BNL-73319-2004-CP

The response of plant carbohydrates to elevated CO_2. What have we learnt from FACE Studies?

Alistair Rogers[1,2], Elizabeth A. Ainsworth[3], Carl J. Bernacchi[4], Yves Gibon[5], Mark Stitt[5], Stephen P. Long[2,6]

[1] Environmental Sciences Department, Brookhaven National Laboratory, Upton, NY 11973, USA.

[2]Department of Crop Sciences, University of Illinois at Urbana Champaign IL 61801, USA

[3]Photosynthesis Research Unit, USDA/ARS, University of Illinois at Urbana Champaign IL 61801, USA

[4]Illinois State Water Survey, University of Illinois at Urbana Champaign IL 61801, USA.

[5]Max-Planck-Institut für Molekulare Pflanzenphysiologie, 14424 Postsdam, Germany.

[6]Department of Plant Biology, University of Illinois at Urbana Champaign IL 61801, USA

Keywords: Carbon, Nitrogen, source-sink balance, FACE

INTRODUCTION

Atmospheric CO_2 concentration ([CO_2]) is expected to rise from a current level of 372 μmol mol^{-1} to about 550 μmol mol^{-1} by the middle of the century (Prentice 2001). Accumulation of foliar carbohydrates is one of the most pronounced and universal changes observed in the leaves of C_3 plants grown at elevated [CO_2] (Drake et al 1997). Carbohydrates are the product of photosynthetic cells and the substrate for sink metabolism. However, carbohydrates are not just substrates, changes in the composition and pool size of foliar carbohydrates have the potential to communicate source-sink balance and a role for carbohydrates in the regulation of the expression of many plant genes is well established (Koch 1996). Importantly, carbohydrate feedback is thought to be the mechanism through which long-term exposure to elevated [CO_2] leads to a reduction in carboxylation capacity (Rogers et al 1998, Long et al 2004). Foliar sugar content has recently been linked to an increased susceptibility of soybeans to insect herbivory (Hamilton et al submitted). In addition increases in the C:N ratio of leaf litter of plants grown at elevated [CO_2] has been implicated in negative feedbacks on ecosystem productivity (Oechel et al 1994). Understanding of the response of foliar carbohydrates will form an important part of our ability to understand and predict the effects of rising [CO_2]

on plants and ecosystems.

As Free-Air CO_2 enrichment technology was emerging, understanding of the link between carbohydrates and plant responses to rising [CO_2] was increasing. However, there were concerns that the hypotheses generated using model system or from studies on mostly juvenile plants grown for relatively short periods of time in controlled environments may not translate to the field. Of particular concern was the effect of a limited rooting volume. Arp (1991) argued that photosynthetic acclimation to elevated [CO_2] was largely an artifact of rooting volume that led to a sink limitation of photosynthesis at elevated [CO_2]. It has been suggested that this "pot effect" was the result of exacerbated nutrient depletion (Korner, 2003), but there is also evidence that physical restriction of root development can cause these feedbacks (Masle et al 1990, Thomas & Strain 1991). Since carbohydrate feedback mechanisms were thought to underlie this response it was unclear whether hypotheses developed in controlled environments would hold up when tested in the field. The central hypothesis around which the uncertainty rested was that the capacity of sinks to utilize the increased carbon supply produced at elevated [CO_2] will determine the response of foliar carbohydrates to growth at [CO_2]. The advent of FACE technology allowed this hypothesis to be tested in the field in open-air conditions where plants lack the constraints that have been considered to limit the response of C_3 plants to rising [CO_2].

MATERIALS AND METHODS

The FACE system for exposing vegetation to elevated [CO_2] in the field has been described previously (Hendrey et al 1999; Miglietta et al 1997; Long et al 2004). Succinctly, an approximately circular plot at least 8 m in diameter is surrounded by a ring of pipes that release CO_2 or air enriched with CO_2 at vertical intervals just above the ground to just above the top of the canopy. Wind direction, velocity and CO_2 concentration are measured at the center of each plot, this information is used to control the flow rate and release point of CO_2 in order to maintain a set point of around 550 $\mu mol\ mol^{-1}$. Foliar carbohydrate content were measured as described by Rogers and Ellsworth (2002) and Rogers et al (2004). Data are presented from work conducted at the SoyFACE experiment. A description of the site and details of the statistical methods used are described by Rogers et al (2004).

