16 The Response of Foliar Carbohydrates to Elevated [CO_2]

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16.1 Introduction

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 concentration (e[CO_2]). Carbohydrates are both the product of photosynthetic cells and the substrate for sink metabolism. However, carbohydrates are not just substrates; and the role of carbohydrates in regulation of the expression of many plant genes, and the activity of many key enzymes, is well established. As free air CO_2 enrichment (FACE) technology was emerging, understanding of the link between carbohydrates and plant responses to growth at e[CO_2] was increasing. However, it remained unclear whether the hypotheses that were being refined in model systems would hold up when tested in open-air field experiments. More than a decade of FACE experiments have provided the answer.

16.1.1 Why is it Important to Understand the Response of Foliar Carbohydrates to Growth at e[CO_2]?

Sucrose is the main product of photosynthesis and in most plants is the main form of translocated carbon (Farrar et al. 2000). Starch and (in the Gramineae) fructan are transitory foliar storage pools for photosynthate, although in some species vacuolar sucrose is the dominant storage carbohydrate (Chatterton et al. 1989; Pollock and Cairns 1991; Zeeman et al. 2004). Due to the relative instability of glucose and fructose and the osmotic problems associated with storing large quantities of hexose, the levels of sucrose and the storage polysaccharides are generally much higher than the levels of free hexose (Isopp et al. 2000b; Rogers et al. 2004).

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Sugars are more than merely substrates and products; and they have important signaling functions throughout all stages of the plant's life cycle. The evidence for a regulatory role for hexoses and sucrose is overwhelming (Sheen 1990; Koch 1996; Bush 1999; Moore et al. 1999; Farrar et al. 2000; Smeekens 2000). Of particular significance is the well characterized feedback inhibition of photosynthetic genes by glucose and sucrose (Krapp et al. 1993; Van Oosten and Besford 1994; Jones et al. 1996; Pego et al. 2000).

The carbohydrate composition of foliage can also have effects beyond the plant. High carbohydrate content is often associated with high flavanoid content (Lindroth 1996). This has implications for plant–herbivore interactions since flavanoids are feeding deterrents for many herbivores. In wheat grown at e[CO_2] using FACE technology, Estiarte et al. (1999) reported that growth at e[CO_2] led to an increased foliar carbohydrate content and elevated flavanoid levels. Hendrix et al. (1994) found increased carbohydrate levels in cotton grown at e[CO_2] and also observed evidence of elevated flavanoid levels. Conversely, Hamilton et al. (2005) showed that soybean grown at e[CO_2] had a higher sugar content and was more susceptible to herbivory by Japanese beetles, for which sugars are a known phagostimulant. Also of particular significance to managed ecosystems is evidence that carbohydrate content may effect herbage palatability. Increased water-soluble carbohydrate content in herbage grown at e[CO_2] correlated positively with an increased organic matter digestibility in ruminants (Allard et al. 2003).

It is clear that an understanding of the response of foliar carbohydrates to growth at e[CO_2] is important if we aim to increase our knowledge of how ecosystems will respond to future e[CO_2] environments.

16.1.2 What Were the Known Effects of e[CO_2] on Foliar Carbohydrates Before FACE?

Accumulation of foliar carbohydrates is one of the most marked and widely observed changes in the leaves of C_3 plants grown at e[CO_2] (Farrar and Williams 1991). The most pronounced increases are in the levels of sucrose and the transient storage polysaccharides, starch and fructan. Long and Drake (1992) summarized the response of foliar carbohydrates to growth at e[CO_2] and found large and significant increases in sucrose and starch content in plants grown at e[CO_2].

In the early 1990s, strong evidence was emerging for the role of sugars in the down-regulation of photosynthetic genes (Sheen 1990); and this mechanism offered an attractive explanation for the emerging reports of a loss of photosynthetic capacity observed in plants grown for extended periods at e[CO_2] where there was also a large accumulation of sucrose (Long and Drake 1992). At this time, a special issue of *Plant Cell and Environment* (vol 14(8), 1991) was published that summarized the current, and predominantly pre-

FACE, knowledge of the response of plants to e[CO_2]. Contributions to this special issue from Stitt (1991), Farrar and Williams (1991) and Arp (1991) summarized the current knowledge of the response of plant carbohydrates to e[CO_2] and emphasized the importance of understanding the role of source–sink relations. Stitt (1991) assessed the evidence for a sink limitation of photosynthesis at e[CO_2] and concluded that the long-term ability of a leaf to maintain high photosynthetic rates is dependent on the source–sink status of the whole plant, i.e. a sustained stimulation of photosynthesis at e[CO_2] is dependent on an adequate sink capacity for the extra photosynthate produced at e[CO_2]. In the same special issue, Arp (1991) also examined the link between source–sink relations and photosynthetic acclimation and concluded that photosynthetic down-regulation was likely an artifact resulting from growing plants with a restricted rooting volume. There was also evidence that physical restriction of root development can cause these feedbacks (Masle et al. 1990; Thomas and Strain 1991). Implicit in Arp's conclusion was the assumption that a marked accumulation of carbohydrates at e[CO_2] was also an artifact of a restricted sink capacity.

