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Partitioning of current photosynthate to different chemical fractions in leaves, stems, and roots of northern red oak seedlings during episodic growth

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Abstract: The episodic or flushing growth habit of northern red oak (*Quercus rubra* L.) has a significant influence on carbon fixation, carbon transport from source leaves, and carbon allocation within the plant; however, the impact of episodic growth on carbon partitioning among chemical fractions is unknown. Median-flush leaves of the first and second flush were photosynthetically labeled with $^{14}CO_2$, and partitioning of ^{14}C into lipids and pigments, sugars, amino acids, organic acids, protein, starch, and structural carbohydrates of source leaves, stem, and roots was determined. In addition, four chemical fractions (sugars, starch, amino acids, and total structural carbohydrates) were quantitatively analyzed in leaves, stems, and roots. Chemical changes in source leaves reflected leaf maturation, changing sink demand during a growth cycle, and leaf senescence. Starch and sugar storage in leaves, stems, and roots during lag and bud growth stages indicate a feedback response of these tissues to decreasing sink strength and temporary storage of both starch and sugar in these plant tissues. Northern red oak, with episodic shoot growth patterns, provides an experimental system in which large changes in sink strength occur naturally and require no plant manipulation. Metabolic changes in leaf, stem, and root tissue of red oak have broad application for other oak species and for both temperate and tropical tree species with cyclic growth habits.

Résumé : La croissance épisodique ou par poussées, typique du chêne rouge (*Quercus rubra* L.), a un impact significatif sur la fixation du carbone, le transport du carbone à partir des feuilles et l'allocation du carbone dans la plante. Cependant, l'impact de la croissance épisodique sur la répartition du carbone parmi les différentes fractions chimiques est inconnu. Les feuilles médianes des première et seconde pousses ont été marquées via la photosynthèse avec du $^{14}CO_2$ et la répartition du ^{14}C dans les lipides et les pigments, les sucres, les acides aminés, les acides organiques et les hydrates de carbone structuraux des feuilles servant de source, de la tige et des racines a été examinée. De plus, quatre fractions chimiques (sucres, amidon, acides aminés et hydrates de carbone structuraux totaux) ont été analysées quantativement dans les feuilles, la tige et les racines. Les changements chimiques dans les feuilles servant de sources suivaient la maturation des feuilles, la variation de la demande venant des puits pendant un cycle de croissance et la sénescence des feuilles. L'emmagasinage d'amidon et de sucres dans les feuilles, la tige et les racines durant les périodes de latence et de croissance des bourgeons témoigne d'une réaction à rebours de ces tissus à la réduction de la force d'attraction des puits et à l'entreposage temporaire à la fois de l'amidon et des sucres dans ces tissus végétaux. Les patrons de croissance épisodique des rameaux du chêne rouge procurent un système expérimental dans lequel d'importants changements dans la force d'attraction des puits surviennent naturellement et ne requièrent aucune manipulation des plantes. Les changements métaboliques dans les feuilles, la tige et les racines du chêne rouge s'appliquent à beaucoup d'autres espèces de chêne et aux espèces d'arbres des régions tempérée et tropicale qui ont des habitudes de croissance cyclique.

[Traduit par la Rédaction]

Introduction

Distribution of photosynthate within a plant represents a coordinated response between photosynthate production by source leaves and assimilate demand by sinks (Geiger 1987). Therefore, both seedling growth habit and stage of leaf and seedling development may affect carbon distribution and metabolism. Northern red oak (*Quercus rubra* L.) has a cyclic growth habit with alternating periods of active shoot growth and apparent rest (Hanson et al. 1986; Dickson 1994). This growth habit is very different from the indeterminate growth of, for example, cottonwood (*Populus deltoides* Bartr.) and should have a significant affect on both carbon allocation within the plant and carbon metabolism.

Previous work in our laboratory with northern red oak seedlings examined leaf and shoot development (Hanson et al. 1986; Tomlinson et al. 1991), photosynthetic rates of leaves in different flushes (Hanson et al. 1988*a*, 1988*b*), carbon metabolism and allocation (Dickson et al. 1990, 2000; Isebrands et al. 1994), and control of episodic shoot growth (Dickson 1994). Detailed investigations with cottonwood have determined ^{14}C-partitioning to different chemical fractions during leaf development (Dickson and Larson 1975,

