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Assimilate partitioning in leaves of the raffinose-storing herb *Lamium album* L.: photosynthesis and carbon partitioning throughout the photoperiod

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ABSTRACT – (Assimilate partitioning in leaves of the raffinose-storing herb *Lamium album* L.: photosynthesis and carbon partitioning throughout the photoperiod). *Lamium album* accumulates starch, sucrose and raffinose-family oligosaccharides (RFO) as the major products of photosynthesis. These products were measured in leaves throughout a sixteen-hour photoperiod and under various irradiance conditions. There was continuous accumulation of sucrose and starch. The rate of gas exchange was higher at 500 $\mu Em^2 s^{-1}$ and 900 $\mu Em^2 s^{-1}$ than at 300 $\mu Em^2 s^{-1}$. The rate of photosynthesis did not decline over the sixteen-hour photoperiod, which suggested that there was no short-term feed back inhibition due to sucrose accumulation in this plant. When the products of photosynthesis were compared at the end of the photoperiod, only sucrose increased in abundance at high irradiance. The RFO pool in leaves was shown to contain raffinose, stachyose and verbascose; galactinol was also present. $^{14}CO_2$ feeding demonstrated that roots and flowers were the major sinks. The middle leaves were major source leaves whilst young leaves acted as both sources and sinks.

Key words - *Lamium album*, partitioning, raffinose family oligosaccharides, starch, sucrose

RESUMO – (Partição de assimilados em folhas de *Lamium album* L., uma planta herbácea que acumula rafinose: fotossíntese e partição de carbono ao longo do fotoperíodo). *Lamium album* acumula amido, sacarose e oligossacarídeos da família rafinósica (OFR) como produtos principais da fotossíntese. Estes produtos foram medidos em folhas durante um fotoperíodo de 16 horas e sob várias condições de irradiância. Houve um acúmulo contínuo de sacarose e amido. As taxas de trocas gasosas foram maiores a 500 e 900 $\mu Em^{-2} s^{-1}$ do que a 300 $\mu Em^{-2} s^{-1}$. A taxa de fotossíntese não declinou durante o fotoperíodo de 16 horas, o que sugere que não existe um mecanismo inibitório de retroalimentação de curto prazo devido ao acúmulo de sacarose nesta planta. Quando os produtos da fotossíntese foram comparados ao final do fotoperíodo, apenas a sacarose aumentou em abundância em alta irradiação. Os OFR foram analisados e são compostos por rafinose, estaquiose e verbascose; galactinol também esteve presente. O fornecimento de $^{14}CO_2$ demonstrou que raízes e flores foram os principais drenos. As folhas do meio foram as principais fontes enquanto folhas jovens atuaram como fonte e dreno ao mesmo tempo.

Palavras-chave - amido, *Lamium album*, oligossacarídeos da família rafinósica, partição, sacarose

Introduction

In mature leaves, carbon fixed in photosynthesis can be considered as having three contrasting short-term fates. It can be exported via the phloem, generally but not exclusively as sucrose; it can be further metabolised or respired or it can be stored for export or metabolism at a later date. The balance between these three fates is dynamic, regulated and sensitive to both external and internal signals (Farrar 1999). The internal signals can be from within the leaf or from distant tissues and the regulatory changes that they induce may alter the rate of carbon fixation as well as the partitioning of fixed carbon (Pollock & Farrar 1996, Stitt 1996). In leaves of C3 dicots such as spinach, tobacco and *Arabidopsis*, there is a complex set of regulatory interactions. This ensures that optimal rates of ribulose bisphosphate regeneration occur even under fluctuating inputs; that the rate of sucrose synthesis is matched both to the instantaneous capacity of the photosynthetic machinery and to the demand status of distant sinks and finally that plastid starch is accumulated to an extent that will sustain carbon export at night and act as a temporary sink for photosynthate when supply exceeds demand (Pollock & Farrar 1996, Stitt 1996). Linked to these regulatory systems is longer-term modulation of gene expression that is sensitive to assimilate abundance in the leaves and that may regulate the abundance of key photosynthetic proteins (Jang & Sheen 1994). Within vascular plants, there are a number of variations on this theme. Temperate C3 forage grasses contain little plastid

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starch and show different patterns of end-product partitioning. The rate of sucrose synthesis does not appear to be sensitive to assimilate abundance, but excess sugars are stored in the vacuole as sucrose or fructans (Cairns *et al.* 2000). The enzymes that synthesise fructans from sucrose are inducible, the induction requires altered gene expression and it is driven by accumulation of sucrose (Cairns *et al.* 2000).