One of the major problems in confidently extrapolating results from controlled environments to the field was the problem of the "pot effect" (Arp 1991). Whilst carbohydrate accumulation was less marked in plants grown with larger rooting volumes (Long and Drake 1992), it was unclear whether the response of foliar carbohydrates to e[CO_2] would prevail in an unlimited rooting volume. Since carbohydrate feedback mechanisms were thought to underlie some important responses of plants to e[CO_2], a truly realistic growth environment was needed in order to test hypotheses developed in controlled environments. The central hypothesis around which much of the uncertainty rested was: *The accumulation of foliar carbohydrates at e[CO_2] is the result of an insufficient sink capacity to utilize the extra photosynthate produced at e[CO_2].*

The advent of FACE technology allowed this hypothesis to be tested in the field in fully open-air conditions where plants lack the constraints that have been implicated as artifacts in many controlled environment studies (Long et al. 2004).

16.2 Do Carbohydrates Accumulate in the Leaves of Plants Grown in the Field Using FACE Technology?

Recent meta-analyses of plant responses to growth at e[CO_2] using FACE technology included an analysis of the response of foliar carbohydrates (Fig. 16.1; Ainsworth and Long 2005; Long et al. 2004). Despite an unrestricted rooting volume, plants grown at e[CO_2] accumulated significantly more sugars and starch than those plants grown at current (c)[CO_2]. In a review that

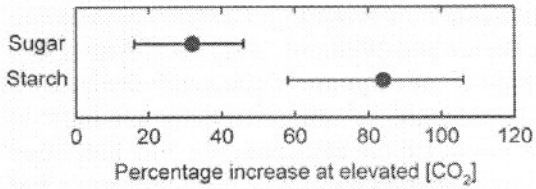

Fig. 16.1 Meta-analysis. The percent increase in the sugar and starch content per unit leaf area in plants grown at e[CO_2] using FACE technology ±95 % confidence interval, n=30 independent observations. Figure redrawn from Long et al. (2004)

preceded most of the work that emanated from FACE studies, Long and Drake (1992) examined the response of plants to e[CO_2] and summarized the impact of rooting volume on carbohydrate accumulation. They compared the ratio of starch in plants grown at e[CO_2] to starch in plants grown at c[CO_2]. They found that plants grown at e[CO_2] had a markedly higher starch content when grown in small pots compared with large pots (small pots, e/c = 3.4; large pots, e/c = 2.2; Long and Drake 1992). The meta-analytical summary of Ainsworth and Long (2005) found sugar and starch accumulation to be markedly lower in plants grown using FACE than in the plants grown in large pots (>10 dm^3) in Long and Drake's (1992) study. This observation is consistent with the results of Robbins and Pharr (1988), who showed that plants grown in a small rooting volume accumulated more starch. However, the trend for reduced carbohydrate accumulation with increased rooting volume may reflect the higher [CO_2] in the studies summarized in Long and Drake's review (range 500–2000 μmol mol^{-1}) compared with that of Ainsworth and Long (range 475–600 μmol mol^{-1}). Another confounding factor is the data in Long and Drake (1992) were expressed on a dry mass basis, which tends to underestimate carbohydrate content. However, despite growing plants in the field where roots were free to develop and forage for nutrients, there was still a marked and significant increase in foliar carbohydrate content in plants grown at e[CO_2].

Soybean offers perhaps the ultimate test of Arp's prediction that field-grown plants will not become sink-limited (Arp 1991). In addition to the unrestricted root development possible in the field, soybean may have an indeterminate vegetative growth pattern and an association with nitrogen-fixing bacteria that significantly increases the sink for photosynthate (Walsh et al. 1987; Vessey et al. 1988). Despite these strong sinks for photosynthate, soybean grown under e[CO_2] in the field still accumulated significantly more glucose, sucrose and most markedly starch (P<0.05, data not shown). Figure 16.2 shows the level of total non-structural carbohydrate (TNC; sum of glucose, fructose, sucrose and starch) in mature soybean leaves sampled at six stages of development within the growth season. Despite a near constant stimulation of diurnal photosynthetic CO_2 uptake (Bernacchi et al., unpublished data), there was a clear trend in TNC content, peaking at the beginning