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1981; Dickson and Shive 1982), during dormancy induction (Dickson and Nelson 1982), and during diurnal changes in photosynthate production and sink demand (Dickson 1987). Cottonwood, with its indeterminate growth habit, has a relatively simple and predictable sequence of leaf production and carbon metabolism. Developing leaves of comparable age at a specific leaf plastochron index (LPI) (see Larson and Isebrands (1971) and Lamoreaux et al. (1978) for discussion of the leaf plastochron index application and use) have essentially the same relative photosynthetic rates, carbon allocation patterns, and carbon partitioning patterns. These patterns are predictable from plant to plant and over time (Dickson 1986). In contrast, red oak, with its episodic growth habit, introduces cyclic patterns of carbon fixation (Hanson et al. 1988*a*) and carbon allocation within the plant (Dickson et al. 2000). Little is known about how these cyclic growth patterns affect carbon metabolism and the partitioning of carbon among different chemical fractions. Therefore, we conducted a study to follow the flow of photosynthetically fixed carbon into the major chemical fractions of leaves, stems, and roots and to determine how these metabolic patterns change during a series of growth cycles. Information obtained from studies of carbon metabolism in red oak is highly significant, because it has broad application, not only for other oak species, but also for both temperate and tropical tree species with cyclic growth habits.

Materials and methods

Plant material

Seedlings were grown from acorns collected from a single parent tree in the Rhinelander, Wis., area and stored according to Teclaw and Isebrands (1987). Stratified and pregerminated seed was sown in 6-L (15 cm diameter by 35 cm high) pots filled with a 1:1 (v/v) mixture of sphagnum peat and sand and maintained in controlled environmental growth chambers (27°C, 16-h day ; 21°C, 8-h night) with an average light intensity (photosynthetically active radiation, PAR) of 300–400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (95% fluorescent, 5% incandescent lighting). Plants were watered two or three times weekly with complete nutrient solution and flushed with tap water once every 2 weeks. The nutrient solution was a modified Johnson solution (Johnson et al. 1957) containing urea and additional KCl to provide a balanced nitrogen source (8 mM NO_3, 8 mM NH_4) and a N:P:K ratio of 16:3:9 more suitable for oak growth. These cultural methods were adequate to maintain rapid growth through at least four growth cycles. Physiological age of the seedlings used in the experiments was defined by the *Quercus* morphological index (QMI) (Hanson et al. 1986; Isebrands et al. 1994), which divides each flush of shoot growth into four developmental stages: bud swell (Bud), linear stem growth (SL), linear leaf growth (LL), and apparent rest (Lag) (Fig. 1 of Dickson et al. 2000 (this issue, p. 1297)).

Plant treatment with $^{14}CO_2$

Plants were labeled with $^{14}CO_2$ in a treatment chamber containing an overhead Sunbrella high intensity light fixture (Environmental Growth Chambers, Chagrin Falls, Ohio) with high-pressure sodium and metal-halide lamps (400 W each), a closed-loop, $^{14}CO_2$ generating and circulating system, and a Plexiglas, water-cooled treatment cuvette. The light fixture provided light intensities ranging from 300 to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ depending on the position of the cuvette below the lights. Plants were taken from the growth chambers 4–6 h after the beginning of the light period and preconditioned in the treatment chamber for at least 1 h before exposure to $^{14}CO_2$. For treatment, a single leaf per plant was enclosed in the cuvette and exposed to 0.9 MBq $^{14}CO_2$ for 30 min. After the 30-min exposure, plants were returned to a growth room for a 48-h transport period. After the 48-h transport period, plants were separated into treated (source) leaf, other leaves (by flush), stems (by flush), taproot, and lateral roots. Plant tissues were oven-dried (70°C), weighed, ground to 40 mesh, and analyzed for ^{14}C. The 48-h transport period was chosen to allow chemical pools with rapid turnover rates (sugars, amino acids) to stabilize and to more closely reflect changes in chemical fractions associated with different QMI growth stages rather than time after ^{14}C treatment (Dickson et al. 1990).

