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Primary partitioning and storage of photosynthate in sucrose and starch in leaves of C_4 plants

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Abstract. A procedure involving pulse labelling of leaves with $^{14}CO_2$ was developed to measure the primary (initial) partitioning of photosynthate between sucrose and starch. Partitioning of photosynthate into sucrose and starch was determined in leaves of C_4 plants and compared with the patterns of storage of carbon in these products during the light period. The ratio of primary partitioning into sucrose and starch varied from about 0.5 in those species that accumulated mostly starch in the leaves (*Amaranthus edulis* L., *Atriplex spongiosa* F. Muell. and *Flaveria trinervia* (Spreng.) C. Mohr) to about 8 in *Eleusine indica* (L.) Gaertn., which accumulated mostly sucrose. No label was detected in free glucose or fructose. Generally there was a reasonable link between the primary partitioning of photosynthate and the type of carbohydrate stored in the leaf during the day. However, the ratio of carbon initially partitioned into sucrose versus starch was about 3 to 4 times higher in leaves of NADP-malic enzyme-type monocotyledonous species compared with phosphoenolpyruvate carboxykinase-type species, although the ratio of sucrose to starch accumulated in leaves during the day was very similar in the two groups. Sucrose and starch were the principal carbohydrates accumulated in leaves during the day. None of the species examined contained significant amounts of fructan and only one species, *Atriplex spongiosa*, contained substantial amounts of hexose sugars. In most of the species studied, the proportion of photosynthate partitioned into starch was greater at the end of the day than at the beginning. With the exception of *Flaveria trinervia*, the rate of CO_2 assimilation did not decline during the day, showing that, under our conditions, accumulation of carbohydrate in the leaves did not lead to feedback inhibition of photosynthesis in these C_4 species.

Key words: Carbohydrate – C_4 photosynthesis – Partitioning – Starch – Sucrose

Chl = chlorophyll; NAD-ME = NAD-malic enzyme; NADP-ME = NADP-malic enzyme; PCK = phosphoenolpyruvate carboxykinase

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Introduction

Sucrose and starch are the principal end products of photosynthesis in most plants, and sucrose is the principal carbohydrate translocated from source to sink tissues (Stitt et al. 1987). During the day leaves accumulate sucrose and starch as well as exporting sucrose to the rest of the plant. At night the sucrose and starch stored during the day are remobilised to maintain export of sucrose to sink tissues and to support respiration in the leaf. Carbohydrate reserves may also provide a buffer against changes in irradiance during the day, being remobilised during periods of low irradiance to supply the rest of the plant (Servaites et al. 1989). This may be especially important in C_4 plants which are subject to larger fluctuations in the rate of CO_2 fixation during natural changes in irradiance during the day (Kalt-Torres et al. 1987).

In C_3 plants the type of carbohydrate stored in the leaves varies between species. In many species (e.g. soybean, potato and tomato) starch is the major leaf storage carbohydrate, whilst other species store either sucrose and starch (e.g. spinach) or mainly sucrose (e.g. wheat and barley) (Stitt et al. 1987). Plants that accumulate sucrose often also store some carbohydrate as fructan or sucrose-derived oligosaccharides such as raffinose (Stitt et al. 1987). Presumably, the different storage strategies adopted by different plants are adaptations to the plant's environment, but the advantages of one strategy over another are unclear (Stitt et al. 1987; Pollock and Chatterton 1988).

Antisense repression of the chloroplast triose-phosphate translocator in transgenic potato plants led to increased synthesis and accumulation of starch in the leaves (Heineke et al. 1994). In these plants the proportion of total photosynthate stored in the leaves during the day and subsequently exported from the leaves at night was greater than in wild type plants (Heineke et al. 1994). Conversely, in transgenic potato plants with reduced leaf ADPglucose pyrophosphorylase activity the rate of starch synthesis was reduced, and the proportion of total photosynthate stored in the leaves during the day and exported at night was lower than in wild type plants (Leidreiter

et al. 1995). These results demonstrate that the initial partitioning of photosynthate between sucrose and starch during photosynthesis has a major influence on both the form in which the leaves store carbohydrate and the diurnal pattern of export of carbohydrate to sink tissues.

The regulatory mechanisms involved in the control of partitioning of newly fixed carbon have been well characterised in spinach, a C_3 plant (see Stitt et al. 1987 for a review). Similar mechanisms appear to operate in maize (Stitt et al. 1987; Kalt-Torres et al. 1987; Usuda et al. 1987; Huber and Huber 1992). In C_4 plants these processes must be coordinated not only between chloroplast and cytosol but also between mesophyll and bundle-sheath cells. In maize, sucrose and starch are synthesised predominantly in the mesophyll and bundle-sheath cells, respectively (Downton and Hawker 1973; Furbank et al. 1985), although under some conditions this compartmentation of sucrose and starch synthesis may be less strict (Ohsugi and Huber 1987). Studies of the localisation of sucrose-phosphate synthase suggest that sucrose synthesis may occur in both cell types in some other C_4 species (Chen et al. 1974; Mbaku et al. 1978; Ohsugi and Huber 1987). Therefore, it is likely that differences will exist between C_4 species in the regulation of photosynthate partitioning depending on whether sucrose or starch is the major leaf storage carbohydrate and in which cell type sucrose and starch are synthesised.