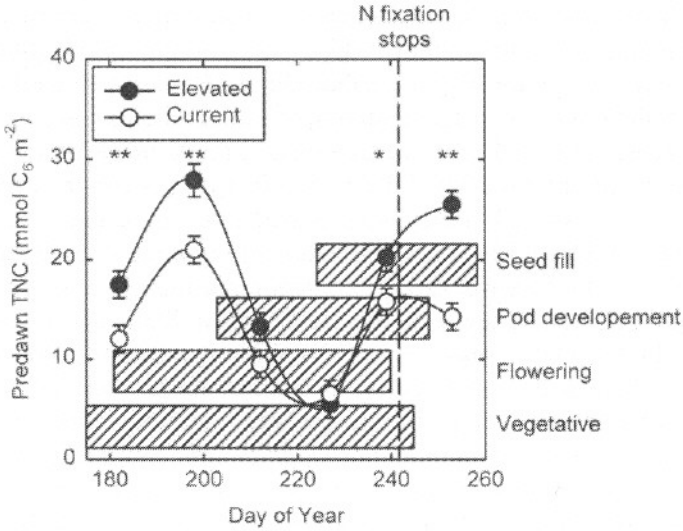

Fig. 16.2 The carbohydrate content of developing soybean grown at the SoyFACE experiment. TNC content calculated as the sum of glucose, fructose, sucrose and starch measured in the lateral leaflets of the most recently fully expanded trifoliate leaves of soybeans grown at e[CO_2] in the field using FACE technology. Samples were taken just before sunrise on three consecutive mornings; and the pre-dawn values for each replicate plot were pooled to give a mean pre-dawn TNC content for each period of measurement (n=4 replicate rings). 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. Across the season, there was a significant 39 % increase in TNC (F=62.73, P=0.0042). The date of measurement was significant (F=42.35, P<0.0001) and there was also a significant interaction between CO_2 treatment and day of year (F=4.24, P=0.0051). *Asterisks* indicate a significant pairwise comparison between elevated and ambient [CO_2] treatments on a specific day of year (* P<0.05, ** P<0.01)

and end of the growth season. At these stages of development, the increase in foliar carbohydrate content at e[CO_2] was maximal. The higher amounts of TNC at the beginning and end of the growth season and the significant CO_2 effect at these times correspond to developmental changes in the source–sink balance. In the middle of the season when TNC levels are lowest and there is no effect of growth at e[CO_2], there is a strong sink for photosynthate. Nitrogen fixation is peaking, vegetative development, flowering and pod set are still underway and seed fill is just beginning (Ritchie et al. 1997). The results presented here are supported by data from the same field site taken in a preceding year on a different cultivar (Rogers et al. 2004). It is clear that carbohydrate accumulation at e[CO_2] occurs even in a plant that lacks the constraints thought to exacerbate carbohydrate accumulation in controlled environments. It is also evident from Fig. 16.2 that sink capacity may be determining the extent and timing of carbohydrate accumulation.

Trees have large sinks for photosynthate and may be expected to avoid foliar carbohydrate accumulation at e[CO_2]. Developing loblolly pines experiencing a step change in [CO_2] at the Duke Forest FACE experiment (Hendrey et al. 1999) did not show an accumulation of carbohydrates when measured at multiple stages during the first season of CO_2 exposure (Myers et al. 1999). Rogers and Ellsworth (2002) did report foliar carbohydrate accumulation later in the experiment, but this was confined to old needles at two points in the season. Herrick and Thomas (2001) did not report carbohydrate accumulation in sun or shade leaves of *Liquidambar styraciflua* (sweetgum) growing at e[CO_2] in the understory at the Duke Forest FACE site (Herrick and Thomas 2001). However, Tissue et al. (2002) did report carbohydrate accumulation at e[CO_2] in the same species at the Oak Ridge National Laboratory FACE site (Norby et al. 2001); and Singaas et al. (2000) reported carbohydrate accumulation in *Acer rubrum*, *Ceris canadensis* and *L. styraciflua* at the Duke site. Clearly carbohydrate accumulation in trees is highly variable and our understanding of the mechanisms underlying the response of foliar carbohydrates to e[CO_2] in trees needs to be increased.

16.3 Manipulations of Source–Sink Balance

One way to test the hypothesis that insufficient sink capacity is causing foliar carbohydrate accumulation is to artificially manipulate the source–sink ratio in order to increase the demand for photosynthate. A simple way to do this is to remove source tissue. By decreasing the amount of photosynthetic tissue, the demand for photosynthate will need to be met by fewer source leaves and the remaining leaves will experience an increase in sink strength. The management practice at the Swiss FACE site (Zanetti et al. 1996) afforded an opportunity to study carbohydrate dynamics following partial defoliation.

Perennial ryegrass is a major C_3 pasture grass of humid and temperate regions that has been selected to be grazed and therefore survive periodic partial defoliation. At the Swiss FACE site, ryegrass was managed as a frequently cut herbage crop. The periodic defoliation abruptly decreased the ratio of source (i.e. photosynthetic tissue) to sink (i.e. roots and pseudostems) and in addition led to an increased demand for photosynthate during the regrowth period.