^{14}C analysis and quantitative analyses of different chemical fractions

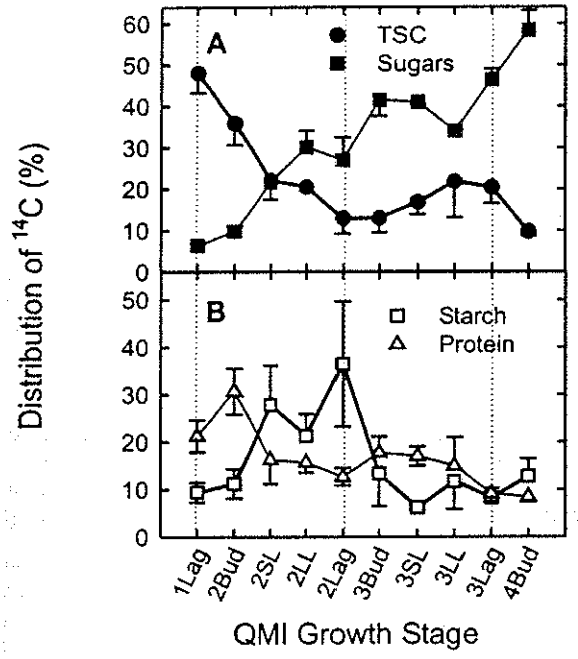
Three individual plants were treated with ^{14}C at each QMI growth stage from 1 Lag to 4 Bud. Carbon-14 in individual chemical fractions was determined on 10- to 50-mg subsamples of leaf, stem, and root tissue with a series of solvent, ion exchange, and enzymatic extractions (Dickson 1979, 1987). Well-mixed subsamples of plant material were extracted with methanol–chloroform–water (MCW) (12:5:3, by vol.), and seven chemical fractions (lipids and pigments, sugars, amino acids, organic acids, protein, starch, and total structural carbohydrates) were separated. Total ^{14}C in each fraction was determined with liquid scintillation spectrometry, and the ^{14}C data were presented as percentages of total ^{14}C recovered (e.g., ^{14}C in sugar/total ^{14}C found in all chemical fractions × 100).

For quantitative chemical analyses, subsamples of leaves, stems, and roots (100 mg) were taken from the same three plants used for analysis of ^{14}C in the chemical fractions and from up to four additional plants at selected QMI growth stages (three to seven individual plants at each QMI growth stage). These tissue samples were extracted with MCW as above, and four chemical fractions (total soluble sugars, amino acids, starch, and total structural carbohydrates) were analyzed. Sugars and amino acids were measured colorimetrically on subsamples of the water-alcohol phase (Nelson and Dickson 1981). Starch in the extracted tissue was hydrolyzed enzymatically with mylase-100 (G.B. Fermentation Ind., Inc., Charlotte, N.C.) and the resulting glucose was measured with *o*-toluidine (Nelson and Dickson 1981). The remaining residue was transferred to tared shell vials, dried at 70°C, and weighed. The residue dry mass (RDM), expressed as total structural carbohydrates (TSC) in this manuscript, was used to calculate the concentration of each chemical fraction in the plant tissue rather than total dry mass. The use of total dry mass as a basis for comparing changes in concentrations of various chemical fractions may be misleading because the chemical fractions are part of the total dry mass. For example, if there is a large buildup of starch in the tissue, the concentration of other chemical fractions could decrease if based on total dry mass but show no change or even increase if based on RDM (see Results; see also Priestley (1973) and Dickson (1987)). The residue portion after extraction is primarily structural carbohydrates and may increase in a tissue with time but should not cycle diurnally or during a shoot growth cycle in oak. Therefore, RDM, like other estimates of plant metabolic fractions such as protein content, should provide a more stable basis for estimating fluctuating metabolic pools.

Statistical analysis

All data are presented as the mean and standard error of the mean from tissue of three different plants (distribution of ^{14}C) or of three to seven different plants (quantitative analysis) harvested at each QMI growth stage. Data for the partitioning of ^{14}C among chemical fractions and for quantitative chemical analyses were also

Fig. 1. Distribution of ^{14}C among chemical fractions of first-flush source leaves at different QMI growth stages 48 h after exposure to $^{14}CO_2$. The percent ^{14}C in each chemical fraction (total structural carbohydrates (TSC), total sugars, starch, protein) is based on the total ^{14}C recovered in all seven chemical fractions analyzed. The percentages in the figures do not add to 100%, because the lipids and pigments, organic acids, and amino acid fractions were each less than 10% of total ^{14}C, changed little with QMI, and were omitted from the figures to avoid confusion. Error bars are SEM. Statistically significant changes with QMI are given in the results. For points without error bars, the SE did not exceed the size of the symbol. (A) Total structural carbohydrates (TSC) and total sugars. (B) Starch and protein. Lag stages are indicated by dotted lines.



analyzed with standard ANOVA techniques on log-transformed data. Log transformation was necessary to meet the assumptions of ANOVA. Means were separated using Tukey's honestly significant differences with pairwise multiple comparisons (Wilkinson 1998).

