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Photosynthate Supply and Utilization in Alfalfa[1]

A DEVELOPMENTAL SHIFT FROM A SOURCE TO A SINK LIMITATION OF PHOTOSYNTHESIS

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ABSTRACT

Long-term carbon dioxide enrichment, $^{14}CO_2$ feeding, and partial defoliation were employed as probes to investigate source/sink limitations of photosynthesis during the development of symbiotically grown alfalfa. In the mature crop, long-term CO_2 enrichment does not affect the rates of net photosynthesis, relative growth, ^{14}C export to nonphotosynthetic organs, or the rates of ^{14}C label incorporation into leaf sucrose, starch, or malate. The rate of glycolate labeling is, however, substantially reduced under these conditions. When the mature crop was partially defoliated, a considerable increase in net photosynthesis occurred in the remaining leaves. In the seedling crop, long-term CO_2 enrichment increased dry matter accumulation, primarily as a result of increases in leaf starch content. Although the higher rates of starch synthesis are not maintained, the growth enhancement of the enriched plants persisted throughout the experimental period. These results imply a source limitation of seedling photosynthesis and a sink limitation of photosynthesis in more mature plants. Consequently, both the supply and the utilization of photosynthate may limit seasonal photosynthesis in alfalfa.

Under optimal plant growth conditions it is generally believed that photosynthesis is limited by the maximum capacity of either the reactions that supply photosynthate (source limited) or those that utilize photosynthate (sink limited) (1, 6, 15, 30). Support for a source limitation of photosynthesis comes primarily from the results of CO_2 enrichment studies. With CO_2 enrichment, an increase in photosynthetic rate is almost invariably observed when plants are first transferred from ambient to enriched atmospheres (7, 21, 23). Also, long-term CO_2 enrichment usually results in an increase in plant dry matter accumulation (summarized in Kimball [14]) or nitrogen content (12).

Sink limitation can be inferred in experiments where long-term CO_2 enrichment does not permanently increase plant photosynthesis or dry matter accumulation (for review, see Kramer [15]) or where an increase in photosynthate demand results in an increase in photosynthetic rate (summarized in Geiger [8]). The increase in sink size rather than source capacity during the evolution of many crop species is also consistent with the rate limiting step being the utilization of photosynthate (10).

Given the wealth of evidence in support of both source and sink limitation, it is apparent that both processes play a major role in determining crop yields (6, 30). What is less apparent is the growth stage(s) at which the source or sink limitation occurs.

There is evidence to suggest that seedling growth may be source limited while latter growth stages may be sink limited. Seedling relative growth rates are often higher than those of mature plants (20, 21, 26). Seedlings also experience the greatest growth enhancement with CO_2 enrichment (15, 21, 26). Consequently, the supply of photosynthate may be more limiting during early stages of plant growth. Lower relative growth rates (6, 21, 26) and the frequent presence of substantial stem carbohydrate reserves following grain fill (6) are consistent with the interpretation that sink activity may be limiting photosynthesis in older plants.

Here we report on experiments designed to test the hypothesis of a shift from source to sink limitation during plant development. To determine the rate-limiting process, we measured the effect of long- and short-term CO_2 enrichment and partial defoliation on the growth rate, net photosynthetic rate, and ^{14}C-labeling kinetics during the development of a symbiotically grown alfalfa crop.

MATERIALS AND METHODS

Plant Material. Alfalfa (*Medicago sativa* L. cv Moapa 69) plants were grown in a gas-tight controlled environment chamber (Conviron PGA 36) under a 12-h photoperiod, a radiant flux density of 800 $\mu E\ m^{-2}\ s^{-1}$, and a 27/20°C, day/night temperature regime. Plants were sown in vermiculite, inoculated with *Rhizobium meliloti* (Nitragin Co., Milwaukee) and watered daily with one-half strength plus N nutrient solution (4) for 5 d. Thereafter plants were watered daily with one-half strength minus N solution (29) brought to pH 6.1 with H_2SO_4. After initial thinning, a plant density of 80 plants m^2 was established. When the crop reached 10% bloom, all leaves and stems above 5 cm were removed.

CO_2 levels within the chamber were monitored and maintained by an IR CO_2 analyzer (Horiba APBA 200E) controlling a CO_2 source. This system was designed to allow a drop in CO_2 levels (through photosynthesis) of 10% before supplementary CO_2 was added.

Growth Measurements. At weekly intervals, 12 plants were removed from the chamber and divided into leaf, stem, tap root, and fibrous root fractions. Following fresh weight determination, plant parts were dried for 48 h at 70°C for dry weight measurements. Leaf area was measured using photocopies of leaves.