Immediately following partial defoliation, the carbohydrate content in the remaining leaf tissue was markedly reduced; and this reduction continued until about 4 days after defoliation, at which time levels began to rise until they peaked just before the next cutting cycle (Fischer et al. 1997). The effect of source–sink manipulation on carbohydrate accumulation is clear; and the trend is exacerbated at e[CO_2]. Foliar carbohydrate accumulation was significantly greater at e[CO_2] immediately before partial defoliation, but following

defoliation the newly developed foliage showed no carbohydrate accumulation at e[CO_2], consistent with the greater demand for photosynthate following defoliation (Fischer et al. 1997; Rogers et al. 1998). Isopp et al. (2000b) showed that the diurnal changes in TNC were largely associated with changes in sucrose content, but the long-term increases in TNC immediately before defoliation, that were exacerbated at e[CO_2], were associated with a marked accumulation of fructan, indicative of an insufficient demand for photosynthate (Fig. 16.3; Isopp et al. 2000b). Rogers et al. (1998) cut one section of the sward early and measured carbohydrate content in both defoliated and undefoliated swards on the same day. They confirmed that the difference in carbohydrate content following a cut was not due to different meteorological conditions on or preceding the day of measurement (Rogers et al. 1998).

Further support for the major role of sink capacity in determining the response of foliar carbohydrates to e[CO_2] comes from the SoyFACE experiment (see Chapter 4), where Ainsworth et al. (2004) grew isogenic lines of soybean that varied by a single gene altering their capacity to utilize photosynthate. Indeterminate soybean cultivars continue vegetative growth after flowering has begun but determinate cultivars do not. Since continued vegetative growth will provide an additional sink for photosynthate, cultivars with a

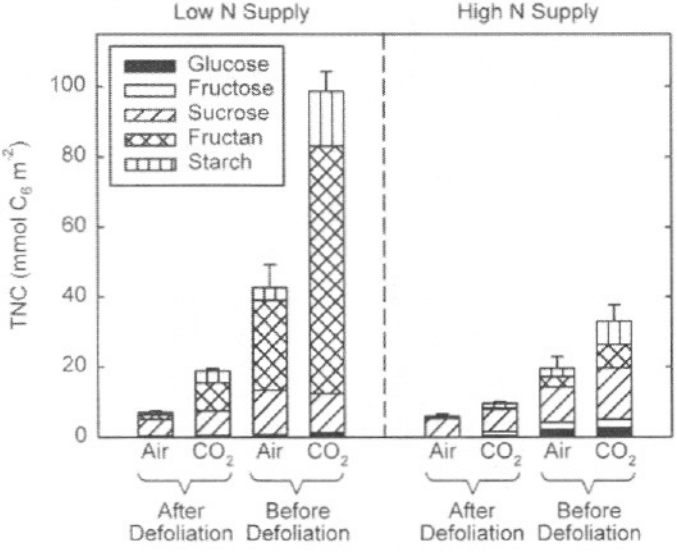

Fig. 16.3 Composition of TNC in the leaves of *Lolium perenne* grown at the Swiss FACE site. Leaves were sampled from plants grown with a high and low nitrogen supply in control (Air) and elevated [CO_2] plots (CO2). Samples were taken at 0700 hours, shortly after and shortly before a planned partial defoliation. Plants harvested shortly after a cut had a markedly lower source:sink ratio than those harvested shortly before defoliation. Error bars represent ±SE of mean TNC (n=3 plots). Figure redrawn from Isopp et al. (2000b)

determinate growth form would be predicted to accumulate greater amounts of foliar carbohydrate. Ainsworth et al. (2004) examined the response of foliar carbohydrates to growth at e[CO_2] in a determinate genotype (Williams *dt1*) of a cultivar with an indeterminate growth form (Williams) and an indeterminate genotype (Elf *Dt1*) of a cultivar with determinate growth form (Elf). All plants with determinate growth forms (Williams *dt1* and Elf) accumulated significantly more sugars at e[CO_2], whereas indeterminate plants (Williams and Elf *Dt1*) showed no additional accumulation at e[CO_2]. Starch content was significantly higher in all plants grown at e[CO_2]. Figure 16.4 shows the levels of TNC in these plants. A single gene mutation to change the indeterminate growth form of Williams to a determinate growth form (Williams *dt1*) resulted in a doubling of the amount of extra carbohydrate accumulated at e[CO_2]. However, the opposite single gene substitution to convert the determinate variety Elf to an indeterminate variety (Elf *Dt1*) did not lead to an exacerbated accu-

