceed this concentration. Furthermore, in the Controlled Ecological Life Support System (CELSS) that the National Aeronautics and Space Administration (NASA) plans to use for extended space travel, the [CO_2] can exceed 10000 μmol mol^{-1} (1%). For example, the US Sky Lab mission recorded [CO_2] up to 6000 μmol mol^{-1} (Ross, 1973). Similarly, the Soviet space station, Mir, routinely encountered levels between 3000 and 7000 μmol mol^{-1} CO_2 (Wheeler *et al.*, 1993; F. Salisbury pers. comm.). Despite an extensive literature on CO_2 enrichment, there are only a few reports on the effect of very high CO_2 concentrations ($>$ 1200 μmol mol^{-1}) and, most of these only studied the CO_2 effect up to 3000 μmol mol^{-1} CO_2 (0·3%). Very few of these studies include a sufficient number of [CO_2] treatments to clearly define the CO_2 effect on plant growth and yield.

Julliffe and Ehret (1985) reported that the maximum increase in total dry weight of bean plants (*Phaseolus vulgaris* L.) was achieved at 1200 μmol mol^{-1} CO_2, and enrichment to 2000 or 3000 μmol mol^{-1} CO_2 had no additional effect. Madson (1974) reported that tomato fruit yield peaked at 1000 μmol mol^{-1} and declined at higher levels up to 3200 μmol mol^{-1} CO_2. Wheeler *et al.* (1993) studied soybean growth at 500, 1000, 2000 and 5000 μmol mol^{-1} CO_2. They found that seed yield and total biomass were greatest at 1000 μmol mol^{-1} for cv. McCall, suggesting

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that higher [CO_2] levels were supraoptimal, but seed yield and total biomass for cv. Pixie showed little difference between [CO_2] treatments. Mackowiak and Wheeler (1996) found that potato yield peaked at 1000 μmol mol^{-1} CO_2, and declined at higher levels. Grotenhuis and Bugbee (1996) studied the effect of 350, 1200 and 2500 μmol mol^{-1} CO_2 on growth and yield of two cultivars of wheat grown hydroponically in growth chambers. They found that the peak of vegetative biomass and seed yield was obtained at 1200 μmol mol^{-1} CO_2. Further elevation of [CO_2] to 2500 μmol mol^{-1} reduced seed yield by 22% in cv. Veery-10, and by 15% in cv. USU-Apogee. They also found that exposing wheat plants to 2500 μmol mol^{-1} 2 weeks before and after anthesis, mimicked the results of a constant high [CO_2] exposure treatment.

Here we report a study of the response of two wheat cultivars to six levels of [CO_2], from ambient up to 10000 μmol mol^{-1} CO_2. We compared the growth and the yield results of the wheat plants grown hydroponically in growth chambers to those of plants grown in a glasshouse in soilless media. In addition, we investigated the deleterious effect of very high [CO_2] on the seed yield of wheat plants.

MATERIALS AND METHODS

The effect of six CO_2 concentrations in the air (350, 1000, 1800, 2600, 5000 and 10000 μmol mol^{-1}) were evaluated on the growth, yield, gas exchange and ethylene production of two wheat cultivars (*Triticum aestivum* L. cvs. 'Veery-10' and 'USU Apogee'). Three trials were conducted in a glasshouse and three in a controlled-environment growth chamber. Each trial had two replicate chambers.

Glasshouse

Twelve plexiglass chambers (36 × 47 × 60 cm) were placed on a 23 cm depth of soilless media (1:1; peatmoss:perlite). Two benches (92 × 184 cm) were used with six chambers on each bench. Two wheat cultivars ('Veery 10' and 'USU-Apogee') were seeded (800 seeds m^{-2}) in two lines along each bench. The plants were irrigated with nutrient solution (Peter's Soluble Plant Food 20-10-20 PL 0·25 g l^{-1}, Fe-EDDHA 10 mM, $CuSO_4$ 0·1 mM and K_2SiO_3 10 mM) twice a day. Two days after emergence, the seedlings were thinned and the chambers were placed on the benches, each chamber placed over the two wheat cultivars equally (giving a total of about 90 plants in each chamber, 45 plants from each cultivar). The plants in each chamber were exposed to one of the six CO_2 levels (350, 1000, 1800, 2600, 5000 and 10000 μmol mol^{-1}), maintained by mixturing ambient air with pure CO_2 from a compressed gas cylinder. The mixture of the ambient air with the pure CO_2 was controlled with rotameters. The CO_2 from the cylinder was passed through two columns of concentrated $KMnO_4$ to prevent any possible contamination. The chambers received 20 l min^{-1} mixture of ambient air and CO_2.