Photosynthetic Rate Measurements. Net canopy photosynthesis was measured in the controlled environment chamber using recorder tracings of the output from the CO_2 analyzer. This design permitted continuous measurement of the photosynthetic rate throughout the experiment. Chamber leakage was measured periodically and never exceeded 15% of photosynthesis. Contributions due to leakage have been subtracted from all rate data.

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Metabolite Labeling and Measurement. An exposure chamber capable of holding five plants in individual compartments was used for ^{14}C studies. Each compartment was connected to a gas exchange apparatus (23) that maintained steady state concentrations of $^{12}CO_2$ and $^{14}CO_2$ throughout the experiment. Environmental conditions, with the exception of a radiant flux density of 550 $\mu E\ m^{-2}\ s^{-1}$, were the same as in the growth chamber. Plants were allowed to photosynthesize for 30 min in either 350 or 1000 $\mu l\ l^{-1}$ $^{12}CO_2$, before the addition of $^{14}CO_2$. After varying lengths of time in $^{14}CO_2$, plants were quickly removed from their compartments and plunged into liquid nitrogen. Plant parts were then separated and extracted (3). Neutral sugars, phosphorylated sugars, and organic acids were analyzed by paper chromatography (16), amino acids by high pressure liquid chromatography (24), and starch by enzymic degradation followed by glucose determination (11). Export of ^{14}C to nonphotosynthetic tissues was measured by determining the radioactivity in the tap root, fibrous roots, and woody stem.

RESULTS

Photosynthetic and Growth Measurements. To determine the effect of long-term CO_2 enrichment on crop photosynthesis, net CO_2 uptake per plant per day was measured for both high and ambient CO_2 treatments. The results (Fig. 1) show that CO_2 enrichment increased the photosynthetic rate per plant throughout the experimental period. However, the relative rates of increase are similar to both treatments. If photosynthetic rates are expressed per unit leaf area (Fig. 2) a reduction in rate with CO_2 enrichment is observed. Again, however, the relative rates of change are similar. These results suggest that CO_2 enrichment accelerates the seedling (less than 5 weeks) growth rate, but is without effect on latter stages of growth. The high CO_2 plants thus appear to experience a growth enhancement of about 1 week. Plant growth measurements (Fig. 3) support this interpretation. High CO_2 grown plants are larger than ambient plants with the growth curves offset by about a week.

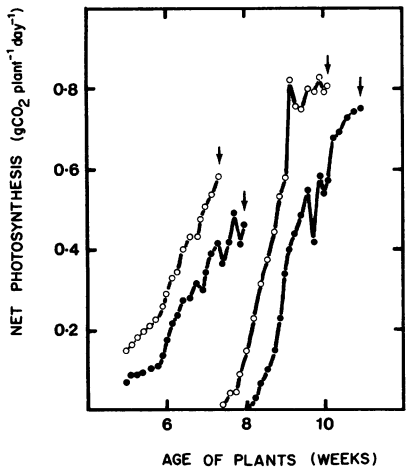

FIG. 1. Net photosynthetic rate per plant of alfalfa grown at 350 $\mu l\ l^{-1}$ (●) and 1000 $\mu l\ l^{-1}$ (○) CO_2. (↓), Partial defoliation of the crop.

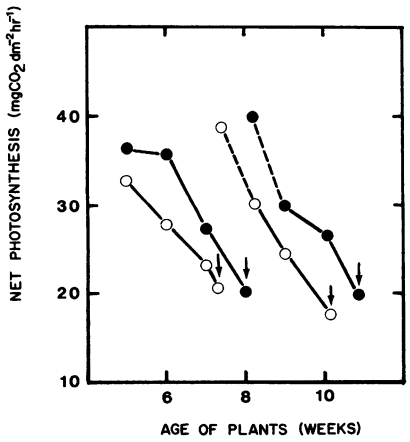

FIG. 2. Net photosynthetic rate per unit leaf area of alfalfa grown at 350 $\mu l\ l^{-1}$ (●) and 1000 $\mu l\ l^{-1}$ (○) CO_2. Arrows indicate partial defoliation of the crop. (- - -), photosynthetic rate measurements taken shortly after defoliation are less precise due to interplant variability in the remaining leaf area.

The results of the photosynthetic and growth rate measurements, when adjusted for a difference in initial growth rate, show that long-term CO_2 enrichment did not enhance crop photosynthesis or growth rate beyond the seedling stage. During the seedling stage, however, growth enhancement by CO_2 is apparent.