The purpose of the present study was to assess the variation in partitioning of photosynthate in different C_4 species representing the three major biochemical subtypes. The initial partitioning of photosynthate into sucrose and starch was measured by a procedure involving short-term pulse labelling with $^{14}CO_2$. These results were compared with data on the type of carbohydrate stored in the leaves during the light period.

Materials and methods

Materials. Plants were grown in soil in a naturally illuminated glasshouse, maintained between 20 and 28 °C. Biochemicals and enzymes were obtained from Boehringer-Mannheim, Australia. Barium [^{14}C]carbonate (1.85–2.20 TBq·mol^{-1}) was obtained from Amersham International.

Extraction of soluble sugars and starch from leaves. Samples of the youngest (top) fully expanded leaves from 3- to 6-week-old plants were harvested at 5 a.m. (before dawn), noon and 5 p.m. The leaves were immediately wrapped in aluminium foil, frozen in liquid N_2 and stored at − 80 °C until extraction. The frozen leaves were plunged into 30 ml of boiling 80% (v/v) ethanol, boiled for 1 min, and then blended at full speed in a 90-ml cup of a Sorvall Omni-Mixer for 60 s. This homogenate was centrifuged at 13 200·*g* (swinging-bucket rotor) for 10 min. The supernatant was decanted and the pellet was resuspended by vortex mixing in 15 ml of 50% (v/v) ethanol and left for 10 min at room temperature, before centrifuging as described above. The supernatant was combined with the 80% ethanol extract and the pellet was re-extracted with 15 ml of water. The ethanol- and water-soluble extracts were combined and partitioned with 15 ml of $CHCl_3$. The aqueous-ethanol phase was taken to dryness in vacuo at 30–35 °C using a rotary evaporator, redissolved in 1 ml of water and assayed for hexoses and sucrose. The chlorophyll content of the $CHCl_3$ phase was measured after conversion of the chlorophyll to phaeophytin as described by Vernon (1960).

The aqueous-ethanol insoluble residue was frozen at − 20 °C then ground to a fine powder in liquid N_2 using a mortar and pestle. The powdered residue was suspended in 5 ml of 0.2 M KOH and placed in a boiling-water bath for 30 min to break down cell wall material. After cooling, 0.9 ml of 1 M acetic acid was added and the starch content of the suspension was measured as described below.

Measurement of hexoses, sucrose and starch. Glucose and fructose were assayed spectrophotometrically as described by Stitt et al. (1980). To measure sucrose, leaf extracts were incubated with an equal volume of 100 mM citric acid-NaOH (pH 4.6) containing 20 units·ml^{-1} of invertase (EC 3.2.1.26) at 30 °C for 60 min, and assayed for glucose and fructose as described above. Equimolar amounts of glucose and fructose were released by treatment with invertase, indicating that the extracts contained little or no β-(2 → 1) linked fructans, which are also readily hydrolysed by invertase (Gonzalez et al. 1989). Starch was measured in the aqueous-ethanol-insoluble residue by incubating 100 µl of the neutralised KOH-treated suspension with 400 µl of 50 mM acetic acid-NaOH (pH 4.8) containing 1 unit of amyloglucosidase (EC 3.2.1.3) and 2 units of α-amylase (EC 3.2.1.1) at 50 °C for 15 h. The incubation mixture was centrifuged at 11 600·*g* for 2 min and an aliquot (50 µl) of the supernatant was assayed for glucose as described above.

Determination of primary photosynthate partitioning in leaves. Two fully expanded leaves from 2- to 6-week-old plants were harvested and the basal end immediately recut under water. Leaves from monocotyledonous species were trimmed at both ends to a length of about 15 cm and the surface of the leaf blotted dry before being placed in a 6-l perspex chamber with the basal end of the leaf immersed in a water-filled trough and the upper end protruding by about 1 cm through a gas-tight foam-rubber gasket in the lid of the chamber. Leaves from dicotyledonous species were suspended inside the chamber with the petiole passing through the the gasket in the lid, and the cut end of the petiole was covered with wet tissue paper. The floor of the chamber was covered with water to maintain high relative humidity and the air in the chamber was circulated by two high-speed fans. The temperature in the chamber, monitored by a thermocouple inserted into the side of the chamber, was maintained at 25 °C. The chamber was flushed with humidified air, supplemented with CO_2 to give a CO_2 concentration between 390–410 µl·l^{-1} in the chamber (total CO_2 about 90 µmol.), at a flow rate of about 5 l·min^{-1}. The leaves were illuminated by a mercury-vapour lamp giving an irradiance of 800 µmol quanta·m^{-2}·s^{-1} at the leaf surface.