Four CO_2 concentration treatments were monitored every 20 min by an IRGA (Voltronics model 2015 CA) connected to a computer. The high [CO_2] treatments (5000 and 10000 μmol mol^{-1}) were monitored manually twice a day by injecting diluted samples into an IRGA (ADC 225 2E). The photoperiod was 24 h. Direct and diffuse sunlight as well as high pressure sodium (HPS) 1000 W lamps provided a photosynthetic photon flux density (PPFD) which varied at the top of the canopy between 350–1200 μmol m^{-2} s^{-1} depending on time of day and stage in the life cycle of the plants. The lamps were turned off for 4 h during the middle of each day, to prevent over heating. Preliminary tests showed that the integrated light intensity coming from the sunlight during the day was the same to each of the 12 chambers. Air temperature was measured in each chamber with a shielded, 24-gauge, Type-E thermocouple and was maintained at 23 ± 2 °C. Humidity in the chambers was maintained at 70 ± 5%. The temperature and dew-points in the chambers were maintained by a water cooled, ventilated, copper heat exchanger. The temperature of the water circulating though the heat exchanger was controlled to ± 0·5 °C.

Three trials were conducted in the glasshouse with two replicates of each CO_2 treatment. Once mature, [as determined by loss of green colour from the seeds (63–64 d)] the plants were harvested and evaluated for: seed yield (g d. wt m^{-2} d^{-1}), vegetative biomass (g d. wt m^{-2} d^{-1}), harvest index [seed yield/total biomass (%)], total heads m^{-2}, number of primary, secondary and tertiary heads m^{-2}, seeds per head and mass per seed (mg), for each treatment. The treatments were randomized among chambers. The respiration and the ethylene production rates of the wheat heads were evaluated in the third trial.

Wheat head respiration measurements

In addition to the dark respiration of the whole canopy measurements (see below), measurements were conducted to examine whether the dark respiration (Rd) of the wheat heads was influenced differentially compared with the whole Rd canopy under high [CO_2]. Therefore, wheat heads (3–4 d post anthesis age) grown in the glasshouse under three different [CO_2]: 350, 1000 and 2600 μmol mol^{-1} were chosen and the Rd of each head (total of 12 heads, four heads from each chamber) was measured after exposing the head each time to one of the three following [CO_2]: 350, 1100 and 2100 μmol mol^{-1}. To measure the head Rd we used a cylinder tube (4 × 17 cm) with inlet and outlet to the air. The Rd was measured using an open, differential gas-exchange system as described by Bugbee (1992). Inside the tube there was a thermocouple to keep measuring the head Rd at 18 ± 0·2 °C. The respiration of the heads was measured during the daytime *in situ*, in the chambers. The heads were put into cylinders shaded by aluminum foil; this resulted in a 5 °C drop in temperature (see discussion). The Rd measurements started 1·5 h from darkness.

Wheat head ethylene production measurements

In order to investigate whether there was any correlation between seed yield reduction and ethylene production by

wheat plants, ethylene biosynthesis was estimated using detached wheat heads (cvs. USU-Apogee and Veery-10). About 4 g f. wt of intact wheat heads were incubated in a 125 ml flask containing 1 ml deionized water to prevent dehydration. After passing air with the desired [CO_2] (between 350–10000 μmol mol^{-1}) through the flasks according to the [CO_2] that the heads experienced in the glasshouse, the flasks were sealed with a septum and transferred to a growth chamber at $18 \pm 0{\cdot}5$ °C. The light intensity within the chamber was chosen so as to keep the photosynthesis and respiration of the heads roughly equal. The [CO_2] in the flasks was measured several times during the experiment, the heights of each flask in the growth chamber were different, to maintain the compensation point and to prevent [CO_2] increasing in the flask. Gas samples (2 ml) were withdrawn from the flasks at intervals of 1 h until 10 h and ethylene was assayed using a gas chromatograph (TRACOR 222) equipped with an alumina column and a flame ionization detector. A total of 12 flasks (with six [CO_2] and two replicates) were used in each trial.