Metabolite Labeling. In the mature alfalfa crop, long-term CO_2 enrichment does not increase the crop growth rate. To determine what effect, if any, long-term CO_2 enrichment has on plant metabolism, $^{14}CO_2$ feeding was employed. One week after partial defoliation, plants from each treatment were exposed to $^{14}CO_2$ (9.5 $\mu Ci/\mu mol$) at the same concentration at which they were grown. After varying lengths of time, plants were harvested and metabolite labeling kinetics determined. The results (Fig. 4) show little difference between treatments in rates of total incorporation, ^{14}C export to nonphotosynthetic tissues, sucrose, starch, and malate labeling and in labeling kinetics for all other compounds tested (data not shown) with the exception of glycolate (Fig. 4). Glycolate labeling rate is substantially reduced by the high CO_2 treatment. Thus, with long-term CO_2 enrichment, a considerable reduction in photorespiration may occur without a concomitant increase in the net photosynthetic rate.

Short-Term CO_2 Enrichment. Several explanations are possible for the lack of response to long-term CO_2 enrichment by the mature crop. If photosynthate requirements are adequately met at ambient CO_2 levels, any increase in supply as a result of CO_2 enrichment might be eventually dampened by the action of a slowly acting regulatory system (8). Alternatively, in the mature crop the supply of photosynthate may be limited by some factor other than the level of CO_2. In this case, increasing the CO_2 levels would not result in an increase in photosynthesis.

To test these alternatives, plants were removed from the ambient CO_2 chamber at intervals of 2 weeks. Photosynthetic rates, before and after the addition of 1000 $\mu l\ l^{-1}$ CO_2, were measured

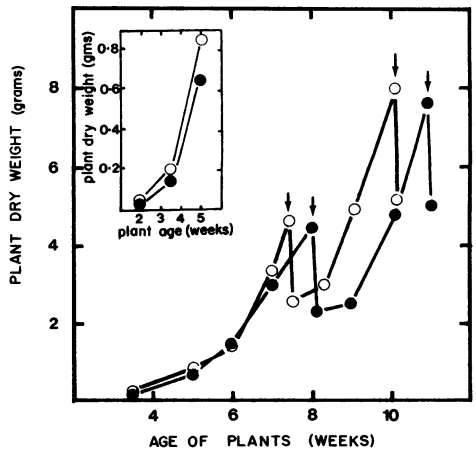

FIG. 3. Dry weight per plant of alfalfa grown at 350 $\mu l\ l^{-1}$ (●) and 1000 $\mu l\ l^{-1}$ (○) CO_2. (↓), partial defoliation of the crop. Inset, growth of seedlings, Note expanded vertical scale.

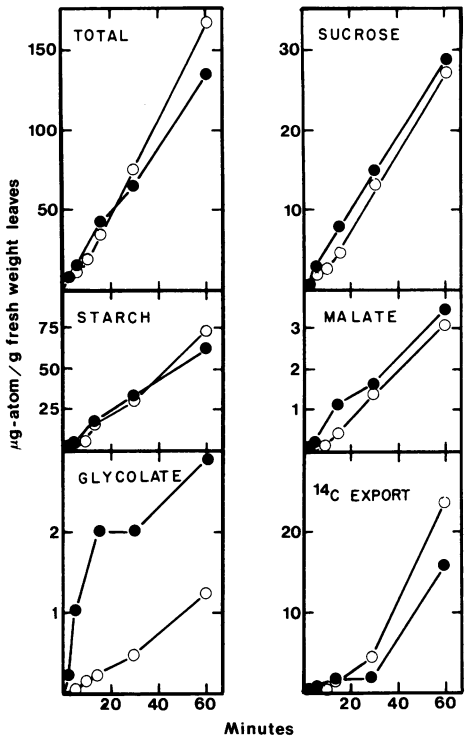

FIG. 4. Total photosynthesis and incorporation of $^{14}CO_2$ into leaf sucrose, starch, malate, and glycolate and ^{14}C translocated to roots and stems in alfalfa grown at 350 $\mu l\ l^{-1}$ (●) and 1000 $\mu l\ l^{-1}$ (○) CO_2.

Table I. *Effect of Short-Term Exposure to 1000 $\mu l\ l^{-1}$ CO_2 on Photosynthetic Rate of Plants Grown at 350 $\mu l\ l^{-1}$*

Results are expressed as percentage increase in photosynthetic rate (mg $CO_2\ dm^{-1}\ h^{-1}$) after 1 h at 1000 $\mu l\ l^{-1}$ CO_2. Values are the mean of five plants per time point.

