ELSEVIER

Basic and Applied Ecology

http://www.elsevier-deutschland.de/baae

Intergenerational above- and belowground responses of spring wheat (*Triticum aestivum* L.) to elevated CO_2

Justin D. Derner[1,*], Charles R. Tischler[2], H. Wayne Polley[2], Hyrum B. Johnson[2]

[1] USDA-ARS, High Plains Grasslands Research Station, Cheyenne, WY USA
[2] USDA-ARS, Grassland, Soil and Water Research Laboratory, Temple, TX USA

Received December 17, 2002 · Accepted August 19, 2003

Abstract

We quantified intergenerational above- and belowground responses of two genotypes of semi-dwarf, hard red, spring wheats (*Triticum aestivum* L.) to elevated (700 µmol mol^{-1}) CO_2. These plants were progeny of seeds produced from previous generation plants grown at elevated CO_2 under well-watered and high nutrient conditions. Because neither genotype in the first generation exhibited enhanced performance with CO_2 enrichment, our objective in this investigation was to assess if exposure to CO_2 enrichment in subsequent generations resulted in temporal changes in the relative enhancement (elevated/ambient) of above- and belowground plant growth. Relative enhancement occurred in both the second and third generations for both above- and belowground variables. Above- and belowground variables were enhanced by similar relative amounts at elevated CO_2 within a generation at each harvest date. Relative enhancement of measured variables was generally greater in the third than second generation when plants were in the seedling or vegetative stage, but not when plants were reproductive. Additional research is needed to investigate physiological or other limitations of translating above- and belowground responses to CO_2 in vegetative growth stages to reproductive performance. Intergenerational above- and belowground responses of this C_3 annual plant to CO_2 enrichment are not driven by genetic change (selection) that occurred between generations, but rather CO_2-induced changes in seeds that affected seedling responses to CO_2 enrichment.

Wir quantifizierten die intergenerationelle ober- und unterirdische Reaktionen von zwei Genotypen mittellangen, hartroten Winterweizen (*Triticum aestivum* L.) auf erhöhtes CO_2 (700 µmol mol^{-1}). Diese Pflanzen waren Abkömmlinge von Samen, die von Pflanzen der vorherigen Generation produziert wurden, welche ihrerseits bei erhöhtem CO_2 und bei ausreichender Wasserversorgung sowie guten Nährstoffbedingungen kultiviert wurden. Weil keiner der beiden Genotypen in der ersten Generation eine verbesserte Leistung bei CO_2-Anreicherung zeigte, war unser Ziel, in der Untersuchung abzuschätzen, ob die Exposition einer CO_2-Anreicherung in den nachfolgenden Generationen zu temporären Veränderungen in der relativen Förderung (erhöht/umgebend) des ober- und unterirdischen Wachstums führte. Eine relative Steigerung fand in der zweiten und in der dritten Generation sowohl bei den ober- als auch unterirdischen Variablen statt. Bei jedem Erntetermin waren die ober- und unterirdischen Variablen innerhalb einer Generation bei erhöhtem CO_2 mit ähnlichen relativen Anteilen positiv beeinflusst. Die relative Steigerung der gemessenen Variablen war im Allgemeinen bei Pflanzen im Keimlings- oder vegetativen Stadium in der dritten Generation größer als in der zweiten, jedoch nicht bei reproduktiven Pflanzen. Zusätzliche Forschung ist notwendig, um physiologische oder andere Limitierungen zu untersuchen, die ober- und unterirdische Reaktionen von vegetativen Wachstumsstadien auf CO_2 in die reproduktiven Leistung übersetzen.

*Corresponding author: Justin D. Derner, USDA-ARS, High Plains Grasslands Research Station, 8408 Hildreth Road, Cheyenne, WY USA 82009, Phone: ++1-307-772-2433, Fax: ++1-307-637-6124, E-mail: jderner@npa.ars.usda.gov

Intergenerationelle, ober- und unterirdische Reaktionen dieser C_3-Pflanze auf CO_2-Anreicherung werden nicht durch genetische Veränderungen (Selektion) im Laufe der Generationen gesteuert, sondern eher durch CO_2-induzierte Veränderungen in den Samen, welche die Reaktion der Keimlinge auf eine CO_2-Anreicherung beeinflussen.

Key words: C_3 plant – carbon dioxide – physiological tradeoffs – relative enhancement – root responses – vegetative vs. reproductive growth

Introduction

The C_3 annual crop species spring wheat (*Triticum aestivum* L.) has been subjected to many elevated atmospheric carbon dioxide (CO_2) studies evaluating aboveground growth and grain yield (Samarakoon et al. 1995, Mulholland et al. 1998, Hudak et al. 1999, McMaster et al. 1999, Pleijel et al. 2000, Grossman-Clarke et al. 2001, Schütz & Fangmeier 2001). Generally, CO_2 enrichment increases leaf area, tillering, shoot growth and yield. However, in-depth investigations of belowground responses to CO_2 enrichment are few. Root biomass has been shown to be unresponsive to elevated CO_2 (Hudak et al. 1999), or to increase relatively more than does shoot biomass (McMaster et al. 1999, Wechsung et al. 1999).