Controlled-environment growth chamber

Three trials were conducted in the growth chamber. One included six CO_2 concentrations. After obtaining the results of this experiment (Fig. 2), which were very similar to the glasshouse results, two further trials were carried out that included two replicates of three CO_2 concentrations. The objectives of these trials were to evaluate the photosynthetic and respiration rate of wheat plants (USU-Apogee cv.), and to see if there was any correlation between the growth and the yield of these plants grown in hydroponic nutrient solution and growth chambers with well controlled environments to those grown in a glasshouse under a combination of sunlight and HPS light and in soilless media. The growth chamber (Percival, Model PT-80, Boone, IA) contained six Plexiglas cylinders (30×62 cm). The CO_2 treatments were randomized among the cylinders. Seeds were direct-seeded into two layers of 0·071 m² plastic hydroponic flats, covered with a 5 mm diameter extruded diatomaceous earth (Isolite, Sundine Enterprises, Arvada, CO, USA), and thinned 48 h after emergence to 70 plants per cylinder (1000 plants m^{-2}). The plants were exposed to a 24 h light period. PPFD at the top of the canopy was 350–600 μmol m^{-2} s^{-1}. The long photoperiod made the total, daily W m^{-2} close to the saturation. Temperature in the cylinders was maintained at $23 \pm 0{\cdot}5$ °C and the relative humidity at 70 ± 5 % throughout the trials. The nutrient solution, hydroponic system, air flow rate, CO_2 controlling system, harvesting time and the parameters calculated at the end of each trial were as described previously (Grotenhuis and Bugbee, 1996).

Every 5 d, from seed emergence until harvesting, the photosynthesis (P) and the dark respiration (Rd) of the canopy were measured, using the open gas-exchange system and a differential IRGA mode (Bugbee, 1992). When Rd was measured, the lights in the growth chamber were turned off, the temperature maintained to $20 \pm 0{\cdot}3$ °C and the Rd measurements started after 1·5 h from darkness.

Protein and chlorophyll content

The protein percentage in the whole flour ground seeds was evaluated using a Near Infra Red Analyzer (NIR) 350, (Technicon, Bran Lueboe Company, NY, USA). The protein concentration in flag leaves (the main leaves on the wheat plants, and usually representative of the other leaves) was evaluated by subtracting the total N measured by combustion on a Leco instrument, with the total NO_3-N determined colorimetrically (subtracting total N with total NO_3-N gives the N-assimilated values in the leaves). The chlorophyll content was assayed according to Monje and Bugbee (1992).

Statistical analysis

Data from common trials were pooled and analysed using the General Linear Models procedure (PROC GLM) of the Statistical Analysis System (SAS, Version 6.1 for Microsoft windows).

RESULTS

General growth parameters

The effect of different [CO_2] on seed yield, harvest index, vegetative biomass, heads m^{-2}, seeds per head and mass per seed for the glasshouse trials, are shown in Fig. 1. The effect of CO_2 on the two wheat cultivars (USU-Apogee and

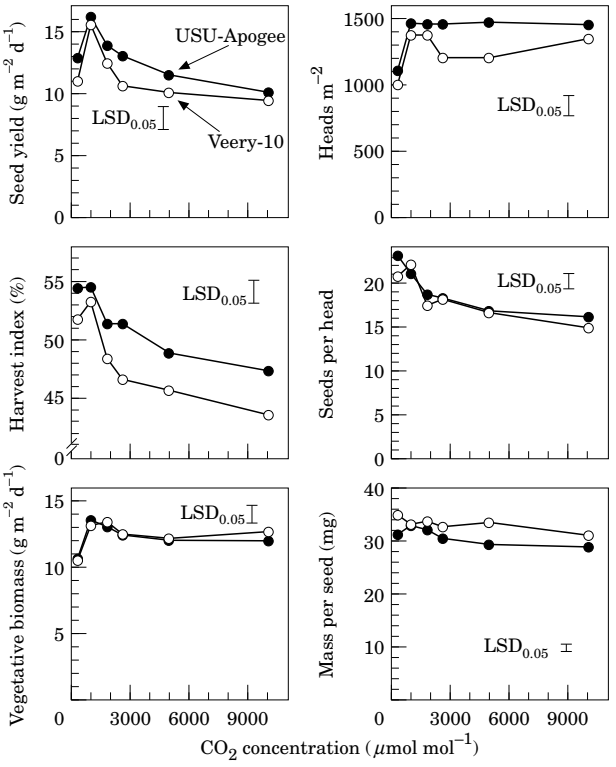

FIG. 1. The effect of CO_2 on seed yield, harvest index and vegetative growth rate of two wheat cultivars (●, USU-Apogee; ○, Veery-10), grown in a glasshouse. Error bars represent the least significant difference at $\alpha = 0{\cdot}05$. The results are based on three trials for each cultivar.