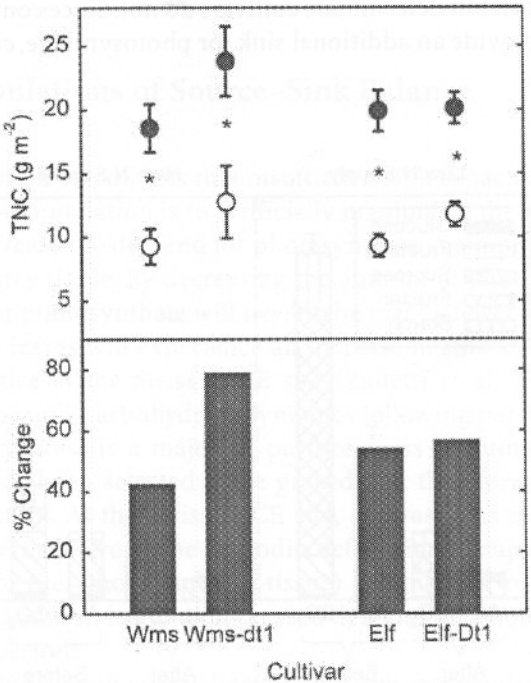

Fig. 16.4 Sink manipulation at the SoyFACE experiment. TNC content of four soybean cultivars ±SE, sampled during pod-fill. Williams (Wms) has an indeterminate growth form and Williams *dt1* (Wms-dt1) is a determinate form of Williams, Elf (Elf) has a determinate growth form and Elf *Dt1* (Elf-Dt1) is an indeterminate form of Elf. The bar charts show percent change in carbohydrate with growth at e[CO_2], i.e. (FACE –control)/control × 100. Pre-planned comparisons of [CO_2] treatments within cultivars were made using linear contrast statements. * $P < 0.05$. Figure redrawn from Ainsworth et al. (2004)

mulation of TNC at e[CO_2] (Ainsworth et al. 2004). This difference in the response of the two determinate lines may lie in the fact that Elf was developed as a determinate variety. For a determinate variety to be competitive, it is likely that breeders selected lines with sufficient potential for pod formation to ensure that yield would not be compromised by an insufficient sink capacity. Therefore, an indeterminate line of Elf may not offer a significant advantage at e[CO_2].

Together these two FACE experiments have provided strong evidence that sink capacity is a key factor in determining the response of foliar carbohydrates to growth at e[CO_2]. The picture is not always so clear. Rogers and Ellsworth (2002) investigated TNC accumulation in the needles of loblolly pine grown at e[CO_2] at the Duke Forest FACE site. They anticipated that when there was a strong proximal sink for carbohydrate (developing buds and new needles), they would not observe carbohydrate accumulation at e[CO_2]. However, carbohydrate accumulation was maximal at this time and minimal when predicted proximal sink activity was lowest. It is possible that other distal carbohydrate sinks may have played a more significant role than the adjacent developing shoots and needles. Developing shoots and needles are nitrogen sinks as well as carbon sinks; and if nitrogen were limiting needle development, then it is possible that the supply of photosynthate at e[CO_2] may have been in excess of sink requirements (Rogers and Ellsworth 2002).

Carbohydrate accumulation in the leaves of trees grown under e[CO_2] in FACE experiments has been difficult to predict and is highly variable. Much of this variation may be due to developmental heterogeneity within the tree and the problems associated with selecting comparable leaf tissue for analysis. More accurate methods of measuring leaf development are now available and should help clarify the responses to e[CO_2] (Schmundt et al. 1998; Taylor et al. 2003). In addition, carbon sinks such as wood formation, and in some species the emission of volatile organic compounds, complicate the understanding of source–sink relations.

16.4 The Effect of Nitrogen Supply on Sink Capacity

When plant growth is limited by nitrogen supply, carbon is in excess and surplus photosynthate often accumulates in leaves (Rogers et al. 1996). The interaction of e[CO_2] and nitrogen supply has been the subject of many studies (Stitt and Krapp 1999). Prior to FACE, many of these studies had been conducted in pots or containers. In addition to the physical constraint imposed by container walls (Arp 1991), enhanced growth under e[CO_2] may lead to more rapid exhaustion of the available nitrogen. In this case, plants growing at e[CO_2] will experience nitrogen limitation sooner, or to a greater extent than

the plants growing at c[CO_2] (Stitt and Krapp 1999; Körner 2003). In the field there is no restriction on root development, and increased exploration of the soil with accelerated growth at e[CO_2] would allow the plant to utilize additional sources of nitrogen as it develops. The FACE experiments provide the opportunity to examine plant responses to e[CO_2] in an open-air environment without the confounding effects of potentially exaggerated nitrogen limitation.

The concept that a low-nitrogen supply could lead to a sink limitation and that this could be exacerbated at e[CO_2] has been investigated at the Swiss FACE site (Fischer et al. 1997; Rogers et al. 1998; Isopp et al. 2000b). In addition to the effect of partial defoliation, Fig. 16.3 also shows the response of foliar carbohydrates to a low- and high-nitrogen supply (Isopp et al. 2000b). High-nitrogen supply reduced foliar carbohydrate content at both c[CO_2] and e[CO_2]. In plants harvested shortly before periodic defoliation, the combination of e[CO_2] and a low-nitrogen supply led to a marked increase in foliar carbohydrate content. This trend was also reported by Fischer et al. (1997) and Rogers et al. (1998). The large increase in carbohydrate content at e[CO_2] and low nitrogen immediately prior to planned periodic partial defoliation was consistent with a severe sink limitation. This severe sink limitation was investigated further towards the end of a growth cycle where day-to-day accumulation of foliar carbohydrate in source leaves was examined. Plant grown with a high-nitrogen supply did not show a significant accumulation of carbohydrate between successive days. However, plants with a low-nitrogen supply accumulated significant amounts of carbon over a 24-h time-course. Figure 16.5 shows the extent of this carbon accumulation in two successive years