Because there is genetic variation within species in responses to elevated CO_2 (Curtis et al. 1996, Schmid et al. 1996, Thomas & Jasienski 1996, Bezemer et al. 1998, Van der Kooij et al. 2000, Andalo et al. 2001, Klus et al. 2001, Goverde et al. 2002), impacts of CO_2 enrichment on spring wheat may be genotype-specific (Mulholland et al. 1998). Genotypic differences in responses of spring wheat to CO_2 enrichment have been recently assessed. Genotypes differ in response to CO_2 enrichment when they vary in gross morphology (e.g., height, Mayeux et al. 1997, De la Puente et al. 2000), but not in leaf physiology (Samarakoon et al. 1995).

Responses of plants to elevated CO_2 have been well-chronicled (Poorter 1993, Bunce 1997, 2001, Cotrufo et al. 1998, Pritchard et al. 1999, Makino & Mae 1999, Wand et al. 1999, Ward et al. 1999, Arnone et al. 2000, Campbell et al. 2000, Ghannoum et al. 2000, Norby & Jackson 2000, Pritchard & Rogers 2000, Zak et al. 2000, BassiriRad et al. 2001, Jablonski et al. 2002, Edwards et al. 2003), but most studies were conducted on plants grown for only a single generation, or more frequently, less. There is a paucity of information on the extent to which plants show intergenerational differences in their response to elevated CO_2. A few studies with C_3 annuals indicate that elevated CO_2 may differentially affect growth across generations (Curtis et al. 1996, Bezemer et al. 1998, Huxman et al. 1998, Ward et al. 2000), with potentially no further increases in plant biomass relative to first generational responses (Ward et al. 2000).

We quantified responses of three generations of two genotypes of spring wheat to CO_2 enrichment in glasshouse experiments. Second and third generations of plants were progeny of seeds produced by plants grown at elevated CO_2 (700 µmol mol^{-1}) under well-watered and high nutrient conditions the previous generation. Neither genotype in the first generation exhibited enhanced biomass or reproductive performance with CO_2 enrichment (see Materials and Methods, below). Because of this, our objective in this investigation was to assess if exposure to CO_2 enrichment in subsequent generations resulted in temporal changes in the relative enhancement (elevated/ambient) of above- and belowground plant growth.

Materials and methods

The CO_2 concentration of air in each of the four glasshouse bays was measured at 4-min intervals with a Li-Cor Model LI-6262 infrared gas analyzer (Li-Cor, Inc., Lincoln, NE). The CO_2 readings were corrected for atmospheric pressure measured with a Druck model DPI 260 pressure indicator (Druck, Inc., New Fairfield, CT). The infrared analyzer was calibrated daily against four CO_2 gas standards and monthly against a Li-Cor LI-610 dewpoint generator. Air temperature, manually set at 25 °C for both day and night, was measured in the center of each bay with fine-wire (25-µm diameter) thermocouples. Pure CO_2 gas was injected into appropriate bays as required to maintain the elevated CO_2 concentration. The CO_2 concentration of air in the ambient and elevated CO_2 treatments averaged 360 and 697 µmol mol^{-1} in 1995, respectively, 363 and 699 µmol mol^{-1} in 1999, respectively, and 365 and 701 µmol mol^{-1} in 2000, respectively. Photosynthetic photon flux density (PPFD) was measured on the greenhouse roof with a quantum sensor (LI-190SB, Li-Cor) and within the glasshouse bays with 1-m long line quantum sensors (LI-191SA, Li-Cor) mounted about plant height. On average, the daily integral of PPFD inside the glasshouse bays was 70% of that measured above the greenhouse.

A rust resistant common wheat (AZ-MSFRS-82RR, Thompson 1983) and a highly productive, commercially available hybrid (DK49, Douglas King) were

studied. Both genotypes are semi-drawf, hard red, spring wheats. Seeds collected from first generation plants (1995 experiment) grown at 700 μmol mol^{-1} CO_2 were pooled and used for second generation plants (1999 experiment). The same approach was used for third generation plants (2000 experiment) as seeds produced from second generation plants grown at 700 μmol mol^{-1} CO_2 were pooled. We justify the use of seed produced under CO_2 enrichment for use at both elevated and ambient CO_2 in subsequent generations as seed produced at elevated and ambient CO_2 differ in composition (e.g., Huxman et al. 1998, review by Jablonski et al. 2002), and thus also in properties of the resulting seedlings.