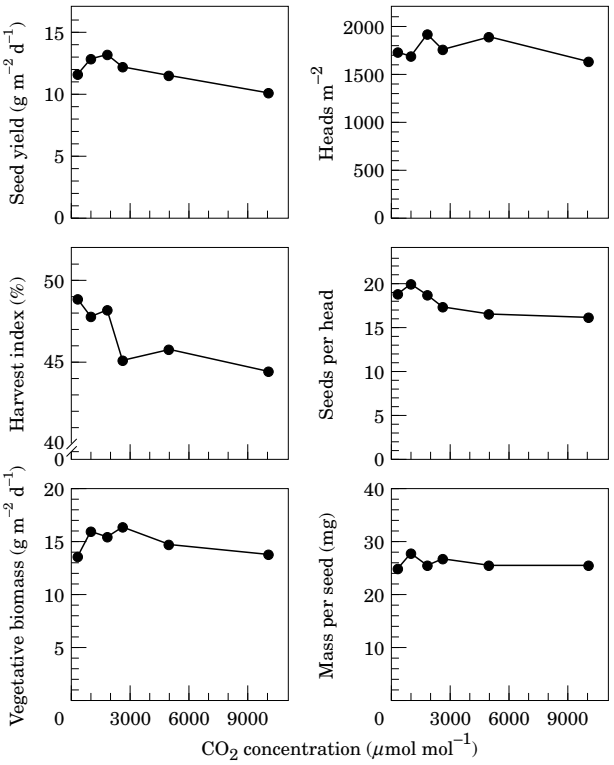

FIG. 2. The effect of different CO_2 concentrations on seed yield and vegetative growth rate of USU-Apogee wheat cultivar, grown in a controlled environment growth chamber. The results are based on one trial.

Veery-10) is similar for all the parameters. Elevation of [CO_2] from 1000 to 10000 μmol mol^{-1} had a clearly negative effect on seed yield, harvest index and seeds per head, but a negligible and non significant effect on vegetative biomass, heads m^{-2} and mass per seed. Elevating CO_2 from 350 to 1000 μmol mol^{-1} increased seed yield by 33% ($P < 0{\cdot}01$). There was a steep decrease (about 26%) in the seed yield of the plants grown in 2600 μmol mol^{-1} CO_2 compared to those grown in 1000 μmol mol^{-1} CO_2 ($P < 0{\cdot}01$). A further increase in [CO_2], from 2600 to 10000 μmol mol^{-1}, caused a less steep decrease (about 18%) in the seed yield. Overall there was an 18% decrease in seed yield in plants grown under 10000 compared to 350 μmol mol^{-1} ($P < 0{\cdot}01$), and a 37% seed yield decrease compared to those grown under 1000 μmol mol^{-1} CO_2 ($P < 0{\cdot}01$).

The harvest index (HI) values were also clearly influenced by high CO_2. Elevating [CO_2] from ambient to 1000 μmol mol^{-1} does not change HI significantly, but further CO_2 elevation decreased HI by 6% at 1800 ($P < 0{\cdot}01$) to 14% at 10000 μmol mol^{-1} ($P < 0{\cdot}01$). The number of seeds per head did not differ significantly between 1000 μmol mol^{-1} and the ambient treatment, but declined by 18% at 1800 ($P < 0{\cdot}01$) and by 29% at 10000 μmol mol^{-1} CO_2 ($P < 0{\cdot}01$), compared to the ambient CO_2 treatment.

The vegetative biomass was 25% higher in 1000 μmol mol^{-1} compared to ambient CO_2 treatment ($P < 0{\cdot}01$). Further increase in [CO_2] only decreased vegetative biomass slightly compared to 1000 μmol mol^{-1} CO_2. There was a non significant declining trend in vegetative biomass at 1800 (2%) and at 10000 μmol mol^{-1} CO_2 (8%). The same effect was seen for the numbers of heads m^{-2}. Heads m^{-2} increased by 34% ($P < 0{\cdot}01$) between 350 and 1000 μmol mol^{-1}, but higher [CO_2] did not change the heads density compared to 1000 μmol mol^{-1}. Mass per seed was not influenced by elevating [CO_2] from ambient to 1800 μmol mol^{-1} CO_2. Higher [CO_2] influenced mass per seed slightly. There was a non-significant decrease at 2600 (3%), a 5% decrease at 5000 ($P < 0{\cdot}01$), and a 9% decrease at 10000 μmol mol^{-1} CO_2 ($P < 0{\cdot}05$) compared to the ambient CO_2 treatment.