Fructans are not the only alternative storage sugars found in plant leaves. Members of the raffinose series of oligosaccharides (RFO) accumulate in a number of plant groups including the Labiateae and Cucurbitaceae, where they are also translocated (Keller & Pharr 1996). In leaves of these plants, they generally accumulate in concert with plastid starch rather than as an alternative. The pathways of RFO biosynthesis have been well characterised. Sucrose acts as an acceptor for the transfer of galactose residues from galactinol and there may also be direct galactosyl transfer from donor to acceptor RFO in order to achieve further chain elongation in a manner analogous to fructans (Bachmann & Keller 1995).

In contrast to grasses and starch-storing dicots, the physiology of the short-term interactions between photosynthesis, sink demand, sucrose synthesis and RFO metabolism is not well studied in members of the Labiateae. Bachmann *et al.* (1994) made both seasonal and developmental measurements of long-term partitioning in leaves of the perennial labiate *Ajuga reptans* L., and demonstrated the involvement of starch, sucrose and RFO. These workers also showed the presence of a significant storage pool of larger RFO, particularly under cold conditions. In this study, we investigated short-term assimilate partitioning in *Lamium album* L. (white dead-nettle). This species was chosen because it is herbaceous, overwintering via rhizomes, and because the leaves contain both starch and RFO. It provides, therefore a good comparison with grasses and other species in which short-term assimilate partitioning has been studied. The aim of the experiments reported in this paper was to characterise the diel patterns of photosynthesis and carbohydrate partitioning within mature source leaves under different irradiance conditions.

Material and methods

Plant material - Cuttings were taken from a single plant of *Lamium album* grown in Cambridge. Stock plants were maintained in a glasshouse under natural irradiance, at temperatures above 15 °C. For experimental work, cuttings were rooted in soil and then and subsequently transferred to nutrient solution (Zeeman & ap Rees 1999). Plants in soil were grown at an irradiance of 300-350 $\mu Em^{-2}s^{-1}$ and hydroponically-grown plants at 500-550 $\mu Em^{-2}s^{-1}$. In all cases the photoperiod was 12 or 16 h, the temperature 20 °C and the relative humidity 70%. Hydroponically-grown plants were transferred to a range of experimental conditions at the five paired-leaf stage one week before the experiment commenced.

Gas exchange measurements - Plants of *L. album* were transferred to a two-compartment perspex chamber that separated shoots and roots by means of a flexible rubber/vaseline seal around the lower stem. Ambient air was pumped through the upper chamber at 470 cm^3 and the outflow monitored by infra-red gas analysis (IRGA 225, ADC Co Ltd, Cambridge). For illumination, four 50 W metal halide bulbs were used, with a 3 mm heat absorbing filter placed below each bulb. Irradiance was altered by varying the distance between the light source and the plant and was measured with a photon sensor (Skye Instruments, Powys, UK). Shielding the leaves with silver foil in the light indicated that net gas exchange from stem tissues was negligible so the values for effluent CO_2 were used to calculate leaf gas exchange.

Metabolite measurements - Leaves (0.2-0.3 g fresh wt.) were killed and extracted in boiling 80% aqueous ethanol as described by Pollock & ap Rees (1975). Ethanol-insoluble material was re-suspended in 2-10 mL deionised water to give the insoluble fraction. Starch was measured in the insoluble fraction following digestion with 12 units of amyloglucosidase and 1.1 units of α-amylase (Sigma UK) according to Stitt *et al.* (1978). Fructose and glucose were measured enzymatically (Beutler 1984, Kunst *et al.* 1984). Sucrose was assayed in the same way after enzymatic hydrolysis (Lyne & ap Rees 1971). Galactose, raffinose and stachyose were separated by descending paper chromatography using Whatman Nº 3 paper eluted with butan-1-ol: pyridine: H_2O (6:4:3 by volume). The galactose content was measured enzymatically using galactose dehydrogenase in eluted material that co-migrated with the relevant marker. Raffinose and stachyose were hydrolysed prior to assay (500 mM HCl for 150 min at 100 ºC; Tanner *et al.* 1968).