Results

Distribution of ^{14}C among chemical fractions of leaves

The percentage of photosynthetically fixed ^{14}C incorporated into the major chemical fractions of first-flush source leaves changed markedly during episodic growth in these oak seedlings (Fig. 1). Leaves at 1 Lag and 2 Bud are fully expanded but physiologically immature. The relatively large amount of ^{14}C incorporated into total structural carbohydrates (TSC) and protein at these stages indicates continued development of vascular and photosynthetic systems. Carbon-14 recovered in each of these two chemical fractions decreased significantly (TSC, $P = 0.0002$; protein, $P = 0.02$) during the second flush to about 15% of total ^{14}C recovered and showed little response to subsequent flushing. In contrast, ^{14}C incorporated into the sugar and starch fractions increased during the second flush. Incorporation of ^{14}C into starch was greatest during 2 Lag when overall seedling sink strength was minimal; it then decreased during active growth of the next flush (2 Lag to 3SL, $P = 0.057$). Incorporation of ^{14}C into sugar generally increased during the second and third flushes (1 Lag to 2 Lag, $P = 0.005$). The large increase in ^{14}C in the sugar fraction at 3 Lag and 4 Bud probably indicates sugar retention in these senescing leaves. Carbon-14 in lipids and pigments, organic acid, and amino acid fractions was less than 10% of recovered ^{14}C in each of these chemical fractions and showed little response to the growth cycles. (Data not included in Fig. 1 and subsequent figures to avoid confusion.) The organic acid and amino acid pools in leaf tissue and in stem and root tissue are relatively small and turn over rapidly after ^{14}C labeling.

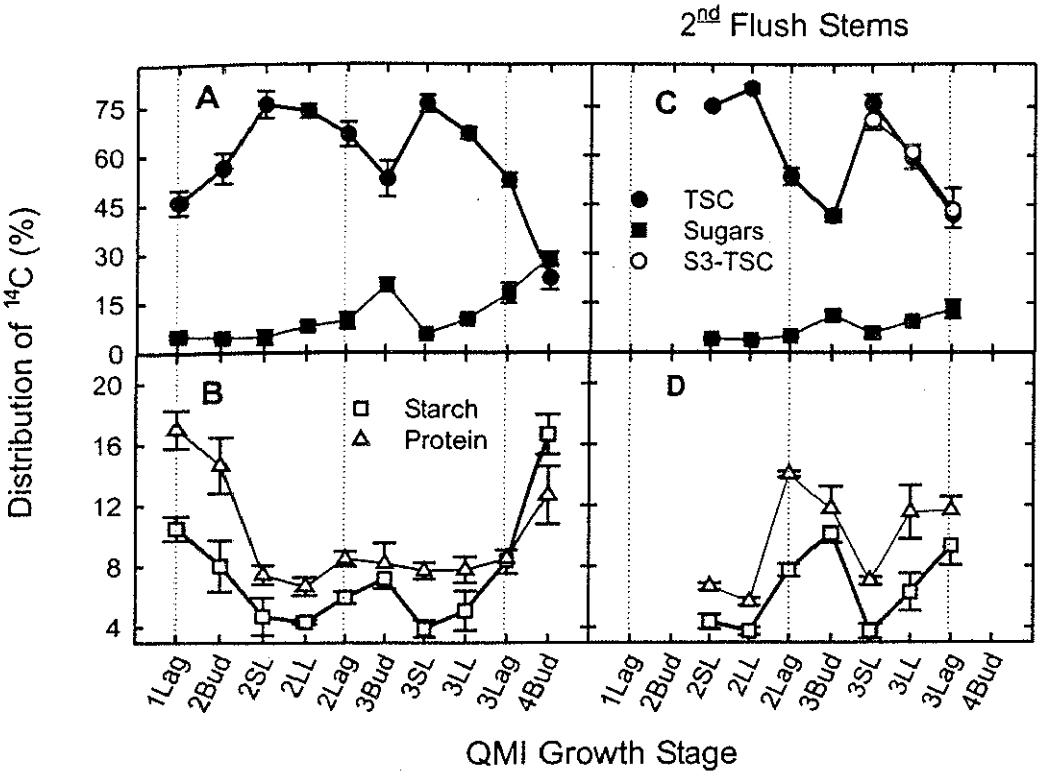
Distribution of ^{14}C among chemical fractions of stem and taproots from ^{14}C-photosynthate translocated from first-flush leaves

The percent distribution of ^{14}C among different chemical fractions of first-flush stems varied considerably during flushing (Figs. 2A and 2B). Incorporation of ^{14}C into TSC increased to 76% during flush development and decreased to 53% during the lag and bud stages (2SL to 3 Bud, $P = 0.007$: 3 Bud to 3SL, $P = 0.006$). In contrast, incorporation into starch and protein increased during the lag and bud stages and decreased during the SL and LL growth stages, with maximum incorporation of ^{14}C at 1 Lag and 4 Bud (starch, 1 Lag to 2L: $P = 0.007$; protein, 1 Lag to 2LL: $P = 0.000\,05$). Similarly, ^{14}C recovered in sugar was significantly greater at 3 Bud, 3 Lag, and 4 Bud compared with 2SL or 2LL and 3SL and 3LL ($P = 0.0001$ and $0.000\,01$, respectively).