RESULTS & DISCUSION

Do plants grown in the field using FACE technology accumulate carbohydrates in their leaves?

A meta analysis of the response of foliar carbohydrates to growth at elevated [CO_2] using FACE technology showed that for 30 independent studies and four different species, foliar sugar content was on average c. 35% ($P<0.05$) higher and starch content c. 85% higher ($P<0.05$) in plants grown at elevated [CO_2]. Field grown N-fixing soybean, with indeterminate flowering might be expected to avoid accumulating foliar carbohydrates because of its strong sinks for photosynthate. However, soybean had significantly ($P<0.05$) higher levels of predawn glucose, sucrose and most markedly starch when grown at elevated [CO_2] <Figure 1>. When combined to give total nonstructural carbohydrate (TNC) content (Figure 1) it is clear that the increase in foliar carbohydrate content is restricted to the beginning and end of the growth season. In the middle of the season there is a strong sink for photosynthate and this may explain the low overall levels of TNC at that time of the year and the lack of a CO_2 effect at this time. The mean night-time temperature preceding the dawn harvests was not significantly different on the days of measurement ($P=0.38$, data not shown) and the daily integral of CO_2 uptake did not appear to effect TNC accumulation (Bernacchi, personal communication). Rogers et al (2004) reported similar trends for a different cultivar of soybean also grown in the field using FACE technology.

Source-sink balance

Lolium perenne is adapted to survive periodic partial defoliation and is managed as a frequently cut herbage crop. Partial defoliation (cutting) abruptly decreases the ratio of photosynthetic tissue to sinks for photoassimilates (roots, pseudo-stems). Leaves in partially defoliated plants will have a greater demand for their photosynthate than similar leaves on intact plants. The accumulation of carbohydrate in source leaves of *Lolium perenne* grown at elevated [CO_2] was eliminated by reducing the source-sink ratio through partial defoliation (Rogers et al 1998). Manipulation of source-sink balance through single gene mutations in soybeans also demonstrated that genetic constraints on sink capacity can result in carbohydrate accumulation at elevated [CO_2]. Soybeans with a determinant growth form accumulated foliar carbohydrates to a greater extent at elevated [CO_2] than identical germplasm with an indeterminate growth form (Ainsworth et al 2003). When we studied loblolly pine grown at elevated [CO_2] we hypothesized that when proximal sink development was maximal carbohydrate accumulation in needles on pines grown at elevated [CO_2] would be minimal. However, carbohydrate accumulation in loblolly pines grown at elevated

[CO_2] did occur despite strong proximal sink development suggesting that distal sinks or interactions with N could be more important in that system (Rogers & Ellsworth, 2002).

Interaction of elevated CO_2 and N supply

When *Lolium perenne* was grown at two N-supply levels and managed as a frequently cut herbage crop it was found that just before a cut plants grown at elevated [CO_2] with a low N supply had a large accumulation of foliar carbohydrates compared to current [CO_2] controls but that plants grown at elevated [CO_2] with a high N supply did not have a significant accumulation of foliar carbohydrates when compared with their current [CO_2] controls (Rogers et al 1998). Later in the course of the overall Swiss FACE experiment we found that just before a cut, plants grown at low N began to accumulate significant amounts of foliar carbohydrate over a 24h time course whereas plants grown at high N did not, this accumulation was exacerbated at elevated [CO_2], clearly demonstrating the importance of N supply on carbohydrate accumulation.

Supply of carbon can also affect nitrogen status. Soybeans grown at elevated [CO_2] had a significantly ($P<0.05$) lower leaf protein content but this effect was confined to the beginning of the season. Later in crop development levels at elevated [CO_2] matched those at current [CO_2] <Figure 2> (Figure 2). The supply of ureides from N-fixation peaked in the middle of the season. At this time plants grown at elevated [CO_2], that had a greater supply of carbon skeletons from photosynthesis (Bernacchi, personal communication) and that showed no evidence of carbohydrate accumulation (Figure 1), had significantly ($P<0.01$, data not shown) lower levels of foliar ureide content. Concomitantly the ratio of free minor amino acids at elevated [CO_2] to that at current [CO_2] was significantly greater (data not shown). We hypothesize that the extra photosynthate available at elevated [CO_2] allowed the plants to better exploit the high levels of ureide that became available in the middle of the season to make amino acids and raise their leaf protein content.