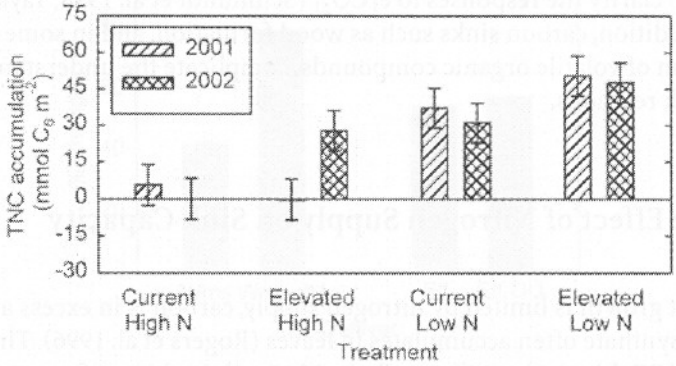

Fig. 16.5 The effect of low nitrogen on sink limitation at the Swiss FACE experiment. Immediately prior to a scheduled defoliation, the accumulation of foliar TNC over a 24-h period was determined in *Lolium perenne* grown at e[CO_2] and c[CO_2] in both low- and high-nitrogen plots. Accumulation was 5-fold greater in plants grown with a low-nitrogen supply (F=7.96, P<0.05). Error bars represent mean ±SE of TNC accumulation (n=3 rings)

of investigation. Together with the results from Fischer et al. (1997), Rogers et al. (1998) and Isopp et al. (2000a, b), it is clear that the low-nitrogen supply treatment at the Swiss FACE site led to a markedly reduced sink capacity and to the accumulation of foliar carbohydrates, a phenomenon that was exacerbated at e[CO_2].

16.5 What Are the Signs of a Limited Sink Capacity?

A higher carbohydrate content at e[CO_2] is not necessarily an indication of replete sinks. According to the Münch hypothesis, if plants are to match increased photosynthetic rates with increased export rates, they need to increase their capacity to load phloem. The plant has to poise its sucrose levels on the trigger of photosynthetic repression to drive symplastic transport at a maximal level (see Chapter 15). So, if an increased carbohydrate content may not be a clear indicator of a source–sink imbalance, what are the signals of sink limitation and how else might the leaf sense an inadequate capacity in the plant for the utilization of additional photosynthate?

The type of carbohydrate accumulated can communicate the capacity of a plant to utilize current photosynthate. When the capacity of sinks to utilize photosynthate decreases, excess carbohydrate is stored in the leaf as either starch or fructan. Figure 16.1 shows the response of sucrose and starch to growth at e[CO_2] using FACE technology. In agreement with prior reports (Farrar and Williams 1991), starch is the main component of the increase in leaf carbohydrates observed in plants grown at e[CO_2]. In the Gramineae, the alternative storage polysaccharide fructan also showed marked accumulation at e[CO_2]. Figure 16.3 clearly shows fructan storage in sink-limited ryegrass grown at the Swiss FACE site (Isopp et al. 2000b).

Another key indicator of a source–sink imbalance is the accumulation: fixation ratio (Rogers et al. 2004). Additional TNC accumulation during a photoperiod at e[CO_2] can be indicative of a limited capacity to utilize photosynthate, particularly if this accumulation is carried over to the next day (Fig. 16.5). However, accumulation during the photoperiod may simply reflect higher photosynthetic rates and a larger transport pool. Accumulation:fixation ratio is a more useful diagnostic parameter. In soybean grown at e[CO_2], Rogers et al. (2004) reported that soybean exported ca. 90 % of fixed carbon, but on one occasion during the season, associated with low temperature and developmental reductions in sink capacity, plants grown at c[CO_2] retained ca. 20 % of their fixed carbon and plants at e[CO_2] retained ca. 50 % (Fig. 16.6).

Moore et al. (1999) offers perhaps the best explanation of how a photosynthetic cell can sense and respond to a source–sink imbalance (Long et al. 2004). Excess sucrose from photosynthesis that accumulates in the vacuole

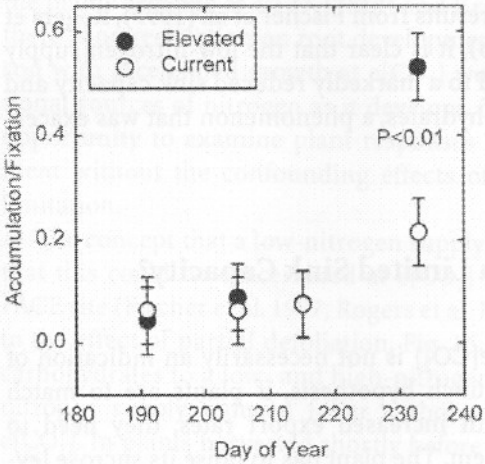

Fig. 16.6 Ratio of foliar carbon accumulation to carbon fixation in the terminal leaflets of the most recently fully expanded trifoliate leaves of soybeans grown in the field at e[CO_2] and c[CO_2] measured on four occasions during development. Carbon accumulation was calculated by subtracting the TNC content at the beginning of the photoperiod from the TNC content at the end of the photoperiod. Carbon fixation is the daily integral of net CO_2 assimilation. There was a significant effect of CO_2 treatment ($P<0.1$), day of year ($P<0.05$) and the interaction of CO_2 × day of year ($P<0.05$). Data are least-square means ±SE of the difference in means; and $P<0.01$ indicates a highly significant difference on day of year = 233, based on a linear pairwise contrast. Figure redrawn from Rogers et al. (2004)