Age of Plant	Increase
weeks	%
3	267
5	171
7	156
9	192

using the steady state apparatus. The results (Table I) show that short-term CO_2 enrichment increases the rate of photosynthesis at all stages of plant growth, with seedling plants being the most responsive. These results suggest that, for short-term exposures, CO_2 and not some other factor limits photosynthesis. That brief CO_2 exposures increase photosynthesis, while long-term enrichment does not, is consistent with the interpretation that, at ambient CO_2 levels, the demand for photosynthate is adequately met by mature crop photosynthetic rates.

Defoliation Experiments. At ambient CO_2 levels, the highest attainable (maximal) photosynthetic rate may be equal to or greater than the rate required to meet the demand for photosynthate. If the maximal photosynthetic rate is greater than the required rate, increasing the demand for photosynthate should increase the rate of photosynthesis. Partial defoliation increases the demand for photosynthate from the remaining leaves, hence their photosynthetic rate should increase.

Plants from both treatments were partially defoliated when the crop reached the 10% bloom stage. At this developmental stage, ambient CO_2 grown plants were 5 d older than those grown at high CO_2, but were similar morphologically, in dry weight (Fig. 3), in tap root starch content (Fig. 5B), and had similar photosynthetic rates (Figs. 1, 2).

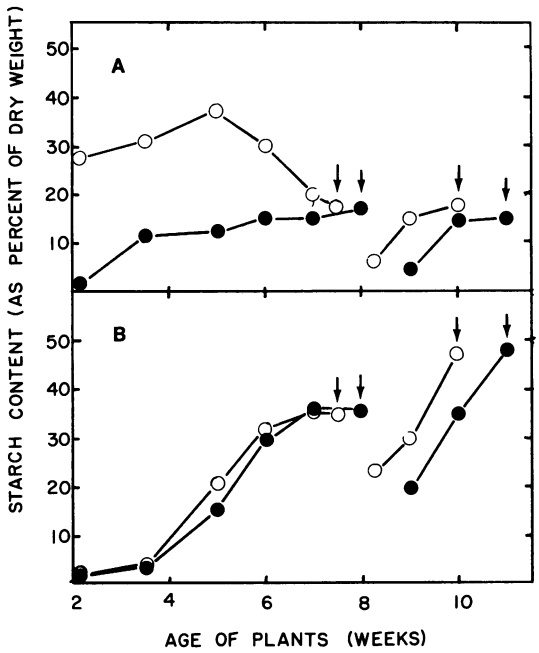

FIG. 5. Leaf (A) and taproot (B) starch content of alfalfa grown at 350 $\mu l\ l^{-1}$ (●) and 1000 $\mu l\ l^{-1}$ (○) CO_2. (↓), partial defoliation of the crop.

Photosynthetic rate measurements (Fig. 2) show that, following defoliation, photosynthesis increased dramatically then gradually declined over a period of weeks to its initial value. The drop in photosynthesis per unit leaf area could be solely the result of shading by the upper leaves. However, the low leaf area index of the crop (under 1.5 for all but the last week) argues against this. A more plausible explanation is that defoliation induces a large increase in photosynthetic rate that gradually declines, at least in part, due to a reduction in demand on individual leaves. The absence of any rate enhancement by high CO_2, even during the period immediately following defoliation, is also consistent with this interpretation. These results suggest that, over much of the life cycle of the crop, growth is sink limited.

Seedlings. The young alfalfa crop appears to respond differently to CO_2 enrichment than do latter stages. Photosynthetic rates are enhanced to a greater degree in seedlings with a short-term pulse of CO_2 (Table I) as is dry matter accumulation under long-term CO_2 enrichment (Fig. 3, inset). The increased dry weight of 2-week-old, high CO_2 grown seedlings is due almost exclusively to an increase in leaf starch content (data not shown). Analysis of leaves (at the beginning of the photoperiod) shows a very high level of leaf starch in high CO_2 plants that gradually declines as the plants mature (Fig. 5A). The tap root starch levels (Fig. 5B), in contrast, are similar for both treatments. Alfalfa seedlings, unlike mature plants, therefore appear to increase their photosynthetic and growth rates in response to long-term elevations in CO_2 levels.

DISCUSSION

The occurrence of a developmental shift from source to sink limitation of photosynthesis during the development of a symbiotically grown alfalfa crop was investigated using long- and short-term CO_2 enrichment and partial defoliation as probes. Long-term CO_2 enrichment does not substantially alter the rate of net photosynthesis (Figs. 1, 2, and 4), dry matter accumulation (Fig. 3), sucrose/starch partitioning (Fig. 4), or ^{14}C export to nonphotosynthetic tissues (Fig. 4), in the mature (older than 5

weeks) crop. This lack of effect occurred in spite of a substantial reduction in the rate of glycolate labeling (Fig. 4). Reduced glycolate synthesis may indicate a reduction in photorespiration (27, 30). Short-term CO_2 enrichment, however, does result in enhanced photosynthesis (Table I) and altered metabolite labeling kinetics (C. Baysdorfer, unpublished data). Increases in the rate of photosynthesis or relative growth with short-term exposure to elevated CO_2, followed by a decline in rate as exposure continues, have also been reported in other species (13, 15, 21, 26).