Conclusion

Despite predictions that field grown plants would not accumulate foliar carbohydrates at elevated [CO_2] we found that an increased carbohydrate content at elevated [CO_2] was commonly observed and that an exacerbated

accumulation of foliar carbohydrates at elevated [CO_2] was consistent with our hypothesis that sink capacity determines the response of foliar carbohydrates to growth at elevated [CO_2].

Acknowledgments—AR was supported by the U.S. Department of Energy Office of Science Contract No. DE-AC02-98CH10886 to Brookhaven National Laboratory.

REFERENCES

Ainsworth EA, Rogers A, Nelson R, Long SP (2004) Testing the "source-sink" hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with single gene substitutions in *Glycine max*. *Agric. For. Meteorol.*. **122**, 85-94.

Arp WJ (1991) Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. *Plant Cell Environ.* **14**, 869-875.

Drake BG, Gonzàlez-Meler MA, Long SP (1997) More efficient plants: A consequence of rising atmospheric CO_2? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48,** 609-639.

Hamilton JG, Dermody O, Aldea M, Zangerl AR, Rogers A, Berenbaum M, DeLucia EH (2004) Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. *Environmental Entomology*. Submitted.

Hendrey GR, Ellsworth DS, Lewin KF, Nagy J (1999) A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biol.* **5**,293-309.

Koch KE. (1996) Carbohydrate-modulated gene expression in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 509-540.

Korner C. (2003) Nutrients and sink activity drive plant CO_2 responses-caution with literature-based analysis. *New Phytol.* **159**, 537-538.

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: Plants FACE the future. *Annu Rev. Plant Biol.* **55**, 591-628.

Masle J, Farquhar GD, Gifford RM. (1990) Growth and carbon economy of wheat seedlings as affected by soil resistance to penetration and ambient partial-pressure of CO_2. *Aust. J. Plant Physiol.* **17,** 465-487.

Miglietta F, Lanini M, Bindi M, Magliulo V (1997) Free air CO_2 enrichment of potato (Solanum tuberosum, L.): design and performance of the CO_2-fumigation system. *Global Change Biol.* **3**, 417-427.

Oechel WC, Cowles S, Grulke N, Hastings SJ, Lawrence B, et al. (1994) Transient nature of CO_2 fertilization in Arctic Tundra. *Nature* **371**, 500-503.

Prentice I, Farquhar G, Fasham M, Goulden M, Heinmann M, et al. (2001) The carbon cycle and atmospheric carbon dioxide. In *Climate Change 2001: The Scientific Basis. Contributions of Working Group I to the Third*

Assessment Report of the Intergovernmental Panel on Climate Change, ed. JT Houghton, Y Ding, DJ Griggs, M Noguer, PJ van der Linden, et al. pp. 183-238. Cambridge, UK: Cambridge Univ. Press.

Rogers A, Ellsworth DS (2002) Photosynthetic acclimation of Pinus taeda (loblolly pine) to long-term growth in elevated pCO_2 (FACE). *Plant Cell Environ.* **25**, 851-858

Rogers A, Fischer BU, Bryant J, Frehner M, Blum H, Raines CA, Long SP (1998) Acclimation of photosynthesis to elevated CO_2 under low N nutrition is effected by the capacity for assimilate utilisation. Perennial Ryegrass under Free-Air-CO_2 Enrichment (FACE). *Plant Physiol.* **118**, 683-689.

Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ, Cornic G, Dermody O, Heaton EA, Mahoney J, Zhu X-G, DeLucia EH, Ort DR, Long SP (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide Enrichment. *Plant Cell Environ.* **27**, 449-458.

Thomas RB, Strain BR (1991) Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon-dioxide. *Plant Physiol.* **96**, 627-634.

