GROWTH AND PHYSIOLOGY RESPOND DIFFERENTLY TO ELEVATED CO_2 IN NAD-ME AND NADP-ME C_4 GRASSES.

D. R. LeCain and J. A. Morgan
USDA/ARS Crops Research Lab, 1701 Center Ave.
Fort Collins, Colorado 80526, USA

ABSTRACT

Plants with C_4 photosynthesis have one of three decarboxylation enzymes in their bundle sheath cells. Reports suggest that bundle sheath leakiness to CO_2 is highest in the NAD-ME group, lowest in the NADP-ME group and intermediate in the PCK group. We investigated the hypothesis that growth and photosynthesis of three NAD-ME grasses would respond more to elevated CO_2 than three NADP-ME grasses. Contrary to our hypothesis, growth of NADP-ME grasses was greater under elevated CO_2, while none of the NAD-ME grasses had a significant response. Increased leaf non-structural carbohydrates were associated with greater growth responses of NADP-ME grasses, while none of the NAD-ME grasses had increased carbohydrates. Assimilation versus intercellular CO_2 curves revealed that none of the grasses was photosynthetically saturated with CO_2 at 350 µL L^{-1}. Blue grama (*Bouteloua gracilis*), an NAD-ME species, was unique in displaying photosynthetic acclimation to elevated CO_2 , a trait often seen in C_3 plants.

KEYWORDS

Acclimation, growth, nitrogen, photosynthesis, total non-structural carbohydrates.

INTRODUCTION

While it is generally believed that plants with the C_4 photosynthetic pathway will have negligible photosynthetic and growth responses to high CO_2, there are several reports of significant growth responses to elevated CO_2 in C_4 species (Hunt et al., 1996; Poorter, 1993; Read and Morgan 1996; Riechers and Strain, 1988).

C_4 plants can be sub-divided into three subgroups, based on the C_4 acid decarboxylation enzyme; NADP-ME (nicotinamide adenine dinucleotide phosphate-malic enzyme) , NAD-ME, and PCK (phosphoenolpyruvate carboxykinase). Each subgroup exhibits a unique stable carbon isotope ratio, which relates to the degree of "leakiness" to CO_2 in the bundle sheath cells (Hattersley, 1982). The NADP-ME subgroup is purported to have the tightest bundle sheath cells, NAD-ME the leakiest, and the PCK group is intermediate.

We hypothesized that CO_2 enrichment might stimulate photosynthesis and growth more in NAD-ME species as compared to NADP-ME species due to carboxylation differences in bundle sheath leakiness. We chose three C_4 grass species from each of the extreme leakiness types, NADP-ME and NAD-ME (Hattersley, 1986) and grew them at 350 and 700 µL L^{-1} CO_2 to investigate this.

MATERIALS AND METHODS

Three grass species from the C_4 acid decarboxylation enzyme subgroups NAD-ME and NADP-ME were used in this study. NAD-ME species included Blue grama (*Bouteloua gracilis* Lag. ex Steud), Buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm.) and Switch grass *(Panicum virgatum* L.) and the NADP-ME species were Big bluestem *(Andropogon gerardii* Vittman), Little bluestem *(Schizachyrium scoparium* (Michx.) Nash), and Indian grass *(Sorghastrum nutans* (L.) Nash) (subgroups referenced in Hattersley, 1986). Seeds were germinated on filter paper and sown (three/pot) into 8 L pots which were filled with a 1:1 mixture of sand and Ascalon fine sandy loam soil. Four replicate pots of each species were placed in growth chambers (Environmental Growth Chambers, Chagrin Falls, Ohio, USA) with a CO_2 concentration of either 350 or 700 µL L^{-1} ($\pm$ 30), and a 14 hour photoperiod at 900 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), 28/16 °C day/night temperature and 35/55 % day/night relative humidity. Pots were irrigated with half-strength Hoaglands nutrient solution (Hoagland and Arnon, 1950) every other day and flushed with water weekly.

Plants were grown for 39 days prior to measurements. Assimilation (A) versus leaf intercellular CO_2 (C_i) measurements were performed using the ADC (Analytical Development Company LTD., Hoddesdon, Herts. England) LCA-3 photosynthesis system at leaf chamber CO_2 concentrations of 50, 100, 200, 350, 500, 700 and 900 µL L^{-1}. Measurement light was 1900 mmol m^{-2} s^{-1}, with a temperature of 28 °C; humidity was not controlled.

Leaves similar to those in the chamber were sampled for carbohydrate analysis (Hendrix, 1993). Upon completion of photosynthesis measurement each pot was harvested. Newly-formed leaves were used for total Kjeldahl nitrogen analysis (Schuman et al., 1973). The remainder of each plant was then cut at the soil surface and soil was washed from the roots. Leaf, stem and root samples were dried at 60 °C for growth analysis.

RESULTS AND DISCUSSION

When analyzed across species within each C_4 acid decarboxylation enzyme group, shoot, root, and total plant dry weight of the NADP-ME species were significantly enhanced by increasing CO_2 from 350 to 700 µL L^{-1}, whereas growth of NAD-ME species was unresponsive to CO_2 (Table 1). Thus, our hypothesis that C_4 species with the NAD-ME decarboxylation enzyme would have a greater growth response to elevated CO_2 than NADP-ME species was not supported. Analysis on a species basis revealed that two of the NADP-ME species, Big bluestem and Indian grass, had significant ($P<0.05$) increases in shoot, root and total plant dry weights when grown at elevated CO_2 (data not shown). There was also a trend for greater shoot dry weight in the NADP-ME grass Little bluestem ($P = .11$). None of the NAD-ME species had a significant growth response to high CO_2. A significant reduction in root to shoot ratio under elevated CO_2 was seen in Switchgrass and Little bluestem (data not shown), but within enzyme subgroup there was no significant difference in root to shoot ratios.