when sinks are unable to utilize all available photosynthate is hydrolyzed by vacuolar invertases to yield glucose and fructose. These hexoses then enter a futile cycle of sucrose synthesis and degradation. Hexokinase catalyzes a key step in this cycle and has a secondary role as a flux sensor, thereby communicating the source–sink imbalance to the cell (Moore et al. 1999). Since there is a good correlation between invertase activity and hexose:sucrose ratio, the end-of-day hexose:sucrose ratio is potentially a useful diagnostic marker for a source–sink imbalance (Moore et al. 1999). Rogers et al. (2004) determined the hexose:sucrose ratio in sink-limited soybean (day of year = 254, Fig. 16.6) and reported a significant and markedly higher hexose:sucrose ratio in plants grown at e[CO_2] compared with those grown at c[CO_2], suggesting that Moore's model may translate to field-grown plants. Further investigation of this model as a possible mechanism that describes how plants sense a source–sink imbalance is required, particularly since a significant proportion of the measured foliar sucrose is in the phloem, where it is not available to a mesophyll-based flux sensor.

16.6 Conclusion

Prior to FACE experiments, there was uncertainty over whether the hypotheses that were being developed from experiments conducted in controlled environments would hold up when tested under fully open-air field conditions.

- Despite predictions that foliar carbohydrates would not accumulate in the leaves of plants grown at e[CO_2] in the field where roots are free to develop and explore the soil for nutrients, plants grown in the field using FACE technology still accumulated carbohydrate. Even N-fixing species with large sink capacities exhibited exacerbated carbohydrate accumulation at e[CO_2] and perennial ryegrass showed evidence of a severe sink limitation.
- FACE experiments confirmed the importance of sink capacity in determining the timing and extent of foliar carbohydrate accumulation; and they provide a valuable field test for key indicators of insufficient sink capacity.
- Many uncertainties still remain. Cross-talk between carbon and nitrogen metabolism in the leaf is extensive and well documented (Stitt and Krapp 1999) and growth at e[CO_2] will have a major impact on carbon and nitrogen metabolism. A full and more mechanistic understanding of the response of foliar carbohydrates to growth at e[CO_2] cannot be realized without parallel and comprehensive investigations of nitrogen metabolism.

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References

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of responses to rising CO_2 in photosynthesis, canopy properties and plant production. New Phytol 165:351–372

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

Allard V, Newton PCD, Lieffering M, Clark H, Matthew C, Soussana J-F, Gray YS (2003) Nitrogen cycling in grazed pastures at elevated CO_2: N returns by ruminants. Global Change Biol 9:1731–1742

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

Bush DR (1999) Sugar transporters in plant biology. Curr Opin Plant Biol 2:187–191

Chatterton NJ, Harrison PA, Thornley WR, Asay KH (1989) Carbohydrate partitioning in 185 accessions of Gramineae grown under warm and cool temperatures. J Plant Physiol 134:169–179

Estiarte M, Penuelas J, Kimball BA, Hendrix DL, Pinter PJ, Wall GW, LaMorte RL, Hunsaker DJ (1999) Free-air CO_2 enrichment of wheat: leaf flavonoid concentration throughout the growth cycle. Physiol Plant 105:423–433

Farrar JF, Williams ML (1991) The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source–sink relations and respiration: commissioned review. Plant Cell Environ 14:819–830

Farrar JF, Pollock CJ, Gallagher J (2000) Sucrose and the integration of metabolism in vascular plants. Plant Sci 154:1–11

Fischer BU, Frehner M, Hebeisen T, Zanetti S, Stadelmann F, Lüscher A, Hartwig UA, Hendrey GR, Blum H, Nösberger J (1997) Source–sink relations in *Lolium perrene* L. as reflected by carbohydrate concentrations in leaves and pseudo-stems during regrowth in a free air carbon dioxide enrichment (FACE) experiment. Plant Cell Environ 20:945–952

Hamilton JG, Dermody O, Aldea M, Zangerl AR, Rogers A, Berenbaum M, DeLucia EH (2005) Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. Environ Entomol 34:479–485

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

Hendrix DL, Mauney JR, Kimball BA, Lewin K, Nagy J, Hendrey GR (1994) Influence of elevated CO_2 and mild water stress on nonstructural carbohydrates in field-grown cotton tissues. Agric For Meteorol 70:153–162

Herrick JD Thomas RB (2001) No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO_2 enrichment at the Duke forest FACE experiment. Plant Cell Environ 24:53–64

Isopp H, Frehner M, Almeida JPF, Blum H, Daepp M, Hartwig UA, Lüscher A, Suter D, Nösberger J (2000a) Nitrogen plays a mjor role in leaves when source-sink relations change: C and N metabolism in *Lolium perrene* growing under free air CO_2 enrichment. Aust J Plant Pysiol 27:851–858