After a period of 30 min illumination to allow establishment of a steady rate of photosynthesis, a 60-ml sample of the gas in the chamber was removed for determining the CO_2 concentration (with an LCA-2 infra-red gas analyser; ADC, Hoddesdon, Herts., UK), then the air flow through the chamber was stopped and the chamber was sealed. Approximately 1 ml of $^{14}CO_2$ (2–3 µmol, 1.85–2.20 TBq·mol^{-1}) was then immediately injected into the chamber and after 10 s a sample (5 ml) of the air in the chamber was taken using a gas-tight syringe for determination of the total amount of $^{14}CO_2$ in the chamber as described by Furbank and Hatch (1987). At intervals after the start of the pulse, usually 75 s and 135 s, one leaf was removed from the chamber and killed by plunging into boiling 80% (v/v) ethanol for 1 min. The extent of the depletion of CO_2 in the chamber was determined by taking a gas sample from the chamber after the last leaf was removed.

The leaves were extracted successively with 80% ethanol, 50% ethanol and water and partitioned with $CHCl_3$ as described above. Samples (100 µl) of the aqueous-ethanol phase were taken for measurement of fixed ^{14}C. The remainder was taken to dryness in vacuo at 30–35 °C using a rotary evaporator and then redissolved in 1 ml of water. Samples of this soluble fraction were chromatographed on Whatman (Maidstone, UK) 3MM paper in ethyl acetate/pyridine/water (8:2:1, by vol.) for about 20 h. In this solvent, sucrose, glucose and fructose are separated from each other and from other labelled compounds, which run as a peak at or near the origin (Hatch and Slack 1966). The position of sucrose, glucose and

fructose, added as a marker mixture in reference tracks, was located using *p*-anisidine; chromatogram strips were dipped in a solution of 1% H_3PO_4 in acetone followed by 0.5% *p*-anisidine with 0.5% aniline in $CHCl_3$ and then heated at 100 °C to detect the location of sugars by the colour developed. The percentage of the total ^{14}C that was in sucrose was determined using a radiochromatogram scanner fitted with a peak integrator.

The insoluble residue remaining after aqueous-ethanol extraction was washed with a further 40 ml of water to remove any remaining water-soluble compounds. This extract contained less than 2% of the total ^{14}C in the soluble fraction and was discarded. The insoluble residue was treated as described above for extraction and measurement of starch. Samples (100 µl) of the neutralised KOH-treated suspension, and the supernatant after digestion with α-amylase and amyloglucosidase, were taken for determination of ^{14}C. Using paper chromatography, essentially all the ^{14}C in this supernatant was found to be in glucose, showing that starch was the only significantly labelled compound in this fraction. In some experiments where leaf starch content was not measured, this washed insoluble fraction was suspended in 80 ml of water and replicate aliquots of the suspended material were collected onto Whatman GF/A glass-fibre filter paper discs (2.5 cm diameter). The discs were washed successively with 10 ml each of water, ethanol and acetone, allowed to dry then placed in 20-ml glass scintillation vials containing 1 ml of Soluene-350 tissue solubiliser (Packard). The vials were capped and incubated with gentle shaking at 40 °C for 3 h. Glacial acetic acid (40–45 µl) was added to each vial to reduce chemiluminescence, before determination of ^{14}C. Radioactivity was measured by liquid scintillation counting using the external-standard method to correct for quenching.

The primary partitioning ratio was calculated as the increase of ^{14}C in sucrose to the increase of ^{14}C in starch over the period between 75 and 135 s. Very similar values were obtained for the ratio of total ^{14}C incorporated into sucrose and starch at 135 s, and this would also be a reasonable alternative for assessing partitioning of photosynthate.