Radiotracer analysis of sources and sinks - Plants grown at 550 $\mu E\ m^{-2}\ s^{-1}$were placed in an airtight perspex container where the roots and shoots were in separate compartments. Plants were illuminated in direct natural sunlight (1,000-1,200 $\mu Em^{-2}\ s^{-1}$). The upper chamber was filled with CO_2-free air, sealed and $^{14}CO_2$ released from 50 μCi of sodium[^{14}C]bicarbonate (0.1 milicurie. $mmole^{-1}$) by addition of excess lactic acid. After 1 h, unassimilated $^{14}CO_2$ was trapped in 10% (w/v) KOH. For the chase period, the chamber was re-sealed and air circulated through the upper chamber at 1 $dm^3\ min^{-1}$. Plants were harvested immediately after the feeding period, and 4 or 24 hours into the chase period (the latter after 12 h darkness). Plants were divided into lower, middle and upper (youngest) leaves; roots; flowers; petioles

and stems. Samples were killed and extracted as described above. Radioactivity in soluble components was measured by scintillation counting of aliquots (20-100 µL). The soluble fractions were separated into neutral, acidic and basic components by ion exchange chromatography on 5 mL tandem columns of Dowex 50 (H^+form) and Dowex-1 (Cl^- form). Neutral compounds were eluted from the tandem columns using 5 volumes of deionised water. The cation column was eluted with 10 mL 1M NH_4OH to give the basic fraction (mainly amino acids) and the anion column with 8 mL 2M formic acid followed by 2 mL 8M formic acid to give the acidic fraction (non-volatile organic acids plus acid-stable phosphorylated intermediates; Harley & Beevers 1963) The neutral components were further separated by thin layer chromatography (TLC) using three ascending developments of Schleicher and Schuell F1500 pre-prepared silica gel plates with butan-1-ol: propan-2-ol: H_2O (3:12:4 by volume; Cairns & Pollock 1988). Radioactivity was localised by autoradiography, quantified densitometrically and products identified by co-chromatography with known markers except as discussed below (Cairns & Pollock 1988). Radioactivity in the insoluble material was measured after digestion of aliquots (50-200 µL) by 0.8 mL of Scintran tissue solubiliser and 0.2 mL of water. This mixture was incubated at 55 °C for 8 hours, cooled and 24 µL of glacial acetic acid was added prior to liquid scintillation counting. Aliquots (500 µL) of the insoluble material from middle leaves were also digested with amylase/ amyloglucosidase as described above. After centrifugation at 13.000 *g* for 5 minutes, the supernatant was separated by TLC as above. Autoradiographs were prepared for densitometry (Cairns & Pollock 1988) and then areas on the plate corresponding to marker glucose were tremoved, washed with deionised water (3 × 2 mL) and the pooled material concentrated and assayed enzymatically for glucose as above. Identification of unknown compounds - Two "unknown" compounds were detected by autoradiography of neutral extracts from leaves. These were further characterised using a non-radioactive neutral extract prepared as above from 30-40 g of glasshouse-grown *L. album* leaves. After separation of aliquots by TLC, relevant areas were eluted and analysed further. This neutral extract (1 µL) was used for TLC separations of the unknown compounds as described above before and after treatment with invertase (BDH, 5 units, 6 h) and α-galactosidase (Sigma UK, 3 units, 6 h). The TLC plates were stained with carbazole-sulphuric acid (all sugars); and urea-HCL (ketose sugars; Churms 1982). Some samples were eluted from TLC plates and analysed further. For gas chromatography/mass spectrometry (GC/MS), extracts were dried, trimethylsilylated using BSTFA: TCMS (10:1 in pyridine, 60 min at 75 °C) and separated on a 30 M × 0.2 mm column coupled to an ion trap mass spectrometer working in electron impact mode (Peterbauer *et al.* 1998). For high performance liquid chromatography (HPLC), extracts were diluted with distilled H_2O and separated by ion exchange (Dionex PA10 eluted with 100 mM NaOH using pulsed amperometric detection; Peterbauer & Richter 1998).