To prevent confounding influences of seed mass on initial seedling development (Villar et al. 1998), seeds of each genotype were weighed prior to planting of the first generation to ensure similar seed mass (0.050–0.060 g $grain^{-1}$). Three seeds of each genotype were planted in commercial potting mix into each of 40, 0.0025 m^3 (0.15 m diameter × 0.14 m deep) pots in mid-February 1995. Although this pot size is small, both of these wheat varieties are spring wheats that mature quickly and have minimal tillering, thereby minimizing the effects of pot size on plant performance. Following planting, pots with each genotype were randomly assigned to one of four glasshouse bays, two maintained at ambient CO_2 and two at elevated CO_2, with 10 pots per genotype in each bay. Upon emergence, seedlings were randomly thinned to one per pot. Soil in pots was wetted three times per week with 200 ml of full-strength Hoagland's solution (Hoagland & Arnon 1950). Positions of pots within each bay were randomized twice weekly.

All plants in the first generation were harvested at physiological maturity, designated when the entire seed head was brown. Neither genotype exhibited differences between CO_2 treatments for aboveground mass (14.36 ± 0.86 vs. 14.87 ± 0.89 g $plant^{-1}$, ambient vs. elevated) or reproductive variables including number of seed heads (6.1 ± 0.4 vs. 6.5 ± 0.3) and seed mass (6.38 ± 0.40 vs. 6.49 ± 0.31 g $plant^{-1}$).

Seeds of each genotype grown under CO_2 enrichment the previous generation were weighed prior to planting to ensure similar seed mass for each generation (0.0450–0.055 g $seed^{-1}$, second generation; 0.025–0.035 g $grain^{-1}$, third generation). These values represent the mean (± 1 SD) seed mass from plants produced the previous generation.

Three seeds of each genotype were planted in mid-February in a sandy loam soil into each of 120 same-sized pots for the second (1999) and third (2000) generations. Emerged seedlings were thinned randomly to result in one per pot. Properties of the soil include: pH = 7.1, organic carbon content = 0.57%, 76.2% sand, 16.2% silt, 7.6% clay, field capacity = 18% on a volumetric basis. This soil was chosen for the second and third generations in contrast to the commercial potting mix because we wanted to examine root responses in these generations. This soil facilitates retrieval of roots through manual root washing, whereas the commercial potting mix presents methodological problems in the recovery of the entire root system. We used the same experimental protocol for the second and third generation plants as for the first generation plants, with the exception that there were 30 pots per genotype in each bay.

In contrast to the single harvest of first generation plants, we harvested one-third of the seedlings for each genotype (n = 40) at 10- and 21-days post-emergence and physiological maturity. At the 10- and 21-day harvests, aboveground biomass was separated into leaf and stem components. Area of each individual leaf blade was measured using a LI-3000A portable leaf area meter (Li-Cor, Inc. Lincoln, NE).

Soil was manually washed from roots, which were then digitally scanned using the WinRHIZO software (Regent Instruments, Inc. Quebec, Canada, version 3.9f) and hardware (Hewlett Packard ScanJet 6100C scanner). This software determines root length, surface area and root volume. Roots were scanned using a 10 cm × 15 cm tray for the 10- and 21-day harvests and a 15 cm × 25 cm tray for the final harvest at high resolution (600 dpi) in all cases. Roots were not stained prior to analyses, which result in underestimations (Bouma et al. 2000), but this was minimized by using high resolution and the WinRHIZO automatic threshold to increase sensitivity to pale roots. Aboveground tissues and roots were dried at 60 °C for 72 h prior to weighing.

Leaf area ratio (LAR) was calculated as total plant leaf blade area per total plant mass. Specific leaf area (SLA) was determined as total leaf blade area per total leaf mass. Leaf mass ratio (LMR) was calculated as total leaf blade mass per total plant mass.

Because the generations were not grown in the same growing season, no statistical tests were conducted between the generations. However, inferences are drawn between second and third generations at each harvest using the term relative enhancement, which was calculated by dividing the mean response of plants with CO_2 enrichment by the mean response of plants with ambient CO_2. Data were analyzed by harvest date each year using a split plot design with CO_2 as the main plot and the genotype as split plots in a balanced design (SAS Institute Inc. 1994). Means were separated by Duncan's multiple range test at the 0.05 level of probability. When needed to normalize residuals, data were logarithmically transformed before analysis; means and standard errors are reported after back-transforming.

Results

Second generation

Aboveground variables, with the exception of number of seed heads, were similar between the two genotypes at all harvests (data not shown). However, belowground variables were greater for DK49 than for AZ at day 10 (31–53%) and the final harvest (17–56%), but not day 21. Interactions between genotype and CO_2 were only observed for root variables at day 21 with greater responses (32–104%) to CO_2 enrichment displayed by DK49 than by AZ (2–27%) (data not shown).