Results

Diurnal accumulation of carbohydrate in leaves of C_4 species. The sucrose, hexose and starch contents of young, fully expanded leaves from a range of C_4 plants were measured at the beginning, middle and end of the photoperiod. The levels of sugars and starch in the leaves at the beginning of the day were generally low (Fig. 1) showing that most of the carbohydrate stored in the leaves during the previous day had been remobilised during the night. Sucrose and starch were the principal carbohydrates accumulated in most species; no evidence was found for accumulation of fructans in any of the species examined and only *Atriplex spongiosa* contained substantial amounts of hexose sugars. Although the proportions of sucrose and starch varied widely the total amount of carbohydrate accumulated in leaves of these different species was similar (range 102–186 µmol hexose·mg^{-1} Chl; Fig. 1). The ratios of starch:sucrose accumulated during the day varied widely from about 1:1 in *Eleusine coracana* and *Eleusine indica* to about 99:1 in *Atriplex spongiosa* (Table 1). A similarly wide range of values has been observed in C_3 plants (Gordon 1986; Huber 1989). There was no obvious correlation between the C_4 decarboxylation subtype and preference for sucrose or starch as the leaf storage carbohydrate. However, the three dicotyledonous species studied (*Flaveria bidentis*, *Amaranthus edulis* and *Atriplex spongiosa*) all showed a marked preference for starch as the leaf storage carbohydrate (Fig. 1, Table 1).

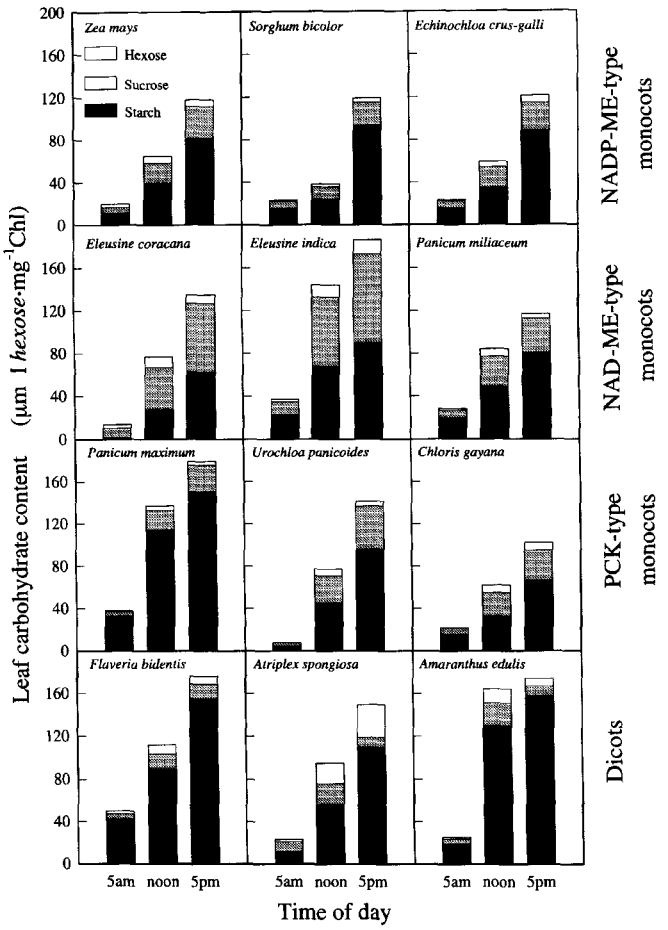

Fig. 1. Diurnal accumulation of carbohydrates in leaves of C_4 plants. Leaves were harvested at 5 a.m., noon and 5 p.m., and their contents of glucose, fructose, sucrose and starch were measured. Data are means of measurements on three separate leaves. The daily dose of photosynthetically active radiation ranged between 35 and 41 mol quanta·m^{-2} for the different experiments. This compares with a potential maximum daily radiation dose for mid-summer in Canberra of about 49 mol quanta·m^{-2} and 15 mol quanta·m^{-2} for mid-winter. *ME*, malic enzyme; *PCK*, phosphoenolpyruvate carboxykinase

In some species, especially those which show a preference for starch as the leaf storage carbohydrate, the sucrose content of the leaves did not increase greatly between noon and the end of the day (Fig. 1). Gordon (1986) found that in several C_3 species that also store starch preferentially, the amount of sucrose in the leaf increased during the first hour of illumination, but remained at a low, relatively constant level throughout the rest of the day. In these species it appears that once the amount of sucrose in the leaf has reached a certain level, perhaps that which is optimal for sucrose export, no further sucrose is accumulated. However, in other C_4 species examined in our studies (Fig. 1) and in some C_3 species (Gordon 1986) the sucrose content of the leaves increases throughout the day.