Chromatography standards - Reference sugars were obtained from Sigma apart from verbascose (Megazyme International, Ireland) and galactinol (gift from Dr. F. Keller, University of Zurich)

Results and Discussion

Photosynthesis and carbohydrate composition during the photoperiod -The carbohydrate composition of mature source leaves was determined over a 16 hours light period (figure 1). Starch and sucrose were the two major products of photosynthesis and their rates of

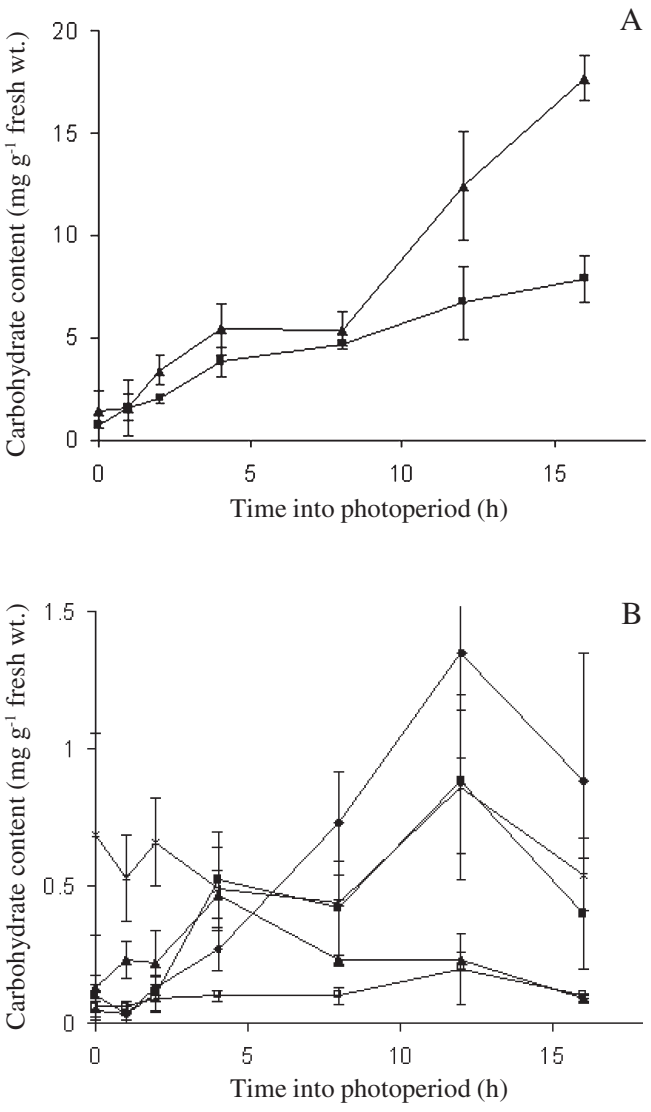

Figure 1. Accumulation of carbohydrates by attached leaves of *Lamium album* during a 16 h photoperiod. A: sucrose (▲) and starch (■). B: glucose (◆), fructose (■), galactose (□), raffinose (▲), and stachyose (×). All values are mean ± sem of triplicate measurements.

accumulation were approximately linear throughout the light period. Hexose contents were lower, reaching a maximum after some 12 h and declining thereafter. Raffinose and stachyose contents were low and showed no discernible trend. Assimilate accumulation was observed throughout the photoperiod, suggesting that sucrose did not significantly inhibit its own synthesis. Goldschmidt & Huber (1992) observed the same in a range of starch- and sugar-storing species. RFO were also present; Kandler (1967) found these oligosaccharides as products of photosynthesis in a number of plants from the Lamiaceae

The rate of photosynthesis was compared with the accumulation of the products of photosynthesis. Gas exchange measurements were made at 330, 500 and 900 $\mu Em^{-2}s^{-1}$ (figure 2). In all cases net CO_2 exchange increased slightly for the first four hours of illumination and remained constant for the rest of the light period. There was no evidence of any decrease in rate late in the photoperiod. An increase in the irradiance from 330 to 500 $\mu Em^{-2}s^{-1}$ caused a marked increase in the rate of net fixation of CO_2. Increasing the irradiance further to 900 $\mu Em^{-2}s^{-1}$ led to a smaller increase in the rate of net CO_2 exchange. In some species, photosynthesis decreases with the accumulation of sucrose (Farrar & Farrar 1985). *L. album* accumulates sucrose (figure 1) but showed no evidence of inhibition of photosynthesis across the photoperid at any of the irradiance values used in the study. At 330 $\mu Em^{-2}s^{-1}$, sucrose and starch were both synthesised simultaneously and continuously up to 16 hours light in *L. album*, suggesting that there was no threshold value for sucrose accumulation before starch synthesis began (Gordon *et al.* 1980).