Total plant mass increased by 21%, 38% and 63% with CO_2 enrichment at day 10, day 21 and the final harvest, respectively (Table 1). Total aboveground mass (31–60%), stem mass (39–77%) and leaf mass (18–27%) also increased with CO_2 enrichment across harvests. CO_2 enrichment decreased LAR, LMR and SLA by 7–22% at day 10 and day 21 (Table 2), but increased leaf area by 13% and 18% day 10 and day 21, respectively (Table 1). Also, the number of seed heads per plant, seeds per plant and seed mass per plant nearly doubled with CO_2 enrichment. CO_2 enrichment increased root mass by 19–88%, root volume by 35–110%, root surface area by 34–40% and root length by 36–44% across harvests.

Third generation

Both above- (15–60%) and belowground (18–36%) growth was greater for genotype DK49 than for AZ at the day 10 harvest, but these differences were not observed at day 21 nor at the final harvest, with the exception of the number of seed heads (data not shown). No interactions between genotype and CO_2 were observed for any variable at any of the harvests.

Total plant mass increased by 53%, 104% and 42% with CO_2 enrichment at day 10, day 21 and the final harvest, respectively (Table 1). Total aboveground mass (43–108%), stem mass (89–121%) and leaf mass (33–103%) exhibited a similar pattern in response to CO_2 enrichment at the day 10 and day 21 harvests. CO_2 enrichment decreased LAR and LMR, but not SLA, by 8–10% at day 10, with no differences displayed at day 21 (Table 2). Leaf area, however, increased with CO_2 enrichment by 26% and 93% at day 10 and day 21, respectively (Table 1). In contrast to vegetative responses to CO_2 enrichment, the number of seed heads per plant, seeds per plant and seed mass per plant were similar between CO_2 treatments. CO_2 enrichment increased root mass by 75–95%, root volume by 45–85%, root surface area by 39–66% and root length by 42–53% at the day 10 and day 21 harvests, but these differences did not persist in the final harvest.

Relative enhancement across generations

Third generation plants displayed increased relative enhancement, compared to second generation plants, of all aboveground variables at day 10 and day 21, but reductions in enhancement of all final harvest parameters (Table 3). Both second and third generation plants displayed similar relative enhancement of root variables at day 10, with the exception of root mass which displayed greater relative enhancement in the third than second generation. In contrast, relative enhancement markedly increased for belowground variables in the third generation at day 21. However, this trend reversed at the final harvest with the third generation exhibiting lower relative enhancement than the second generation. Plants exhibited a greater growth response at elevated than ambient CO_2 for all variables in both generations. Overall, relative enhancement was similar between above- and belowground variables within a generation at each harvest date.

Discussion

Intergenerational above- and belowground plant growth of spring wheat (*T. aestivum*) to elevated CO_2 exhibited intriguing trends. First, relative enhancement occurred in both the second and third generations for both above- and belowground variables. Second, above- and belowground variables were enhanced by similar relative amounts at elevated CO_2 within a generation at each harvest date. Third, relative enhancement of measured variables was generally greater in the third than second generation when plants were in the seedling or vegetative stage, but not when plants were reproductive. However, the occurrence of absolute reductions in plant size and production from the first to third generation, at both ambient and elevated CO_2 concentrations, advises that these results should be cautiously interpreted. The responsiveness of subsequent generations may be attributable to a compensatory response to limited growing conditions, such as the relatively nutrient-poor soil used. Even though supplemental nutrients were provided three times a week for the second and third generation plants, this soil was not comparable to the commercial potting mix used for the first generation plants. Thus, this discrepancy in soils likely contributed to the reduction in plant size and production in the second and third generation plants. Nutrient stress in C_3 plants tends to reduce responses to elevated CO_2 (Wand et al. 1999, Kimball et al. 2002). That we observed significant trends despite absolute reductions in plant and seed size and biomass production from the first to third generations does, however, suggest that elevated

Table 1. Mean ((SE, n = 40) above- and belowground responses to ambient CO_2 (A) or CO_2 enrichment (E)(360 or 700 µmol mol^{-1}, respectively) of second and third generation spring wheat (*Triticum aestivum*) plants, averaged across two genotypes. Plants were grown from seeds derived from plants grown during the previous generation at elevated CO_2. Asterisks indicate a significant ($P < 0.05$) difference between CO_2 treatments within a harvest.