The distribution of ^{14}C imported from first-flush leaves among chemical fractions in second- flush stems was similar to that just described for first-flush stems (Figs. 2C and 2D). Incorporation of ^{14}C into TSC of second-flush stems decreased from 80% of the total ^{14}C in all chemical fractions at 2LL to 40% at 3 Bud, increased again at 3SL, then decreased to 40% at 3 Lag. In contrast, percent ^{14}C in sugars, starch, and protein was greatest at 2 Lag to 3 Bud, and at 3 Lag. Incorporation of ^{14}C into protein peaked at 2 Lag and 3 Bud in these second-flush stems, effectively the same ontogenetic stage as 1 Lag and 2 Bud in first-flush stems (protein, 2 Lag vs. 2SL: $P = 0.0012$; 2 Lag vs. 3SL: $P = 0.0019$).

The patterns of ^{14}C incorporation into the chemical fractions of taproot tissue were similar to those found in stems, except the cyclic incorporation into TSC was smaller in taproots during the second flush and not statistically significant (Fig. 3). The ^{14}C recovered in TSC decreased from 63% at 2 Bud to about 55% at 2 Lag and 3 Bud compared with a decrease from 76 to 53% in first-flush stems and 80 to 40% in second-flush stems (Fig. 2). The decrease in ^{14}C in TSC from 3SL to 4 Bud was, however, significant ($P = 0.006$). Cyclic ^{14}C incorporation into the starch fraction of taproots was greater than that found in stems (compare Figs. 2 and 3), with maximum ^{14}C incorporation at the lag and bud stages of the flush cycle as in stems (2LL to 2 Lag: $P = 0.049$; 3 Bud to 3SL: $P = 0.033$). Incorporation of ^{14}C into sugars of roots also peaked at the lag and bud stages as in stems; however, the increase was only marginally significant

Fig. 2. Distribution of ^{14}C among chemical fractions of stems at different QMI growth stages 48 h after exposure of first-flush leaves to $^{14}CO_2$. (A) TSC and sugars in stem tissue of the first flush. (B) Starch and protein in stem tissue of the first flush. (C) TSC and sugars in stem tissue of the second flush and TSC stem tissue of the third flush. (D) Starch and protein in stem tissue of the second flush.



(P = 0.096). In contrast, ^{14}C incorporation into protein of roots (about 15%) varied relatively little over the flush cycle (Fig. 3).

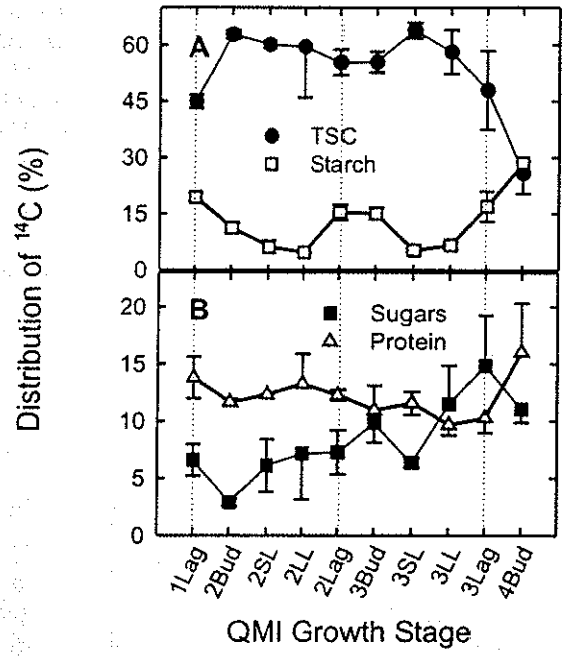
Distribution of ^{14}C among chemical fractions of stem and taproots from ^{14}C-photosynthate translocated from second-flush leaves