Measurement of primary photosynthate partitioning in C_4 leaves. As indicated in the *Introduction*, the primary

Table 1. Diurnal accumulation of carbohydrates in leaves of C_4 plants. The increases in leaf contents of hexose, sucrose and starch shown represent the difference between the levels at 5 a.m. and 5 p.m; calculated from the data in Fig. 1. For details of the full daily radiation dose received see Fig. 1

Species	Diurnal increase in the leaf (μmol hexose·mg^{-1} Chl)		
	Hexose	Sucrose	Starch
NADP-ME-type			
Zea mays	2	25	70
Sorghum bicolor	3	15	79
Echinochloa crus-galli	5	20	73
Flaveria bidentis[a]	6	9	112
NAD-ME-type			
Panicum miliaceum	3	25	61
Eleusine indica	12	71	67
Eleusine coracana	5	55	61
Atriplex spongiosa[a]	28	< 1	99
Amaranthus edulis[a]	6	4	139
PCK-type			
Panicum maximum	2	21	118
Chloris gayana	7	22	51
Urochloa panicoides	4	37	92

[a] Dicotyledonous species. The remainder are grasses

Table 2. Effect of varying time of preillumination on photosynthate partitioning in maize. Third maize leaves (counted from the base) were harvested from plants which had been kept in the dark for 14 h. The leaves were illuminated at an irradiance of 1200 μmol quanta·m^{-2}·s^{-1} for various periods of time before measuring the rate of fixation of $^{14}CO_2$ and incorporation of ^{14}C into sucrose and starch, and the sucrose content of the leaves. Data are measurements from single leaf samples, the means of the two samples are shown in parentheses

Preillumination time (min)	Rate of CO_2 assimilation (μmol·min^{-1}·mg^{-1} Chl)	Ratio of ^{14}C partitioning sucrose/starch	Leaf sucrose content (μmol hexose·mg^{-1} Chl)
0	n.d.[a]	n.d.[a]	(3)
5[b]	7.5, 6.7 (7.1)	2.8, 2.0 (2.4)	10, 12 (11)
18	6.9, 7.8 (7.3)	2.5, 2.4 (2.4)	12, 16 (14)
33	8.6, 7.9 (8.2)	2.3, 2.3 (2.3)	21, 22 (21)
50	8.4, 7.3 (7.8)	1.9, 2.6 (2.2)	29, 33 (31)

[a] Not determined

[b] Leaves were illuminated at an irradiance of 200 μmol quanta·m^{-2}·s^{-1} for 25 min then at 1200 μmol quanta·m^{-2}·s^{-1} for 5 min.

partitioning of photosynthate between sucrose and starch is an important factor in determining the proportions of sucrose and starch that accumulate in the leaves during the day (Huber 1981; Huber 1989) and the diurnal pattern of export of carbohydrate from the leaves to the sink tissues (Heineke et al. 1994; Leidreiter et al. 1995). In view of the importance of this initial partitioning process we developed a procedure for measuring the partitioning of photosynthate between sucrose and starch in leaves under physiological conditions. The method was based on the earlier observations (Hatch and Slack 1966; Hatch 1971) that when leaves of C_4 species are provided with $^{14}CO_2$ in the light, labelling of sucrose and starch begins within 30 s, and that by about 60 s the ratio between the rates of incorporation into sucrose and starch remains essentially constant. In this procedure (see *Materials and methods* for details), leaves photosynthesising at a steady rate in air are supplied with $^{14}CO_2$, and sampled at intervals in the range from 60 to 140 s after the start of the pulse to determine the incorporation of ^{14}C into sucrose and starch.

Gas-exchange measurements on a range of C_4 leaves showed that the rate of photosynthesis did not reach a steady state until 10–20 min after illumination under the conditions used for our partitioning analysis (data not shown); accordingly an illumination period of at least this order would be required prior to supplying $^{14}CO_2$ to determine partitioning. To check the effect of the time of preillumination of detached leaves on the subsequent rates of CO_2 fixation, and particularly the partitioning of photosynthate, we illuminated maize leaves at high light for periods of 5, 18, 33 or 50 min before supplying $^{14}CO_2$. The partitioning of photosynthate between sucrose and starch was not significantly affected by varying the length of time the leaves were illuminated before supplying the $^{14}CO_2$ (Table 2). However, the rate of CO_2 fixation was slightly higher after 33 min illumination compared with 5 or 18 min (Table 2). On the basis of these results we adopted a standard illumination period of 30 min before $^{14}CO_2$ was added.

For technical reasons it was most convenient to work with detached leaves; however, we compared partitioning in detached *Sorghum* leaves and in leaves still attached to the plant to check whether detaching the leaves from the plant had any effect. The absolute incorporation of ^{14}C into sucrose in the detached leaves was slightly lower than in the attached leaves, but the ratios between the rates of incorporation of ^{14}C into sucrose and starch in the detached and attached leaves were almost identical (Fig. 2), as were the rates of CO_2 fixation. Similar results were obtained for attached and detached leaves of *Echinochloa crus-galli*.

The effect of leaf age on partitioning was investigated in detached maize leaves (Table 3). Partitioning was measured in the third leaf (counted from the base) taken from 19- to 29-d-old plants on the same day so that all the plants had the same recent environmental history. The ratios of partitioning into sucrose and starch were very similar in leaves of different ages. The effect of varying leaf number on partitioning was also investigated in maize using the second, third and fifth leaves after each leaf had just reached full expansion (again taken on the same day from plants of different ages). The ratios of incorporation of ^{14}C into sucrose and starch were very similar in the different leaves (Table 3). These observations suggested that measurements of partitioning in any mature leaf would be reasonably representative of the pattern of partitioning in the other photosynthetically active leaves on that plant.