	2nd generation						3rd generation					
	Day10		Day 21		Final		Day 10		Day 21		Final	
Variable	A	E	A	E	A	E	A	E	A	E	A	E
Total Mass (g)	0.077 (0.009)	0.093* (0.009)	0.258 (0.016)	0.355* (0.018)	6.270 (0.404)	10.135* (0.325)	0.040 (0.001)	0.061* (0.005)	0.069 (0.005)	0.141* (0.009)	1.417 (0.231)	2.010* (0.235)
Aboveground												
Plant mass (g)	0.046 (0.002)	0.061* (0.005)	0.175 (0.010)	0.229* (0.013)	5.829 (0.365)	9.306* (0.289)	0.028 (0.001)	0.040* (0.004)	0.049 (0.004)	0.102* (0.008)	1.297 (0.212)	1.856* (0.256)
Stem mass (g)	0.013 (0.001)	0.023* (0.004)	0.059 (0.004)	0.082* (0.005)			0.009 (0.000)	0.017* (0.004)	0.014 (0.001)	0.031* (0.003)		
Leaf mass (g)	0.033 (0.001)	0.039* (0.002)	0.116 (0.007)	0.147* (0.008)			0.018 (0.001)	0.024* (0.001)	0.035 (0.003)	0.071* (0.006)		
Leaf area (cm^2)	12.83 (0.64)	14.52* (0.61)	42.61 (2.57)	50.63* (2.70)			7.83 (0.20)	9.88* (0.29)	11.94 (0.94)	22.99* (1.40)		
Seed heads (number)					2.3 (0.2)	4.3* (0.1)					1.3 (0.1)	1.5 (0.1)
Seed count (seeds/plant)					69 (4)	118* (4)					22 (3)	32 (5)
Seed mass (mass/plant)					1.74 (0.12)	3.23* (0.12)					0.560 (0.095)	0.706 (0.112)
Belowground												
Root mass (g)	0.031 (0.008)	0.037* (0.003)	0.083 (0.008)	0.126* (0.009)	0.441 (0.035)	0.829* (0.047)	0.012 (0.001)	0.021* (0.004)	0.020 (0.002)	0.039* (0.002)	0.120 (0.021)	0.154 (0.021)
Volume (m^3)	0.51 (0.05)	0.72* (0.05)	1.49 (0.14)	2.01* (0.12)	6.93 (0.57)	14.55* (1.49)	0.20 (0.01)	0.29* (0.02)	0.40 (0.04)	0.74* (0.04)	1.60 (0.26)	1.85 (0.25)
Surface area (cm^2)	42.7 (3.4)	59.7* (3.7)	124.5 (9.4)	166.8* (8.5)	346.8 (17.9)	480.9* (16.9)	22.6 (1.2)	31.4* (1.4)	37.3 (3.0)	61.8* (2.5)	134.4 (19.3)	155.5 (17.8)
Length (cm)	311 (22)	443* (28)	939 (60)	1280* (72)	2088 (125)	3005* (164)	217 (10)	308* (11)	330 (21)	504* (19)	1228 (196)	1509 (186)

CO_2 is beneficial to spring wheat relative to ambient concentrations.

Our finding that biomass, reproductive output and other measurements of above- and belowground plant growth remained greater at elevated than ambient CO_2 through three generations contrasts with results from the few previous studies because this investigation tested for maternal change (no genetic change) while others analyzed for effects of genetic change. For example, second generation plants of the C_3 annual grass *Bromus rubens* that were selected at elevated CO_2 did not differ in shoot mass, leaf area, seedling mass, root mass, LMR, LAR, or SLA between elevated and ambient CO_2 concentrations (Huxman et al. 1998). In addition, plants of *Arabidopsis thaliana* selected at elevated CO_2 had similar plant mass as control plants, because of a faster life cycle (Ward et al. 2000). Yet, second generation plants of *Poa annua* produced more tillers in elevated than ambient CO_2 (Bezemer et al. 1998). Additionally, contrasting results between this investigation and previous studies may be attributed, in part, to differences among species studied. Spring wheat is a cultivated crop species that has undergone genetic selection to enhance plant growth, while the C_3 annual species used in previous investigations have not been selected by humans for yield.

Commercially available and common wheat genotypes responded similarly to CO_2 enrichment suggesting that resulting evolution of plant traits is not specific to CO_2, but rather is a response to characteristics common at both ambient and elevated CO_2 (Klus et al. 2001). Unfortunately, few studies have determined whether genotypes respond differently when grown under CO_2 enrichment or ambient levels (Bunce 2001, Klus et al. 2001). In contrast to our results, Klus et al. (2001) found that physiological parameters and tissue chemistry, but not mass allocation, of both families and populations of *Plantago lanceolata* responded differently to CO_2, suggesting that evolution of some plant traits is CO_2-specific.

Table 2. Mean (SE, n = 40) leaf area ratio (LAR, $cm^2\ g^{-1}$), leaf mass ratio (LMR, $g\ g^{-1}$) and specific leaf area (SLA, $cm^2\ g^{-1}$) responses to ambient CO_2 (A) or CO_2 enrichment (E) (360 or 700 $\mu mol\ mol^{-1}$, respectively) of second and third generation spring wheat (*Triticum aestivum*) plants, averaged across two genotypes. Plants were grown from seeds derived from plants grown during the previous generation at elevated CO_2. Asterisks indicate a significant ($P < 0.05$) difference between CO_2 treatments within a harvest.