The distribution of ^{14}C among chemical fractions of younger tissue above second-flush source leaves was similar to that of second-flush stems above first-flush source leaves (data not shown). The distribution of ^{14}C among chemical fractions in tissue below second-flush source leaves followed essentially the same pattern in lower second-flush stems and first-flush stems (Fig. 4), and taproots (Fig. 5). Incorporation of ^{14}C into TSC was greatest during active growth of the third flush (3SL and 3LL) and least during lag and bud stages (Fig. 4A, TSC decrease 3SL to 3 Lag, P = 0.026). Incorporation into sugar and starch fractions differed from TSC: being low during active shoot growth and high during Lag and Bud stages (Fig. 4A, sugar increase 3SL to 3Lag, P = 0.065) The pattern of ^{14}C incorporation into protein was similar to that found for starch and sugar but the cycles were not as large. The decrease found during shoot growth was statistically significant only in second-flush stem tissue (Fig. 4D, 3 Bud to 3LL, P = 0.065). The ^{14}C incorporation cycles of the different chemical fractions were timed somewhat differently, e.g., maximum and minimum incorporation into protein seemed to lag starch and sugar by about one QMI growth stage. The cyclic incorporation of ^{14}C into starch of taproots was particularly large, ranging from about 30% of the ^{14}C recovered at the lag and bud stages to 7% at 3SL (Fig. 5A).

Changes in total chemical content of plant parts during flushing

When leaves, stems, and roots were analyzed to determine the total amounts of starch, sugar, amino acids, and structural carbohydrates, cyclic patterns again were observed and associated with the different growth stages (Figs. 6 and 7). In general, the amino acid concentration in leaves and stems was higher in the young tissue and decreased with age (leaves, 1 Lag to 2LL: P = 0.023; stems, 1 lag to 2 Lag: P = 0.001), but in roots there were no consistent patterns, decreasing in taproots and increasing in lateral roots during the third flush (Figs. 6C and 6D). Sugar and starch concentrations in all tissues were higher at lag and bud growth stages and decreased during shoot flushing (e.g., starch in taproot increased from 2LL to 3 Bud, P = 0.002 then decreased 3 Bud to 3LL, P = 0.001). Total structural carbohydrates (and other structural compounds) generally increased with tissue age in leaves and lower stems but cycled in roots (Fig. 7) (a small cycle also was present in stems). This apparent change in the total amount of TSC in the root tissue results primarily from an increase in starch concentration (Fig. 6C). Structural carbohydrate concentration was calculated from total dry mass of the tissue. Both starch and TSC (and all other

Fig. 3. Distribution of ^{14}C among chemical fractions of taproots at different QMI growth stages 48 h after exposure of first-flush leaves to $^{14}CO_2$. (A) TSC and starch in taproot tissue. (B) Sugars and protein in taproot tissue.



chemical fractions) contribute to total dry mass. Therefore, when starch concentrations increased, structural carbohydrate relative concentration or TSC as a percent of total dry mass decreased (e.g., 3 Bud; Figs. 7C and 7D).

Discussion

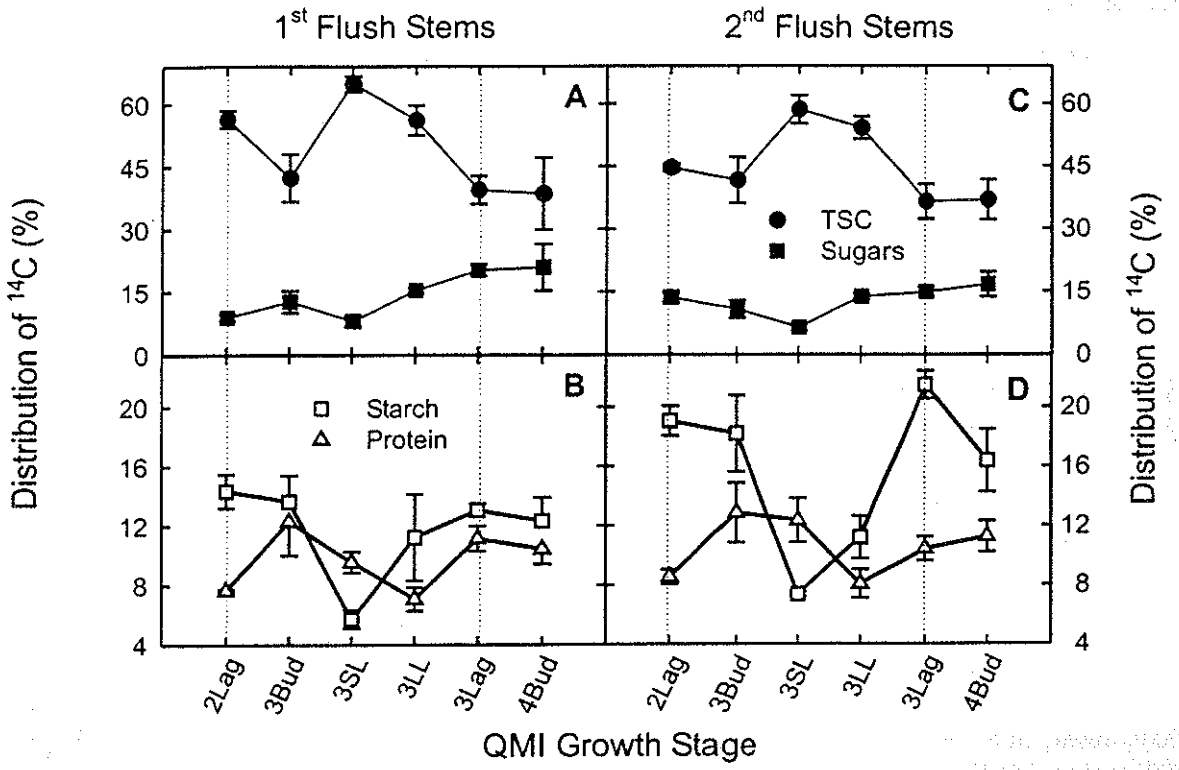
Carbon metabolism in source leaves of northern red oak