The effect of high [CO_2] on wheat plants grown in a controlled environment growth chamber, is shown in Fig. 2. Here too, the main effect of high [CO_2] on seed yield, HI and heads per chamber was significant. It can be seen that the pattern of influence of high [CO_2] on the different parameters shown in Figs 1 and 2 are very similar, although the growth conditions were different.

Protein and chlorophyll contents

Percent protein content in the seeds and in the flag leaves, chlorophyll concentration (mg m^{-2}), and stomatal density, were checked in the wheat plants grown under different [CO_2] in the glasshouse. The results are shown in Fig. 3A–C. Any interaction between [CO_2] and these parameters can easily influence the photosynthetic rate, and accordingly the growth and the yield of the plants. The protein content in seeds and flag leaves (Fig. 3A), and stomatal density (Fig. 3B), were not affected significantly by [CO_2]. The chlorophyll concentration in flag leaves (Fig. 3C) was 20% higher in leaves grown under 1000 μmol mol^{-1} CO_2 than those grown in ambient air. Leaves that were exposed to [CO_2] higher than 1000 μmol mol^{-1} had almost the same chlorophyll concentration as those grown under 1000 μmol mol^{-1} CO_2.

Ethylene contents

Flag leaves and heads from both wheat cultivars were examined for their ability to produce ethylene at different CO_2 levels. The flag leaves produced less than 1/10 ethylene compared to the wheat heads. Flag leaves did not show any difference in their ethylene production rate between the different CO_2 treatments (results not shown). During the measurements, we noticed that the ethylene production rate by heads was strongly dependent on the head development stage. The ethylene production started about 3 d before the onset of anthesis and reached a maximum rate about 3 d after the onset of anthesis (results not shown).

Two to 3 d after the onset of anthesis, heads were examined for their ability to produce ethylene. The results in Fig. 3D, show that there was no positive correlation between CO_2 treatments and ethylene production, and high [CO_2] does not stimulate ethylene production.

Photosynthetic and dark respiration rates

The photosynthetic rate (P) and the dark respiration rate (Rd) of USU-Apogee canopy grown under three different

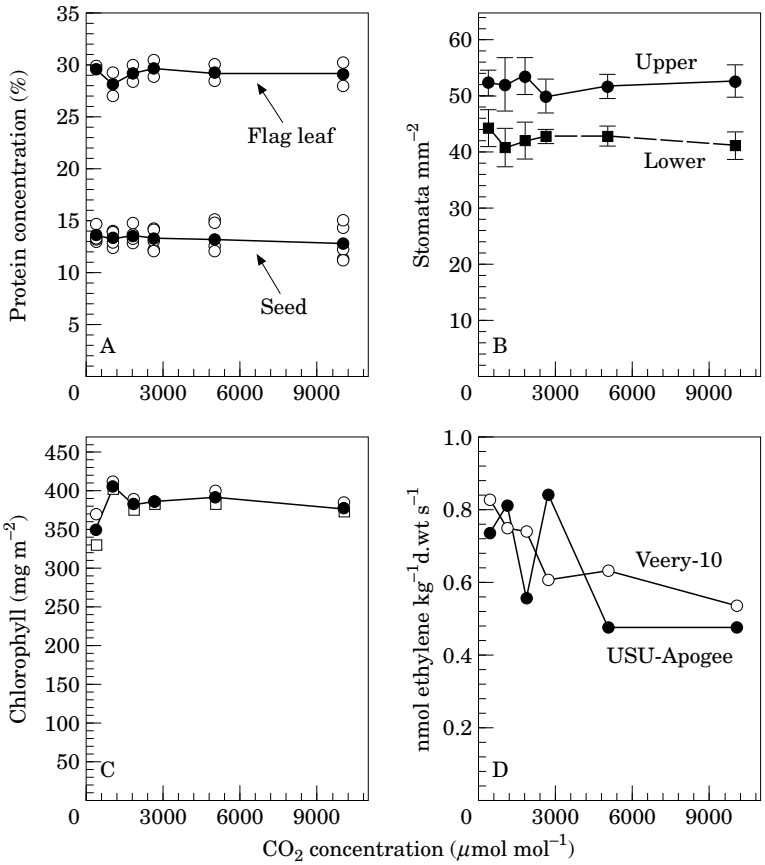

FIG. 3. The effect of different CO_2 levels on protein concentration in seeds, on chlorophyll concentration and stomatal density in flag leaves, and on ethylene production rate by wheat heads, from two wheat cultivars (●, USU-Apogee; ○, Veery-10).