	LAR		LMR		SLA	
Harvest	A	E	A	E	A	E
Second Generation						
Day 10	197.6 (8.0)	154.4* (5.7)	0.477 (0.016)	0.409* (0.013)	412.7 (6.0)	377.4* (4.6)
Day 21	170.1 (5.0)	144.9* (4.0)	0.458 (0.011)	0.417* (0.008)	370.4 (3.8)	346.1* (3.9)
Third Generation						
Day 10	196.5 (3.6)	176.9* (6.3)	0.462 (0.007)	0.426* (0.015)	425.0 (4.0)	417.8 (6.6)
Day 21	172.2 (3.4)	166.0 (3.7)	0.517 (0.008)	0.494 (0.011)	334.6 (6.3)	336.4 (6.9)

Table 3. Relative (elevated/ambient) enhancement of above- and belowground growth at three harvest dates for second and third generation spring wheat (*Triticum aestivum*) plants grown from seeds produced at elevated CO_2.

	Relative Enhancement					
	Day 10		Day 21		Final	
Variable	Second	Third	Second	Third	Second	Third
Total Mass (g)	1.21	1.53	1.38	2.04	1.63	1.42
Aboveground						
Plant mass (g)	1.33	1.43	1.31	2.08	1.60	1.43
Stem mass (g)	1.77	1.89	1.39	2.21		
Leaf mass (g)	1.18	1.33	1.27	2.03		
Leaf area (cm^2)	1.13	1.26	1.18	1.93		
Seed heads (number)					1.87	1.25
Seed count (seeds/plant)					1.71	1.45
Seed mass (mass/plant)					1.86	1.26
Mean enhancement	*1.35*	*1.48*	*1.29*	*2.06*	*1.76*	*1.35*
Belowground						
Root mass (g)	1.19	1.75	1.52	1.95	1.88	1.28
Volume (m^3)	1.41	1.45	1.35	1.85	2.10	1.16
Surface area (cm^2)	1.40	1.39	1.34	1.66	1.39	1.16
Length (cm)	1.42	1.42	1.36	1.53	1.44	1.23
Mean enhancement	*1.35*	*1.50*	*1.39*	*1.75*	*1.70*	*1.21*

Comparable carbon allocation to above- and belowground growth likely contributed to the display of similar relative enhancement of above- and belowground parameters within a generation at each harvest date. Relative enhancement of aboveground responses in the seedling and early vegetative harvests can be attributed to the direct effect of elevated CO_2 increasing growth. In contrast, relative enhancement of aboveground responses at the final harvest was likely driven by tillering differences of plants which is consistent with previous investigations of spring wheat and CO_2 enrichment (Gifford 1977, du Cloux et al. 1987, Samarakoon et al. 1995, Hudak et al. 1999, McMaster et al. 1999, Pleijel et al. 2000). Stimulation of root responses to elevated CO_2 at the seedling and early vegetative stage contrasts with previous work on C_4 grass seedlings (Derner et al. 2001), suggesting that differences in C_3/C_4 physiology and carbon allocation patterns may be manifest in differential belowground responses as for well-studied aboveground responses (e.g., Poorter 1993).

Biomass was enhanced by CO_2 enrichment relatively more in the third than second generation while plants were in seedling and vegetative stages, but the reverse was true for plants in the reproductive stage. However, absolute biomass decreased for both ambient and elevated CO_2 grown plants. Physiological or other limitations prevented biomass responses from being translated into greater reproductive growth and yield. Unfortunately, most previous studies have evaluated responses to CO_2 enrichment only at single, final harvest date (Bezemer et al. 1998, Huxman et al. 1998, Ward et al. 2000). Though biomass responded positively to CO_2 enrichment for successive generations, reproductive growth in the third generation may have been inhibited by environmental factors (e.g., light influence on tillering) that constrained the ability of plants to fully display their genetic potential. This view is supported by observations that growing points shifted from vegetative to reproductive structures within 3 weeks of emergence (Tischler, personal observations). Therefore, potential seed number was set before season-long effects of elevated CO_2 could be manifest on other characteristics, such as aboveground mass or seed mass, another component of grain yield. Data in Tables 1 and 3 supports this view.

Both genotypes of the C_3 annual spring wheat exhibited CO_2 responsiveness for multiple generations, suggesting that CO_2 enrichment effects on seed compositional changes (higher C:N ratio, Huxman et al. 1998, Jablonski et al. 2002) contributed to greater seedling growth in subsequent generations, especially under well-watered and high nutrient conditions. As a result of greater relative seedling growth in each generation, responsiveness to CO_2 enrichment was manifest at subsequent harvests. Intergenerational above- and belowground responses of this C_3 annual plant to CO_2 enrichment are not driven by genetic change (selection) that occurred between generations, but rather CO_2-induced changes in seeds that affected seedling responses to CO_2 enrichment.