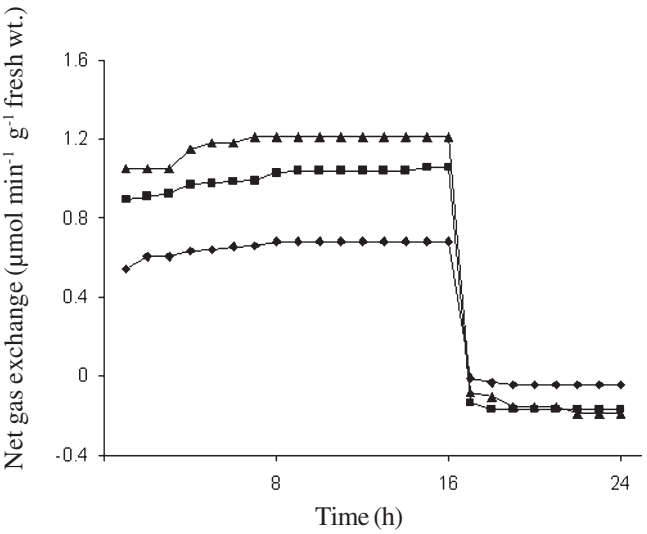

Figure 2. Rate of net carbon exchange in attached leaves of *Lamium album* during a 16 h light period and subsequent 8 h dark period. The net rate of photosynthesis was measured at 330 (◆), 500 (■), and 900 (▲) $\mu Em^{-2}\ s^{-1}$

Carbohydrate accumulation at higher irradiance - In order to see whether elevated photosynthetic rates (figure 2) altered partitioning, the products of photosynthesis were measured at the end of a 16 hour light period at 330 and 500 $\mu Em^{-2}s^{-1}$ and the differences compared. A statistically significant increase in sucrose accumulation was observed at 500 $\mu Em^{-2}s^{-1}$. No significant increases in starch or RFO were observed at increased irradiance (table 1). In soybean leaves that were acclimated to high irradiance, an increase in sucrose was observed, whereas starch contents remained the same (Silvius *et al.* 1979). However, in species such as tomato (Ho 1976), *Sorghum sudanense* (Piper) Stapf. (Wardlaw & Marshall 1976), and barley (Gordon *et al.* 1980), simultaneous increases in starch and sucrose contents were observed.

Partitioning of ^{14}C after whole plant feeding - The distribution of sources and sinks within *L. album* was studied by measuring redistribution of radioactivity between plant parts following a 1 h feed (table 2). Young leaves and flowers functioned as strong sinks, with roots, stems and petioles being weaker, transient sinks. The maximum decline in soluble and insoluble radioactive fractions during the chase period was observed in middle leaves, which indicated that these were the major source leaves. Lower leaves also acted as a source leaves. Young leaves also fixed carbon but, since there was a

Table 1. Differences in leaf carbohydrate content in middle leaves of *Lamium album* harvested after 16 h illumination either at 330 or 500 $\mu Em^{-2}s^{-1}$. The values represent the increase in content at 500 $\mu Em^{-2}s^{-1}$ and are means ± sem of 5 determinations. The values for sucrose were significant at the 0.1% level; the values for the other carbohydrates were not significant at the 5% level (student's T-test).

	Starch	Sucrose	Raffinose	Stachyose
Enhancement in carbohydrate content attributable to exposure to500 $\mu Em^{-2}s^{-1}$ (mg g^{-1}fresh wt.)	1.0 ± 0.9	6.7 ± 0.8	0.5 ± 1.2	1.1 ± 1.0

Table 2. Distribution of ^{14}C as a percentage of the total radioactivity measured in plants of *L.album* harvested at various times after feeding $^{14}CO_2$. Before assay, each plant was divided into Root, LS (lower stem), US (upper stem), LL (lower leaf), ML (middle leaf), YL (young leaf), FL (flower) and P (petioles). Values are mean of duplicates.