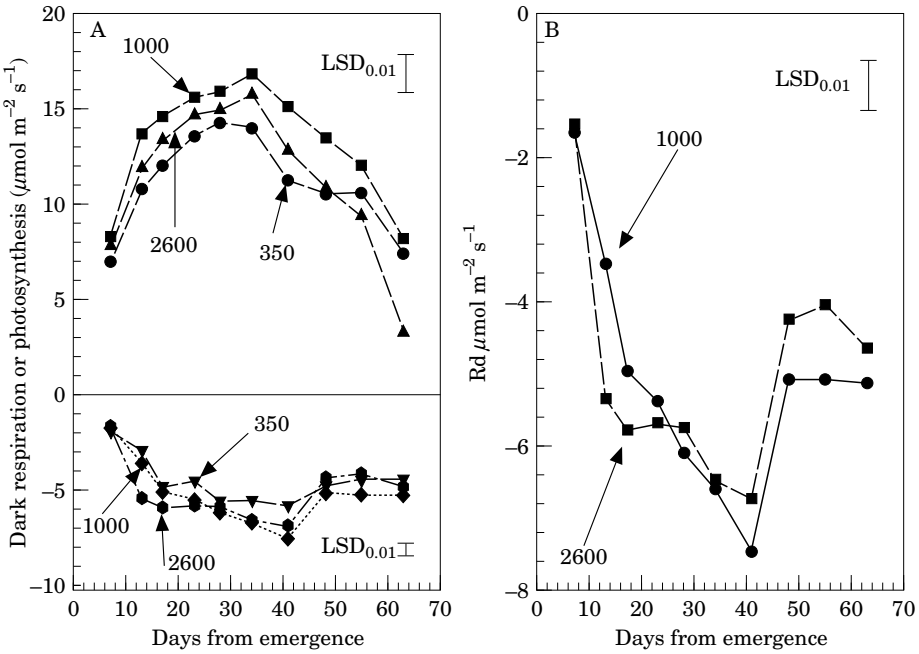

FIG. 4. Photosynthetic and dark respiration rates of USU-Apogee canopy, grown under three different CO_2 concentrations in a growth chamber.

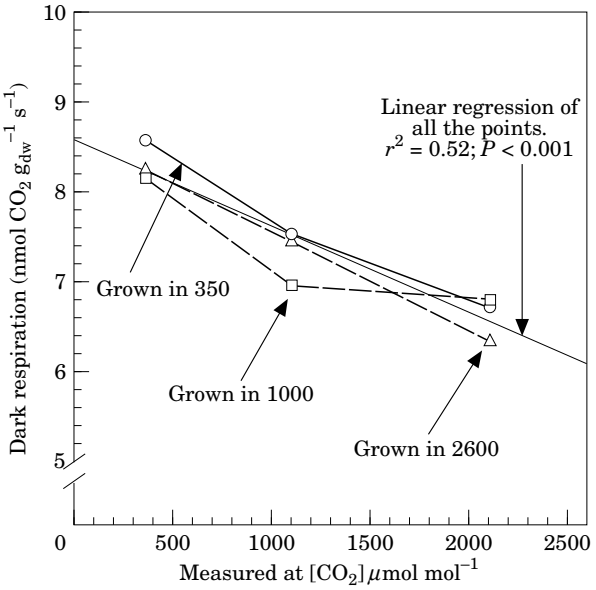

FIG. 5. Respiration response of Apogee wheat heads grown under three different CO_2 regimes (○, 350; □, 1000; and △, 2600 μmol mol^{-1}) to CO_2 concentration. Each point is the average of four replicate heads, each head was exposed to the three CO_2 concentrations.

[CO_2] are summarized in Fig. 4. The P rate of the plants grown in 350 μmol mol^{-1} (P_{350}) was significantly lower than those grown at 1000 μmol mol^{-1} ($P < 0{\cdot}01$) or 2600 μmol mol^{-1} CO_2 ($P < 0{\cdot}05$). The P rate of the plants grown under 2600 μmol mol^{-1} (P_{2600}), compared to those grown under 1000 μmol mol^{-1} (P_{1000}), was significantly lower (8%, or more) during the entire life cycle of the plants ($P < 0{\cdot}01$). Rd of the plants grown under 350 μmol mol^{-1} CO_2 (Rd_{350}) was lower during most of the life cycle than Rd_{1000} or Rd_{2600} ($P < 0{\cdot}01$). Until day 26 Rd_{2600} was higher than Rd_{1000}, but Rd_{2600} was lower for the rest of the plant life cycle (Fig. 4).