The variation in the ratio of ^{14}C partitioning into sucrose and starch for replicate leaves from the same set of plants was relatively small (see Tables 2, 3). The partitioning ratio also remained relatively constant for leaves of particular species grown in the glasshouse at different

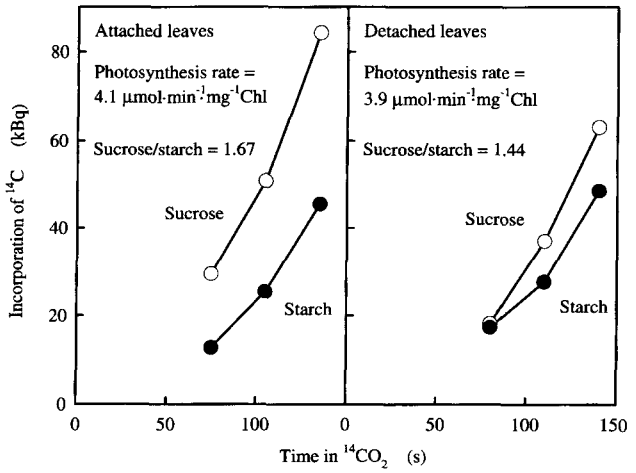

Fig. 2. Partitioning of photosynthate in attached and detached leaves of sorghum. Three detached leaves and three attached leaves of sorghum (*Sorghum bicolor* cv NK-150) undergoing steady-state photosynthesis were supplied with $^{14}CO_2$ at the same time in the same chamber, and sampled at 75, 105 and 135 s after the start of the pulse, to determine the rates of incorporation of ^{14}C into sucrose and starch (see *Materials and methods* for details). The values shown were normalised using the mean rate of CO_2 fixation of the three leaves as the reference

Table 3. Effect of varying leaf number and leaf age on the photosynthesis rate and photosynthate partitioning in maize. The rates of fixation of $^{14}CO_2$ and incorporation of ^{14}C into sucrose and starch were measured in the second, third and fifth leaves (counted from the base) of maize plants of different ages (see *Materials and methods* for details). The third leaf from the 19-d-old plant was not fully expanded. For the comparison of leaves 2, 3 and 5, each of the leaves was taken from plants at the stage when they were just fully expanded. The rates of CO_2 fixation shown are the mean ± SD of measurements from three leaves. The ratios of ^{14}C partitioning shown were derived from measurements on two leaves as described in *Materials and methods*

Leaf number counted from the base	Age of plant (d)	Rate of CO_2 assimilation (μmol·min^{-1}·mg^{-1} Chl)	Ratio of ^{14}C partitioning sucrose/starch
Expt. 1			
3	19	6.5 ± 0.9	3.4
3	22	6.2 ± 0.1	3.7
3	26	6.0 ± 1.0	2.3
3	29	5.8 ± 0.3	3.0
Expt. 2			
2	19	4.9 ± 0.5	2.6
3	22	5.4 ± 0.1	2.7
5	36	5.3 ± 0.3	2.7

times of the year. For example, in four separate experiments on maize (two in summer and two in winter), the ratio of photosynthate partitioning measured in the third leaf under identical temperature and light conditions ranged from 2.3 to 4.0, with a mean ratio of 3.0 ± 0.5 (mean ± SD, $n = 8$). Under our glasshouse conditions the third leaf of maize reaches full expansion in about 13 d in mid-summer compared with about 20 d in mid-winter. As will be illustrated below, the variation in the partitioning ratio between different C_4 species was more than 15-fold.

Survey of primary partitioning of photosynthate in leaves of C_4 species. The rates of photosynthesis, primary partitioning of photosynthate between sucrose and starch, and the hexose, sucrose and starch contents of leaves were measured in a range of C_4 species at the beginning and end of the day (Table 4). The partitioning of photosynthate between sucrose and starch was found to vary widely between species. The ratios of incorporation of ^{14}C into sucrose and starch ranged from about 0.5 in those species that accumulated mostly starch in the leaves (*Atriplex spongiosa*, *Amaranthus edulis* and *Flaveria trinervia*) to about 8 in *Eleusine indica*, which accumulated mostly sucrose. Between these extremes the correlation between initial partitioning of photosynthate and the type of carbohydrate stored in the leaves was less consistent. For example, the primary partitioning of photosynthate into sucrose versus starch was about 3 to 4 times higher in the NADP-ME-type monocotyledonous species compared with the PCK-type species, but the ratios of sucrose to starch accumulated during the day were very similar in both groups (Table 4).