Source–sink interactions have been extensively studied for many years and recently reviewed in much detail (Geiger 1987; Dickson 1991; Geiger and Servaites 1991; Luxmoore et al. 1995). Sinks are net importers of assimilate, and sink strength is the ability of the sink organ to import assimilate relative to other sinks in the plant. Sink strength is related to sink size, growth rate, metabolic activity, and respiration rate. Most experimental studies of changes in source or sink strength have involved manipulation of the plant (e.g., removing fruiting structures, removing other source leaves, shading). Such manipulations often change the normal assimilate allocation patterns within the plant and may provide misinformation about adaptive responses to source–sink changes. Geiger (1987) and Geiger and Servaites (1991) have pointed out that such studies should be conducted with whole plants and with a minimum of disturbance. Northern red oak, with its episodic growth habit, produces a series of shoot flushes that significantly increase sink strength during the flush. It also provides an experimental system that requires no manipulation in which whole-plant and individual sinks change naturally during a growth cycle.

Our red oak seedlings were grown in controlled environmental chambers under optimal and constant environmental conditions through four cycles of growth. Changes in carbon flow among different chemical fractions, therefore, should be in response to changes in seedling ontogeny and changing sink demand within seedlings and not to abrupt environmental changes or seedling manipulation. The incorporation of ^{14}C into various chemical fractions of first-flush source leaves exhibited patterns related to leaf maturation and senescence as well as changing sink demand of the whole plant. Northern red oak leaves at 1 Lag are fully expanded but physiologically immature (Dickson et al. 1990). Such immaturity is clearly shown by the amount of photosynthetically fixed ^{14}C retained in the source leaves and the incorporation of ^{14}C into the different chemical fractions. Source leaves at 1 Lag retained 70–80% of the ^{14}C fixed after a 48-h transport period (Dickson et al. 1990). Retention of recently fixed carbon in source leaves is characteristic of young developing leaves of both flushing plant species (Sleigh et al. 1984) and indeterminate plant species (Dickson and Larson 1981; Dickson and Shive 1982). Carbon fixed by the young leaf is used in situ for the production of functional and structural compounds required for leaf maturation. The large percentage of ^{14}C found in TSC, protein, chlorophyll, and other such compounds indicates continued maturation of leaf vascular and photosynthetic tissue after full expansion (see Fig. 1, 1 Lag; Joly and Hahn 1989; Dickson et al. 1990). In contrast, after 48 h, relatively little ^{14}C was found in sugar, starch, and amino acids, compounds that are rapidly metabolized in this developing tissue.

Partitioning response of ^{14}C to changing sink demand of the whole plant is shown by the cyclic incorporation of ^{14}C into the starch of first-flush source leaves (Fig. 1B). During active shoot growth (e.g., 3 Bud to 3LL), little ^{14}C was recovered in starch and more ^{14}C was incorporated into sugars for export to the expanding sink tissues. In the absence of shoot growth (2 Lag) more ^{14}C was incorporated into starch for storage. Such changes in starch and sugar production are commonly associated with changing sink/source ratios and reflect starch storage during low sink demand and production of transport sugars during high sink demand. A decrease in photosynthetic rate and increase in starch production is a common response to a decreased sink/source ratio but is by no means universal. Plaut et al. (1987) grew seven crop species under the same environment conditions; they found that when the sink/source ratio decreased, photosynthesis decreased in only three species; starch and sugar increased in two species; and in common bean, starch increased, but there were no affect on photosynthesis or source leaf sugar concentration. Carlson and Brun (1985) found similar results in soybean; when the sink/source ratio decreased, ^{14}C incorporation into source leaf starch increased, but there were no change in ^{14}C content of leaf sugars. In a study with *Prunus cerasus*, severe defoliation increased photosynthetic rate, decreased starch, and increased sucrose and sorbitol of the remaining source leaves (Layne and Flore 1993, 1995). These inconsistent responses among plant species may have several causes (e.g., different environmental conditions, degree of sink/source change, plant developmental stage, effects of manipulation) but seem to be strongly related to inherent metabolic characteristics, such as what is utilized as the primary storage compound (starch or sucrose) in response to stress or sink–source changes (Foyer 1987; Geiger and Servaites 1991).