The results shown in Figs 1 and 4B, suggest that the main high CO_2 effect occurs between head boot stage (the time when the wheat head starts to emerge from the flag leaf) and seed set. It can be seen that after day 22 (Fig. 4B), the respiration of the whole plants was lower in plants exposed to 2600 than in those exposed to 1000 μmol mol^{-1} CO_2. This was about the time that the heads started to develop seeds. We therefore checked the Rd rate of the USU-Apogee heads grown at three CO_2 concentrations (350, 1000 and 2600 μmol mol^{-1}) day and night in the glasshouse. During the Rd measurements each head was exposed to three different [CO_2] and the Rd was evaluated at each CO_2 exposure. Figure 5 shows that Rd_{1000} was 12% lower than Rd_{350} ($P < 0{\cdot}01$), and Rd_{2100} was 20% lower than Rd_{350}. If we extrapolate the regression line to a [CO_2] of 2600 μmol mol^{-1}, then Rd_{2600} equals 6·1 nmol CO_2 g^{-1} d. wt s^{-1}, which is about 25% lower than Rd_{350} ($P < 0{\cdot}01$). On the other hand, the Rd rate was similar in the same [CO_2] measurement, regardless of the previous [CO_2] growth condition.

DISCUSSION

Elevating [CO_2] from 350 to 1000 μmol mol^{-1} increased seed yield (by 33%), vegetative biomass (by 25%) and heads m^{-2} (by 34%) (Fig. 1). These results can be explained by a higher photosynthetic rate (20% or more) in the enriched [CO_2] treatment, during all stage of the wheat life cycle (Fig. 4A). The results are similar to many other studies which report higher crop yield and vegetative biomass in wheat and other plants grown under enriched [CO_2], up to 1200 μmol mol^{-1} CO_2, compared to ambient [CO_2] treatment (Krenzer and Moss, 1975; Fischer and Aguilar, 1976; Sionit *et al.*, 1980; Havelka, Wittenbach and Boyle, 1984*a*; Havelka *et al.*, 1984*b*; Wheeler *et al.*, 1993; Grotenhuis and Bugbee, 1996). Elevation of [CO_2] from 1000 to 10000 μmol mol^{-1} had a clear negative effect on seed yield, HI and seeds per head (Fig. 1). The main negative effect was at [CO_2] between 1000 to 2600 μmol mol^{-1}. Further elevation to 5000 and 10000 μmol mol^{-1} CO_2 caused smaller negative effects. Elevation of CO_2 from 1000 to 10000 μmol mol^{-1} only caused a small reduction in the vegetative biomass (Fig. 1). The response of the plants grown in the glasshouse to high [CO_2] was similar to that of plants grown in the growth chamber (Figs 1 and 2), regardless of the growth medium, radiative environment, nutrient solution or diurnal temperature. These results are supported by previous reports of the influence of supraoptimal [CO_2] on wheat (Grotenhuis and Bugbee, 1996), soybean (Wheeler *et al.*, 1993), and potato (Mackowiak and Wheeler, 1996).

From the reduction in number of seeds per head (Figs 1 and 2), we can learn that [CO_2] above 1000 μmol mol^{-1} interfered with the formation or the development of seeds in wheat heads, and this caused reduction in seed yield and HI. There is much experimental evidence indicating that the detrimental effect of high [CO_2] may be mediated by ethylene. Several reports indicate that [CO_2] below 2% can induce ethylene synthesis. Similarly, there is a positive correlation between elevated [CO_2] and ethylene synthesis (Dhawan, Bassi and Spencer, 1981; Bassi and Spencer, 1982; Grodzinski, Boesel and Horton, 1982; Grodzinski, 1984; Philosoph-Hadas, Aharoni and Yang, 1986; Zhi-Yi and Thimann, 1989). Kao and Yang (1982) showed that the stimulation of ethylene production by CO_2 is rapid, and that half-maximal activity in ethylene production was about 600 μmol mol^{-1} CO_2. Philosoph-Hadas *et al.* (1986) found that ethylene synthesis was saturated at about 2% CO_2. Ethephon (2-chloroethyl phosphonic acid), a commercial product used to induce ethylene synthesis in plants, is also a potent inhibitor of pollen formation (Bennet and Hughes, 1972). Several authors found that ethephon can induce full male sterility in wheat if applied at the right concentration at the right time (Rowell and Miller, 1971). Moes and Stobbe (1991) reported that ethephon reduced the number of seeds per head by up to 26% when used to prevent lodging in barley. The results in this report do not show a positive correlation between high [CO_2] and ethylene production by leaves (results not shown), or by wheat heads (Fig. 3D). Therefore the reduction in the seed set in wheat plants exposed to high [CO_2] was not mediated by ethylene.