In most of the species examined, partitioning of photosynthate into starch was greater at the end of the day than at the beginning but, with the exception of *Flaveria trinervia*, the rates of CO_2 fixation were similar at both times of day (Table 4). Apparently, there was no substantial feedback inhibition of photosynthesis by carbohydrate accumulation in the leaves of these C_4 plants even with the high level of photosynthetically active radiation that the plants received (see legend to Table 4) and the high levels of sucrose accumulated. Blechschmidt-Schneider et al. (1989) found that the rate of CO_2 assimilation in *Amaranthus edulis* showed little change during the day as carbohydrate, mostly starch, accumulated in the leaves. They only observed a decline in the rate of CO_2 assimilation when translocation was slowed by chilling the petiole, so that the sucrose content of the leaf increased severalfold over that normally found. Our results and those of Blechschmidt-Schneider et al. (1989) suggest that many C_4 species may avoid feedback inhibition of photosynthesis as carbohydrate accumulates in the leaves in the short term by adjusting the partitioning of photosynthate between sucrose and starch. However, this does not exclude the possibility that feedback inhibition may occur under other conditions, especially in the longer term where sink demand for carbohydrate is reduced (Herold 1980; Azcón-Bieto 1983; Goldschmidt and Huber 1992). It is notable in this regard that Goldschmidt and Huber (1992) found evidence that increases in sucrose or starch may not be directly responsible for feedback inhibition of photosynthesis, and suggested that some other, closely related metabolite may be involved. There is increasing evidence that carbohydrates can affect the expression of genes coding for photosynthetic and respiratory enzymes (Sheen 1989; Krapp et al. 1993; Krapp and Stitt 1994), and that such regulation may play a role in source-sink relationships.

Discussion

The primary partitioning of photosynthate between sucrose and starch is determined by the relative fluxes

Table 4. Diurnal changes in photosynthate partitioning and accumulation of carbohydrates in leaves of C_4 species. The carbohydrate content and the rates of $^{14}CO_2$ fixation and incorporation of ^{14}C into sucrose and starch were measured in leaves harvested at the beginning of the day (a.m.) and after about 8 h illumination in a naturally lit glasshouse (p.m.). The daily dose of photosynthetically active radiation ranged between 25 and 46 mol quanta·m^{-2} (except about 12 mol quanta·m^{-2} for *A. edulis*). The potential maximum daily radiation for the mid-summer period in Canberra (December/January) is about 49 mol quanta·m^{-2} compared with 15 mol quanta·m^{-2} for mid-winter. The rates of CO_2 fixation and leaf carbohydrate content shown are the mean ± SD of measurements from three leaves. The ratios of ^{14}C partitioning shown were derived from measurements on two leaves as described in *Materials and methods*.

Sub-group and species	Rate of CO_2 assimilaion (μmol·min^{-1}·mg^{-1} Chl)		Ratio of ^{14}C partitioning sucrose/starch		Leaf carbohydrate content (μmol hexose·mg^{-1}Chl)		Ratio of sucrose to starch accumulated during the day
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	
Monocotyledenous species							
NADP-ME-type							
Zea mays	4.6 ± 0.5	4.2 ± 0.4	4.0	2.6	14 ± 1	93 ± 1	0.41
Sozghum bicolor cv. TE-33	3.2 ± 0.3	3.3 ± 0.2	5.3	3.0	14 + 0	86 ± 1	0.35
Echinochloa crus-galli	5.5 ± 0.1	5.2 ± 0.1	3.2	2.6	25 ± 1	124 ± 12	0.36
NAD-ME-type							
Panicum miliaceum	4.7 ± 0.5	6.0 ± 0.6	3.0	1.6	20 ± 3	141 ± 20	0.31
Eleusine indica	3.9 ± 0.4	4.5 ± 0.5	8.4	3.1	26 ± 1	94 ± 14	1.03
Eleusine coracana	4.2 ± 0.3	3.8 ± 0.5	2.2	2.1	18 ± 2	120 ± 31	0.94
PCK-type							
Panicum maximum	4.3 ± 0.5	5.4 ± 0.3	1.0	0.60	14 ± 3	157 ± 17	0.27
Chloris gayana	4.5 ± 0.7	4.3 ± 0.5	1.7	0.95	16 ± 1	164 ± 41	0.42
Urochloa panicoides	6.2 ± 0.7	5.9 ± 0.2	1.1	0.72	20 ± 4	152 ± 26	0.45
Dicotyledonous species							
NADP-ME-type							
Flaveria bidentis	5.6 ± 0.6	5.7 ± 0.5	2.3	1.4	24 ± 3	94 ± 20	0.11
Flaveria trinervia	5.8 ± 0.7	2.9 ± 0.3	0.49	0.45	24 ± 1	138 ± 22	0.02
NAD-ME-type							
Atriplex spongiosa	5.0 ± 0.7	4.4 ± 1.0	0.48	0.46	21 ± 4	96 ± 15	0.06
Amaranthus edulis	4.0 ± 1.2	4.3 ± 0.1	0.91	0.43	27 ± 9	104 ± 8	0.03