Acknowledgements. Alicia Naranjo, Kyle Tiner, Holly Harland, Steve Hurtle, Adrian Lopez and Judy Corriston assisted with data collection and entry. Evelyne Childs translated the German abstract. We gratefully thank Travis Huxman and James Bunce for their reviews of a previous version.

References

Andalo C, Goldringer I, Godelle B (2001) Inter- and intragenotypic competition under elevated carbon dioxide in *Arabidopsis thaliana*. Ecology 82: 157–164.

Arnone III JA, Zaller JG, Spehn EM, Niklaus PA, Wells CE, Körner C (2000) Dynamics of root systems in native grasslands: effects of elevated atmospheric CO_2. New Phytologist 147: 73–86.

BassiriRad H, Gutschick VP, Lussenhop J (2001) Root system adjustments: regulation of plant nutrient uptake and growth responses to elevated CO_2. Oecologia 126: 305–320.

Bezemer TM, Thompson LJ, Jones TH (1998) *Poa annua* shows inter-generational differences in response to elevated CO_2. Global Change Biology 4: 687–691.

Bouma TJ, Nielsen KL, Koutstall B (2000) Sample preparation and scanning protocol for computerized analysis of root length and diameter. Plant and Soil 218: 185–196.

Bunce JA (1997) Variation in growth stimulation by elevated carbon dioxide in seedlings of some C_3 crop and weed species. Global Change Biology 3: 61–66.

Bunce JA (2001) Are annual plants adapted to the current atmospheric concentration of carbon dioxide? International Journal of Plant Science 162: 1261–1266.

Campbell BD, Stafford Smith DM, GCTE Pastures and Rangelands Network members (2000) A synthesis of recent global change research on pasture and rangeland production: reduced uncertainties and their management implications. Agriculture, Ecosystems and Environment 82: 39–55.

Cotrufo MF, Ineson P, Scott A (1998) Elevated CO_2 reduces the nitrogen concentration of plant tissues. Global Change Biology 4: 43–54.

Curtis PS, Klus DJ, Kalisz S, Tonsor SJ (1996) Intraspecific variation in CO_2 responses in *Raphanus raphanistrum* and *Plantago lancelata*: Assessing the potential for evolutionary change with rising atmospheric CO_2. In: Körner C, Bazzaz FA (eds) Carbon Dioxide, Populations, and Communities. Academic Press, London, pp 13–22.

De la Puente LS, Perez PP, Martinez-Carraasco R, Morcuende RM, Del Molino IMM (2000) Action of elevated CO_2 and high temperatures on the mineral chemical composition of two varieties of wheat. Agrochemica 44: 221–230.

Derner JD, Polley HW, Johnson HB, Tischler CR (2001) Root system response of C_4 grass seedlings to CO_2 and soil water. Plant and Soil 231: 97–104.

du Cloux HC, Andre M, Daguenet A, Massimino J (1987) Wheat response to CO_2 enrichment: growth and CO_2 exchanges at two plant densities. Journal of Experimental Botany 38: 1421–1431.

Edwards GR, Clark H, Newton PCD (2003) Soil development under elevated CO_2 affects plant growth responses to CO_2 enrichment. Basic and Applied Ecology 4: 185–195.

Ghannoum O, Von Caemmerer S, Ziska LH, Conroy JP (2000) The growth response of C_4 plants to rising atmospheric CO_2 partial pressure: a reassessment. Plant, Cell and Environment 23: 931–942.

Gifford RM (1977) Growth pattern, carbon dioxide exchange and dry weight distribution in wheat growing under differing photosynthetic environments. Australian Journal of Plant Physiology 4: 99–110.

Goverde M, Arnone JA, Erhardt A (2002) Species-specific reactions to elevated CO_2 and nutrient availability in four grass species. Basic and Applied Ecology 3: 221–227.

Grossman-Clarke S, Pinter Jr PJ, Kartschall T, Kimball BA, Hunsaker DJ, Wall GW, Garcia RL, LaMorte RL (2001) Modelling a spring wheat crop under elevated CO_2 and drought. New Phytologist 150: 315–335.

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347: 1–39.

Hudak C, Bender J, Weigel H, Miller J (1999) Interactive effects of elevated CO_2, O_3, and soil water deficit on spring wheat (*Triticum aestivum* L. cv. Nandu). Agronomie 19: 677–687.

Huxman TE, Hamerlynck EP, Jordan DN, Salsman KJ, Smith SD (1998) The effects of parental CO_2 environment on seed quality and subsequent seedling performance in *Bromus rubens*. Oecologia 114: 202–208.

Jablonski LM, Wang X, Curtis PS (2002) Plant reproduction under elevated CO_2 conditions: a meta-analysis of reports on 79 crop and wild species. New Phytologist 156: 9–26.