Tissue	Root	LS	US	LL	ML	YL	FL	P (Sol+Insol)	Total Radioactivity (KBq)
After 1 h pulse									
Soluble	0.7	5.0	5.0	9.4	28.9	15.6	4.6	19.6	323
Insoluble	0.1	0.4	0.5	1.4	5.9	2.1	0.8		
After 4 h chase									
Soluble	2.2	2.3	5.1	8.3	17.7	13.7	14.0	22.6	308
Insoluble	0.9	1.2	0.9	0.8	2.8	5.4	1.9		
After 24 h chase									
Soluble	3.2	2.6	2.8	4.5	10.6	10.0	14.0	22.9	165
Insoluble	3.3	2.6	2.3	1.2	4.7	8.2	4.9		

marked increase in the insoluble component, they behaved as net sinks. Flowers and roots also acted as sinks. Flowers were the strongest sinks, since radioactivity in the soluble as well as in the insoluble fractions increased over time. These results were in accordance with data from soybean, showing that diurnal variation in storage was greatest in the leaf and decreased in the order leaf > petiole > stems > roots (Kerr *et al.* 1985).

Further analysis of the soluble fraction of mature source leaves indicated that radioactivity in neutral material declined both in absolute terms and as a proportion of the total retained radioactivity (table 3). Within the neutral fraction, TLC/autoradiography showed that a range of neutral compounds became heavily labelled during the feeding period, and that radioactivity declined in all of these during the chase period. Loss of label was most pronounced in sucrose, with other compounds showing greater retention, consistent with lower rates of respiration, metabolism or export (table 3). Two strongly-labelled components of the neutral extract required further characterisation. Following TLC, the more mobile component stained with a ketose-specific stain and was degraded by both α-galactosidase and invertase. A tentative identification as verbascose was confirmed by co-chromatography. The more mobile

Table 3. Distribution of radioactivity in acidic, basic and neutral compounds extracted from the middle leaves of *Lamium album* at different times after administration of $^{14}CO_2$. Results are the means of duplicate determinations and are expressed as a percentage of the total water-soluble radioactivity at each harvest. Compounds in brackets represent provisional identifications (see text).

Fraction	Distribution (%) after 1 h pulse	Distribution (%) after 4 h chase	Distribution (%) after 24 h chase
acidic	23.6	43.5	43.2
basic	26.6	18.8	34.0
neutral	49.8	37.7	22.8
(verbascose)	5.2	5.8	4.4
stachyose	10.4	6.3	2.8
(galactinol)	9.7	3.6	6.6
raffinose	8.7	9.0	5.7
sucrose	14.2	11.6	0.8
hexose	2.0	1.4	2.5

component did not contain fructose, was unaffected by invertase treatment but was degraded by α-galactosidase. GC-MS analysis produced a single peak with primary mass fragments at m/z 204, 305, 345, 361 and 433. On this basis it was identified as galactinol. The retention of label in RFO suggests that, as in the case of *Ajuga reptans,* they are stored as well as translocated (Bachmann *et al.* 1994).

We also analysed further the insoluble components of middle (source) leaves. We were not able to detect glucose release following treatment with amyloglucosidase/ α-amylase using either radiometric or enzymatic assay. This apparent absence of starch contrasts strongly with the high rates of accumulation observed at 330 $\mu Em^{-2}s^{-1}$ (figure 1). Synthesis of sucrose was enhanced at 550 $\mu Em^{-2}s^{-1}$ when compared to 330 $\mu Em^{-2}s^{-1}$, whilst there was no increase in starch (table 1). We conclude that, at high irradiance (>1000 $\mu Em^{-2}s^{-1}$), carbon partitioning appears to shift almost completely to sucrose and RFO synthesis. This could result in the plant making oligosaccharides instead of starch as a reserve carbohydrate. Galactinol and verbascose were detected by chemical staining only in TLC separations of extracts from leaves exposed to high irradiance, which is consistent with such a shift in partitioning (data not shown).

We propose that assimilate partitioning in leaves of *L. album* depends on irradiance, with a flexible balance of partitioning between starch, sucrose and RFO. In this respect it is distinctive from the patterns in C3 temperate grasses in which the ratio of starch to sucrose and fructan remains relatively constant over a wide range of storage conditions (Housley & Pollock 1985).

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