Isopp H, Frehner M, Long SP, Nösberger J (2000b) Sucorse-phosphate synthase responds differently to source–sink relations and to photosynthetic rate: *Lolium perenne* L. growing at elevated pCO_2 in the field. Plant Cell Environ 23:597–607

Jones PG, Lloyd JC, Raines CAR (1996) Glucose feeding of intact wheat plants represses the expression of a number of Calvin cycle genes. Plant Cell Environ 19:231–236

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

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

Krapp A, Hofmann B, Schafer C, Stitt M (1993) Regulation of the expression of *Rbcs* and other photosynthetic genes by carbohydrates: a mechanism for the sink regulation of photosynthesis. Plant J 3:817–828

Lindroth RL (1996) CO_2-meadiated changes in tree chemistry and tree–lepidoptera interactions. In: Koch GW, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. Academic, San Diego, pp105–120

Long SP, Drake BG (1992) Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. In: Baker NR, Thomas H (eds) Crop photosynthesis spatial and temporal determinants. Elsevier, Amsterdam, pp 69–103

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

Moore BD, Cheng SH, Sims D, Seemann JR (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. Plant Cell Environ 22:567–582

Myers DA, Thomas RB, DeLucia EH (1999) Photosynthetic capcity of loblolly pine (*Pinus taeda* L.) trees during the first year of carbon dioxide enrichmant in a forest ecosystem. Plant Cell Environ 22:473–482

Norby RJ, Todd DE, Fults J, Johnson DW (2001) Allometric determination of tree growth in a CO_2-enriched sweetgum stand. New Phytol 150:447–487

Pego JV, Kortstee AJ, Huijser C, Smeekens SCM (2000) Photosynthesis, sugars and the regulation of gene expression. J Exp Bot 51:407–416

Pollock CJ, Cairns AJ (1991) Fructan metabolism in grasses and cereals. Annu Rev Plant Physiol Plant Mol Biol 42:77–101

Ritchie S, Hanaway J, Thompson H, Benson G (1997) How a soybean plant develops – special report no. 53. Iowa State University, Ames

Robbins NS, Pharr DM (1988) Effect of restricted root growth on carbohydrate metabolism and whole plant growth of *Cucumis sativus* L. Plant Physiol 87:409–413

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 utilization. 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 lifecycle under free-air carbon dioxide enrichment. Plant Cell Environ 27:449–458

Rogers GS Milham PJ Gillings M, Conroy JP (1996) Sink strength may be the key to growth and nitrogen responses in N-deficient wheat at elevated CO_2. Aust J Plant Physiol 23:253–264

Schmundt D, Stitt, M, Jähne B, Schurr U (1998) Quantitative analysis of the local rates of growth of dicot leaves at a high temporal and spatial resolution, using image sequence analysis. Plant J 16:505–514

Sheen J (1990) Metabolic repression of transcription in higher-plants. Plant Cell 2:1027–1038

Singsaas EL, Ort DR, DeLucia EH (2000) Diurnal regulation of photosynthesis in understory saplings. New Phytol 145:39–49

Smeekens S (2000) Sugar-induced signal transduction in plants. Annu Rev Plant Physiol Plant Mol Biol 51:49–81

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ 14:741–762

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ 22:583–628

Taylor G, Trickler PJ, Zhang FZ, Alston VJ, Miglietta F, Kuzminsky E (2003) Spatial and temporal effects of free-air CO_2 enrichment (POPFACE) on leaf growth, cell expansion, and cell production in a closed canopy of poplar. Plant Physiol 131:177–185

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

Tissue DT, Lewis JD Wullschleger SD, Amthor JS, Griffin KL, Anderson OR (2002) Leaf respiration at different canopy positions in sweetgum (*Liquidambar styraciflua*) grown in ambient and elevated concentrations of carbon dioxide in the field. Tree Physiol 22:1157–1166

Van Oosten JJ, Besford RT (1994) Sugar feeding mimics effect of acclimation to high CO_2 – rapid down regulation of RubisCO small subunit transcripts but not of the large subunit transcripts. J Plant Physiol 143:306–312

Vessey JK, Walsh KB, Layzell DB (1988) Oxygen limitation of N_2 fixation in stem-girdled and nitrate-treated soybean. Physiol Plant 73:113–121

Walsh KB, V essey JK, Layzell DB (1987) Carbohydrate supply and N_2 fixation in soybean: the effect of varied daylength and stem girdling. Plant Physiol 85:137–144

Zanetti S, Hartwig UA, Lüscher A, Hebeisen T, Frehner M, Fischer BU, Hendrey GR, Blum H, Nösberger JA (1996) Stimulation of symbiotic N2 fixation in *Trifolium repens* L. under elevated atmospheric pCO_2 in a grassland ecosystem. Plant Physiol 112:575–583

Zeeman SC Smith SM, Smith AM (2004) The breakdown of starch in leaves. New Phytol 163:247–261