through the highly regulated series of reactions leading from the chloroplast triose-phosphate pool to these end products. The regulation of these reactions has been well characterised in spinach and maize (Stitt et al. 1987; Kalt-Torres et al. 1987; Usuda et al. 1987; Huber and Huber 1992). Only a few attempts have been made to measure primary partitioning in leaves and for C_4 plants only maize has been examined. Stitt (1985) assessed partitioning by measuring the relative rates of accumulation of sucrose and starch in maize leaf segments, which were unable to export sucrose. In another study, Usuda and Shimogawara (1992) deduced the pattern of partitioning in maize leaves from measurements of the photosynthesis rate, storage of carbohydrates and the rate of sucrose export. Huber (1981) assessed partitioning more directly by feeding $^{14}CO_2$ to protoplasts from C_3 leaves and measuring the incorporation of ^{14}C into sucrose and starch. A similar radiotracer-based procedure was used to assess partitioning in C_3 leaves or leaf discs in a leaf-disc oxygen-electrode chamber or similar apparatus (Kruckeberg et al. 1989; Neuhaus et al. 1990; Sharkey et al. 1992). Except for the latter study these analyses were not conducted under totally steady-state conditions and high, saturating concentrations of CO_2 were employed. In our procedure, segments of grass leaves up to 15 cm long or whole leaves of dicotyledonous species were exposed to $^{14}CO_2$ under steady-state conditions for photosynthesis and with near ambient levels of CO_2. With two species we showed that patterns of ^{14}C incorporation into sucrose and starch were very similar in detached leaves and leaves still attached to the plant. We also showed that the pattern of primary partitioning was not affected by varying the period of illumination prior to adding $^{14}CO_2$ in the range from 5 to 50 min, even in leaves that had been darkened overnight (Table 2). There was good agreement between the ratios of photosynthate partitioning observed in replicate leaves, both within experiments (Tables 2, 3), and when leaves from a given species were analysed under identical conditions in different experiments. From these results we suggest that our procedure gives a reliable and reproducible measure of the intrinsic primary partitioning of photosynthate in leaves under physiological conditions.

Our survey of C_4 species showed a wide range of partitioning behaviour with ratios of incorporation of radioactive photosynthate into sucrose and starch varying more than 20-fold (0.4 to 8, see Table 4). Generally, those species that partitioned more photosynthate into sucrose accumulated higher ratios of sucrose to starch in the leaves during the day and vice versa. Much of the sucrose synthesised in the leaves during the day is exported from the leaf to the rest of the plant, and this export would account for the discrepancy between the ratios of partitioning of photosynthate into sucrose and starch and the ratios of sucrose and starch accumulated in the leaves during the day (Table 4). These results also illustrate the point that measurements of the sucrose and starch contents of the leaves would generally be a poor indicator of the primary partitioning of photosynthate. For instance, the three NADP-ME-type monocotyledonous species and the three PCK-type species we examined stored similar proportions of sucrose to starch in the leaves during the light period, but the ratio of primary partitioning of

photosynthate into sucrose versus starch was 3 to 4 times higher in the NADP-ME-type species (Table 4). It would appear that the NADP-ME-type species exported a larger proportion of the sucrose synthesised during the day than the PCK-type species. Further studies will determine whether these distinct differences between the C_4 subgroups are related to either differences in the cellular location of sucrose and starch synthesis (mesophyll versus bundle-sheath cells) or the different mechanisms of C_4 photosynthesis operating in these species.

With the exception of *Flaveria trinervia*, no evidence was found for feedback inhibition of photosynthesis as carbohydrate accumulated in the leaves during a full day of photosynthesis at high light. However, a shift in partitioning favouring starch synthesis was seen in most species as the levels of sucrose and starch increased in the leaf during the day. Stitt (1985) found that partitioning of photosynthate into starch in maize leaves showed a positive correlation with the leaf content of fructose 2,6-bisphosphate, which in turn was correlated with the sucrose content of the leaf. However, we found that the partitioning of photosynthate between sucrose and starch was the same in detached maize leaves with sucrose contents ranging from about 35 to 100% of that found at the end of the day (30 μmol hexose·mg^{-1} Chl; Fig. 1) (Table 2). This result suggests that accumulation of sucrose in the leaf may not directly influence the partitioning of photosynthate between sucrose and starch. Further work will be directed towards elucidating the regulatory mechanisms controlling the partitioning of photosynthate in C_4 leaves.

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