FIGURE LEGENDS

Figure 1. Total non-structural carbohydrate content calculated as the sum of glucose, fructose, sucrose and starch measured in the lateral leaflets of the most recently fully expanded trifoliate leaves of soybeans grown at elevated [CO_2] in the field using Free-Air CO_2 Enrichment technology to raise the concentration of CO_2 from 370 to 550 μmol mol^{-1}. Samples were taken just before sunrise on three consecutive mornings and the values within each replicate plot pooled to give a mean predawn TNC content for each period of measurement. Data are mean ± lsmean for the 4 replicate control (current) and elevated [CO_2] (elevated) plots. Horizontal bars indicate the periods of vegetative growth, flowering, pod fill and seed fill. The broken vertical line indicates the stage in development when N-fixation stops.

Figure 2. Leaf protein content of soybeans grown at current and elevated [CO_2] as described in Figure 1. Data are expressed on a dry weight basis corrected for the mass of TNC.

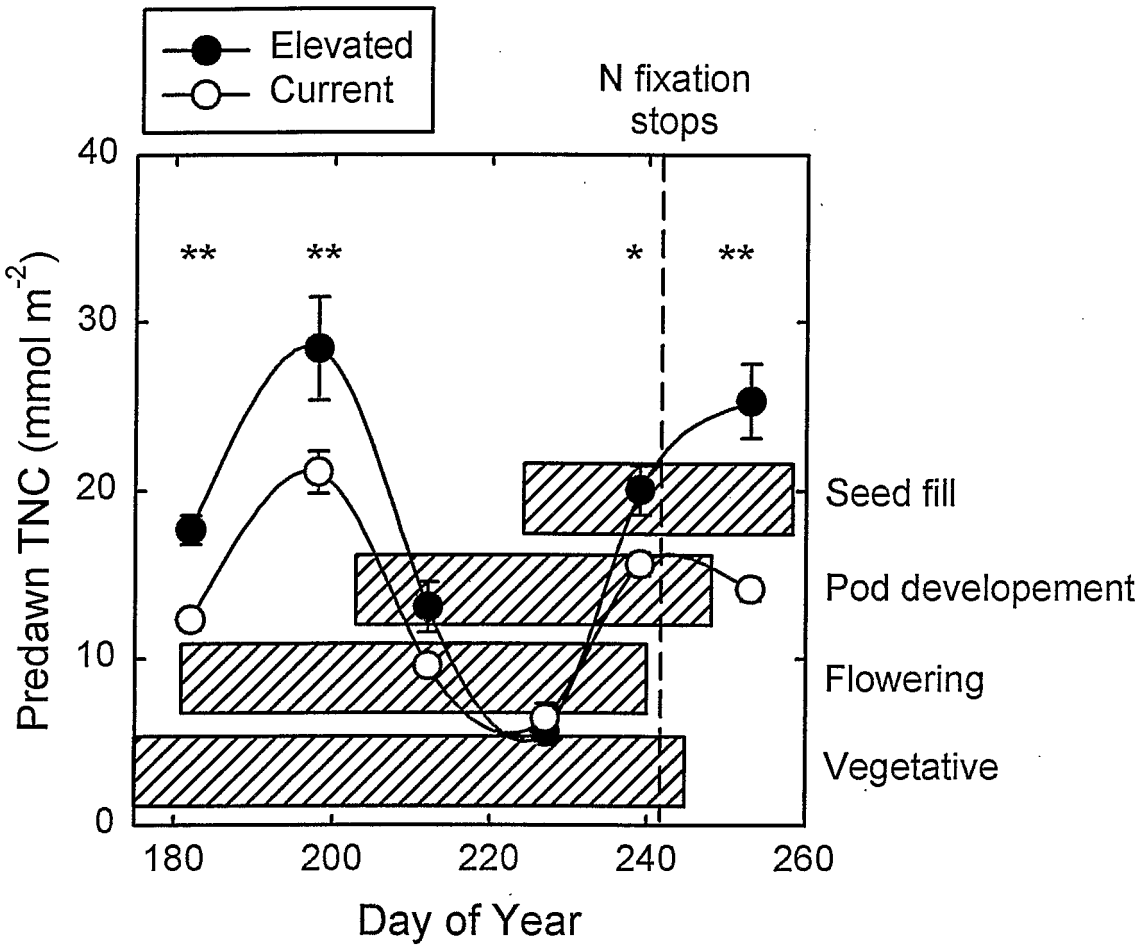
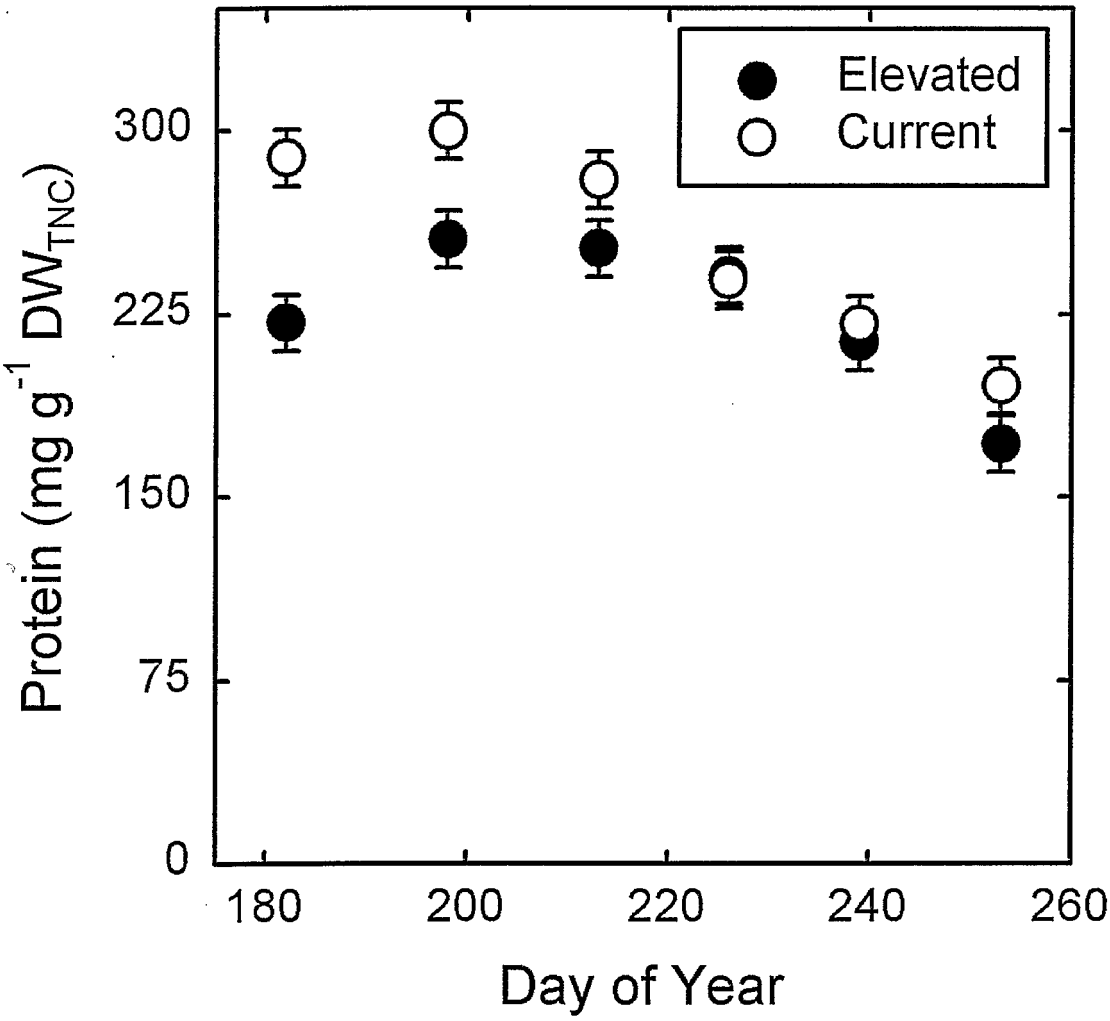

Elevated
Current
N fixation stops
Predawn TNC (mmol m-2)
40
30
20
10
0
**
**
*
**
Seed fill
Pod developement
Flowering
Vegetative
180
200
220
240
260
Day of Year

Figure 1

Figure2


Elevated
Current
300
225
150
75
0
180
200
220
240
260
Protein (mg g-1 DWTNC)
Day of Year