We measured the Rd of the canopy and the wheat heads at a lower temperature (see methods); however, although this resulted in a lower rate of respiration, previous work with alfalfa plants found that the CO_2 effect on Rd was not affected by temperature (Reuveni and Gale, 1985). From the canopy gas exchange measurements, it can be seen that Rd_{2600} began to be lower than Rd_{1000} from day 25 (Fig. 4B); this day is parallel to the head boot stage period in wheat plants. Lower Rd can be explained either by lower assimilate levels in the plant, or by partial suppression of the Rd by high [CO_2] in the air (Gifford, Lambers and Morison, 1985; Reuveni and Gale, 1985; Bunce and Caulfield, 1991; Amthor, Koch and Blum, 1992; Ziska and Teramura, 1992; Reuveni, Gale and Zeroni, 1997). Although the light regime and the irrigation type were different in the growth chamber compared to the glasshouse, the growth and the yield were similar (Figs 1 and 2), so we believe that the gas exchange measurements in the growth chamber are relevant to the glasshouse grown plants.

Considering the Rd of wheat heads only, and their response to three different [CO_2] levels (Fig. 5), shows that Rd_{1000} is 12% smaller than Rd_{350}, and Rd_{2600} is about 25% smaller than Rd_{350} (Fig. 5, after extrapolating the regression line). Developing seeds have a very high metabolic rate; they are undergoing intense activity, synthesizing complex polysaccharides, proteins and fats from simple components. This entails the need for considerable energy supplied by Rd (Penning de Vries, 1972); so reproductive growth may be more sensitive to a reduction in Rd than vegetative growth. Quebedeaux and Hardy (1973) reported a large reduction in the development of soybean and sorghum seeds when the plants were grown at subatmospheric concentrations of O_2. Gale (1974) reported that the rate of Rd of pods and seeds of soybean plants was positively correlated with O_2 concentration and he suggested that the reported depression of seed growth due to subatmospheric O_2 concentration was caused by depression of Rd. Reuveni *et al*. (1997) reported that suppression of Rd by exposing *Xanthium strumarium* plants to a high level of CO_2 (900 μmol mol^{-1}) during the night, decreased the plant's tolerance to being grown under salt stress. They concluded that high [CO_2] at night suppressed part of the functional Rd that is needed to overcome the salt stress. Reuveni *et al*. (1995) reported that there is no evidence for the hypothesis that high [CO_2] suppresses the non-phosphorylating alternative respiration pathway. Palet *et al*. (1991, 1992) reported that the ATP-producing cytochrome pathway in plants was partially suppressed by high [CO_2].

In conclusion, the reduction in the number of seeds set in this study (Figs 1 and 2) can be explained by the reduction in Rd in the wheat heads (Fig. 5), when most of the respiration is functional, and is needed for seed development. Although seed set increased between 350 and 1000 μmol mol^{-1} CO_2, this was a result of the higher rate of photosynthesis which more than compensated for any reduction in respiration. Above these CO_2 levels, respiration reduction became the more dominant factor. This conclusion can also explain the results of Grotenhuis and Bugbee (1996), who found that reducing [CO_2] 2 weeks before and after wheat anthesis eliminated the adverse effects of supraoptimal CO_2. Similarly, elevating [CO_2] to 2500 μmol mol^{-1} over the same time period mimicked the results of constant treatment with 2500 μmol mol^{-1} CO_2. The steep reduction in numbers of seeds per head and seed yield cannot be explained solely by a reduction in photosynthetic rate at high [CO_2]. The P_{2600} rate was about 8% smaller than P_{1000} (Fig. 4A), and the reduction in seed yield and seeds per head were > 20% higher in the plants grown under 1000 μmol mol^{-1} CO_2. Furthermore, if the only explanation for the reduction was the smaller P rate in higher [CO_2], we would have expected a reduction of only 7% in seed yield, similar to the reduction in vegetative biomass in plants grown at 2600 μmol mol^{-1} *vs*. 1000 μmol mol^{-1} CO_2 (Fig. 1). It is possible, therefore, that both processes combine to reduce seeds per head and seed yield. The lower P rate results in a smaller amount of assimilates in the plants and, more importantly, the reduction in Rd in the wheat heads reduces the energy supply that is so essential to successful seed development.

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