In the mature alfalfa crop, therefore, short-term photosynthetic rates are limited by CO_2 and not by the capacity of the photosynthetic apparatus. That these increased rates are not maintained over the long term, in spite of a reduction in photorespiration, suggests that supply exceeds demand under these conditions.

Partial defoliation provides a second, independent probe for source or sink limitation of photosynthesis. In the mature crop, partial defoliation dramatically increased photosynthesis in the remaining leaves (Fig. 2). These rates gradually declined as the canopy regrew. Although shading by the upper leaves could account for part of this response, the low leaf area index of the crop suggests that a significant proportion (28) of the rate increase was due to the increased demand on the remaining leaves. In general, long-term increases in the rate of photosynthesis in response to increased photosynthate demand have been reported in the literature (8, 22).

The results of the CO_2 enrichment and defoliation experiments support the conclusion that photosynthesis in the mature alfalfa crop is sink limited. This does not appear to be the case for alfalfa seedlings. Two weeks after planting, high CO_2-grown seedlings are already larger than ambient-grown plants (Fig. 3, inset) with increased starch storage in the leaves accounting for most of this increase (Fig. 5A). Although the difference in the level of starch disappears by the 7th week, the growth advantage of the high CO_2 seedlings is maintained throughout the experimental period.

Seedlings are generally more responsive to short-term CO_2 enrichment than are mature plants (15, 21, 26; Table I). The increase in dry weight observed in this study suggests that long-term CO_2 enrichment produces a sustained increase in seedling photosynthetic rate as well. These results imply that alfalfa seedlings are photosynthesizing at their maximal rate in ambient CO_2. Seedling photosynthesis may therefore be source limited.

The results of this study, if applicable to other species, could offer one explanation for the apparent limitation of crop yields by both the supply and demand for photosynthate. Enhanced growth as a result of CO_2 enrichment occurs primarily in seedlings, and the growth enhancement they achieve appears to persist into later developmental stages (Fig. 3). Comparisons of plant dry weights, therefore, usually show an enhancement by long-term CO_2 enrichment (14). Enhanced dry weight does not necessarily mean that increased growth occurs over the entire developmental profile of the plant (5). To separate current from previous CO_2 effects, growth rate constants or photosynthetic rates should be measured. In mature plants these parameters often (15, 26) but not always (2) show little change with CO_2 enrichment.

The shift from source to sink limitation in alfalfa could be caused by a number of factors. Seedling growth in symbiotically grown legumes is reported to be limited by the supply of combined nitrogen (17, 18). In the present study, the high leaf starch content of the enriched plants begins to decline after the 5th week (Fig. 5A). Photosynthetic rates are unresponsive to long-term CO_2 enrichment by this time as well (Fig. 2). Thus, source limitation appears to occur prior to the 5th week, at a time of presumed nitrogen limitation. This apparent contradiction may have been resolved by the work of MacDowall (19) who showed that while seedling growth coefficients were unresponsive to CO_2 enrichment, the coefficient of nitrogenase development was dependent on the CO_2 level. Nodule development in seedlings may be hindered by low rates of carbohydrate translocation from immature leaves while leaf growth (and increased translocation) may be limited by the rate of nodule development. This physiological double bind appears to be gradually overcome as the percentage of mature, exporting leaves in the population increases. In enriched leaves, the transition occurs more rapidly, perhaps because of their higher carbohydrate status. If the shift from source to sink limitation in alfalfa seedlings occurs as a result of an increased percentage of mature exporting leaves, then source limitation could be ascribed to the low photosynthetic rate or export capacity (9, 25) of immature leaves.

Plant carbohydrate status also appears to be important in the period immediately following defoliation. Tap root starch levels which are high initially, decline as regrowth starts (Fig. 5B). These root reserves apparently prevent a source limitation of growth at a time when photosynthate requirements are high and immature leaves dominate the population.

In summary, under conditions of optimal water and nutrient supply, alfalfa seedling photosynthesis appears to be limited by the capacity of the reactions that supply photosynthate. For the mature crop, however, photosynthesis appears to be limited by the rate at which photosynthate is utilized.

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