An analysis of C_i response curves of A revealed that photosynthesis of none of the six species was saturated at present ambient CO_2 concentrations. Consistent with previous work (Morgan et al. 1994, Read et al. 1996), C_i response curves of Blue grama displayed photosynthetic acclimation to growth at elevated CO_2. A lower initial slope and a lower plateau were seen in A:Ci curves of high CO_2 grown Blue grama (Fig. 1). The other grasses showed no photosynthetic acclimation. At high C_i Big bluestem plants grown in elevated CO_2 had higher photosynthesis rates than low CO_2 grown plants (Fig. 1). Big bluestem also had the largest growth response to high CO_2.

Total non-structural carbohydrates in leaves sampled during the photosynthesis measurements were greater in all three NADP-ME species grown at elevated CO_2 (Table 1), but were unaffected by CO_2 in the NAD-ME grasses. However, while leaf N concentrations appeared greater in the NAD-ME species, neither photosynthetic group showed a response of leaf N to CO_2 growth regime.

Our hypothesis that bundle sheath leakiness would affect the growth response of C_4 plants to elevated CO_2 was not supported in

this study. In what way the decarboxylation enzyme NADP-ME is related to greater growth at elevated CO_2 is unclear at this time. In general, the species which had a positive growth response to elevated CO_2 had significantly greater leaf carbohydrates. This response is commonly reported in C_3 plants, but there have been few reports with C_4's (Read and Morgan, 1996). It is interesting that the accumulation of leaf carbohydrates, often correlated with the occurrence of CO_2-induced photosynthetic acclimation in C3 species (Sage, 1994), occurred in plants whose photosynthesis rates appeared unaffected by long-term growth at elevated CO2. It is also interesting that leaves of Blue grama, which clearly displayed photosynthetic acclimation, showed no changes in leaf metabolite levels due to growth at high CO_2.

REFERENCES

Hattersley, P.W. 1982. d^{13} values of C_4 types in grasses. Aust. J. Plant. Physiol. **9**:139-154.

Hattersley, P.W. 1986. Variations in photosynthetic pathway. Int. Grass Symp. 1986:49-64.

Hendrix, D.L. 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. Crop Science **33**:1306-1311.

Hoagland, D.R. and D.I Arnon 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347.

Hunt, H.W., Elliott, E.T., Detling, J.K., Morgan, J.A., and Chen, D.-X. 1996. Responses of a C_3 and a C_4 perennial grass to elevated CO_2 and climate change. Global Change Biology. **2**:35-47.

Morgan, J.A., H.W. Hunt, C.A. Monz and D.R. LeCain 1994. Consequences of growth at two carbon dioxide concentrations and two temperatures for leaf gas exchange in *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4).

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

Read, J.J., J.A. Morgan, N.J. Chatterton and P.A. Harrison 1996. Gas exchange and carbohydrate and nitrogen concentrations in leaves of *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) at different carbon dioxide concentrations and temperatures. Annals of Botany (in review).

Read, J.J. and J.A. Morgan 1996. Growth and partitioning in *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) as influenced by carbon dioxide andtemperature. Annals of Botany **77**:487-496.

Riechers, G.H. and B.R. Strain 1988. Growth of blue grama *(Bouteloua gracilis)* in response to atmospheric CO_2 enrichment. Can. J. Bot. **66**:1570-1573.

Sage, R.F. 1994. Acclimation of photosynthesis to increasing atmospheric CO_2: The gas exchange perspective. Photosynthesis Research 39:351-368.

Schuman, G.E., M.A. Stanley and D. Knudsen 1973. Automated total nitrogen analysis of soil and plant samples. Soil Sci. Soc. Amer. J. **37**:480-481.

Table 1

Shoot dry weight, root dry weight, total dry weight, root to shoot ratio, leaf nitrogen concentration (per structural dry weight) and leaf non-structural carbohydrates of three NAD-ME and three NADP-ME C_4 species pooled by decarboxylation enzyme subgroup (n=12). Plants were grown at either 350 or 700 mL L^{-1} CO_2 for 39 days prior to harvest.

CO_2 trt.	NAD-ME	NADP-ME
	Shoot dry weight (g)	
350 mL L^{-1}	5.7	2.1
700 mL L^{-1}	5.7	3.9
P > f	0.97	0.033
	Root dry weight (g)	
350 mL L^{-1}	3.1	1.8
700 mL L^{-1}	2.7	3.0
P > f	0.52	0.062
	Total dry weight (g)	
350 mL L^{-1}	8.8	3.9
700 mL L^{-1}	8.5	7.0
P > f	0.78	0.04
	Root to shoot ratio	
350 mL L^{-1}	.57	.92
700 mL L^{-1}	.50	.78
P > f	0.31	0.11
	Leaf non-structural carbohydrates (g kg^{-1})	
350 mL L^{-1}	182	107
700 mL L^{-1}	183	207
P > f	0.97	0.001
	Leaf nitrogen (g kg^{-1})	
350 mL L^{-1}	43.1	30.5
700 mL L^{-1}	41.5	30.0
P > f	0.54	0.67

FIGURE 1