Kimball BA, Kobayashi K, Bindi M (2002) Responses of agricultural crops to free-air CO_2 enrichment. Advances in Agronomy 77: 293–368.

Klus DJ, Kalisz S, Curtis PS, Terri JA, Tonsor SJ (2001) Family- and population-level responses to atmospheric CO_2 concentration: gas exchange and the allocation of C, N, and biomass in *Plantago lanceolata* (Plantaginaceae). American Journal of Botany 88: 1080–1087.

Makino A, Mae T (1999) Photosynthesis and plant growth at elevated levels of CO_2. Plant Cell Physiology 40: 999–1006.

Mayeux HS, Johnson HB, Polley HW, Malone SR (1997) Yield of wheat across a subambient carbon dioxide gradient. Global Change Biology 3: 269–278.

McMaster GS, LeCain DR, Morgan JA, Aiguo L, Hendrix DL (1999) Elevated CO_2 increases wheat CER, leaf and tiller development, and shoot and root growth. Journal of Agronomy and Crop Science 193: 119–128.

Mulholland BJ, Craigon J, Black CR, Colls JJ, Atherton J, Landon G (1998) Growth, light interception and yield responses of spring wheat (*Triticum aestivum* L.) grown under elevated CO_2 and O_3 in open-top chambers. Global Change Biology 4: 121–130.

Norby RJ, Jackson RB (2000) Root dynamics and global change: seeking an ecosystem perspective. New Phytologist 147: 3–12.

Pleijel H, Gelang J, Sild E, Danielsson H, Younis S, Karlsson P, Wallin G, Skarby L, Sellden G (2000) Effects of elevated carbon dioxide, ozone and water availability on spring wheat growth and yield. Physiolgia Plantarum 108: 61–70.

Poorter H (1993) Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio 104/105: 77–97.

Pritchard SG, Rogers HH (2000) Spatial and temporal deployment of crop roots in CO_2-enriched environments. New Phytologist 147: 55–71.

Pritchard SG, Rogers HH, Prior SA, Peterson CM (1999) Elevated CO_2 and plant structure: a review. Global Change Biology 5: 807–837.

Samarakoon AB, Muller WJ, Gifford RM (1995) Transpiration and leaf area under elevated CO_2: effects of soil water status and genotype in wheat. Australian Journal of Plant Physiology 22: 33–44.

SAS Institute Inc. (1994) SAS/STAT User's Guide, Release 6.03, 4th Edition. SAS Institute, Inc. Cary, North Carolina.

Schmid B, Birrer A, Lavigne C (1996) Genetic variation in the response of plant populations to elevated CO_2 in a nutrient-poor, calcareous grassland. In: Körner C, Bazzaz FA (eds) Carbon Dioxide, Populations, and Communities. Academic Press, London, pp 31–50.

Schütz M, Fangmeier A (2001) Growth and yield responses of spring wheat (*Triticum aestivum* L. cv. Minaret) to elevated CO_2 and water limitation. Environmental Pollution 114: 187–194.

Thomas SC, Jasienski M (1996) Genetic variability and the nature of microevolutionary responses to elevated CO_2. In: Körner C, Bazzaz FA (eds) Carbon Dioxide, Populations, and Communities. Academic Press, London, pp 51–81.

Thompson RK (1983) Registration of AZ-MSFRS-82RR1 Rust resistant common wheat germplasm. Crop Science 23: 605.

Van der Kooij TAW, De Kok LJ, Stulen I (2000) Intraspecific variation in the response of *Arabidopsis thaliana* lines to elevated atmospheric CO_2. PhytonAnnales Rei Botanicae 40: 125–132.

Villar R, Veneklaas EJ, Jordano P, Lambers H (1998) Relative growth rate and biomass allocation in 20 *Aegilops* (Poaceae) species. New Phytologist 140: 425–437.

Wand SJE, Midgley GF, Jones MH, Curtis PS (1999) Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. Global Change Biology 5: 723–741.

Ward JK, Antonovics J, Thomas RB, Strain BR (2000) Is atmospheric CO_2 a selective agent on model C_3 annuals? Oecologia 123: 330–341.

Ward JK, Tissue DT, Thomas RB, Strain BR (1999) Comparative responses of model C_3 and C_4 plants to drought in low and elevated CO_2. Global Change Biology 5: 857–867.

Wechsung G, Wechsung F, Wall GW, Adamsen FJ, Kimball BA, Pinter Jr PJ, LaMorte RL, Garcia RL, Kartschall T (1999) The effects of free-air CO_2 enrichment and soil water availability on spatial and seasonal patterns of wheat root growth. Global Change Biology 5: 519–529.

Zak DR, Pregitzer KS, King JS, Holmes WE (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytologist 147: 201–222.