Fig. 4. Distribution of ^{14}C among chemical fractions of stems at different QMI growth stages 48 h after exposure of second-flush leaves to $^{14}CO_2$. (A) TSC and sugars in stem tissue of the first flush. (B) Starch and protein in stem tissue of the first flush. (C) TSC and sugars in stem tissue of the second flush. (D) Starch and protein in stem tissue of the second flush.



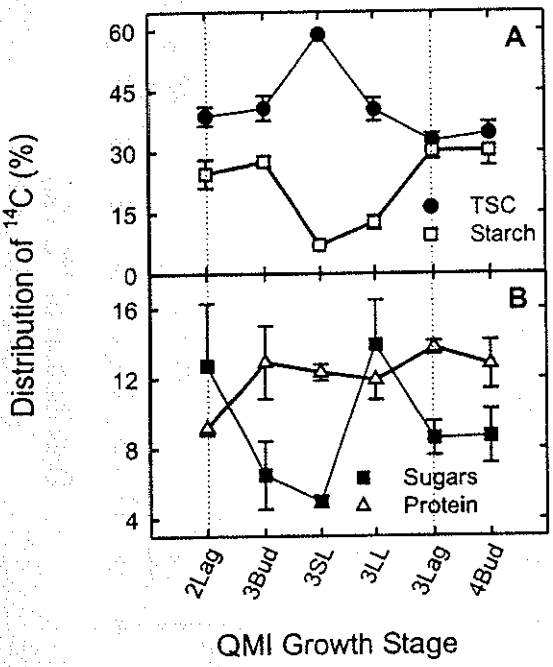
There is little information on what controls these adaptive changes in source leaf metabolism in response to changing sink/source ratios of red oak. However, the metabolic changes and control systems involved have been studied in other plants and recently reviewed (Herold 1980; Foyer 1987, 1988; Geiger 1987; Geiger and Servaites 1991; Sheen 1994; Luxmoore et al. 1995). The adaptive changes in red oak involve cyclic metabolic changes in source leaves, because no change in source leaf area is present. Changes in photosynthetic rate, translocation from the source leaves, starch/sugar ratios, and other chemical changes are involved (Dickson 1991; Isebrands et al. 1994). A recent study with *Quercus robur* L. found decreases in sucrose concentrations but no change in starch concentration of first-flush leaves during the second flush (Alaoui-Sosse et al. 1996). In this study with *Q. robur*, the activities of enzyme systems involved in sugar metabolism favored sucrose production. Sucrose phosphate synthase activity was high but changed relatively little during the second flush, while sucrose synthase activity increased. Thus, the response of first-flush leaves to increased sink strength of the second flush was primarily the production and transport of sucrose. In the first-flush leaves of our red oak seedlings, the incorporation of ^{14}C into both sugar and starch increased during the second flush (Fig. 1). Thus, both sugar and starch metabolism responded to the increased sink strength of the developing flush. Some of the changes that occurred may be related to the continuing development of first-flush leaves rather than increased sink strength associated with the second flush. However, changing photosynthetic rates (Hanson et al. 1988*a*) and translocation rates (Dickson et al. 2000) indicated that first-flush leaves responded to the changing sink strength of the second flush.

By the end of the third flush, first-flush leaves were beginning to senesce as indicated by interveinal chlorosis. In addition, transport of recently fixed ^{14}C from these leaves decreased (Dickson et al. 2000) and the ^{14}C found in sugar after the 48-h transport period increased (Fig. 1A). Increased retention of current photosynthate in source leaves, decreased photosynthetic rates, and increased partitioning of ^{14}C into soluble sugars are typical responses of senescing leaves (Dickson and Nelson 1982). However, little or no first-flush leaf loss was observed in the current study through four growth cycles of development.

Carbon metabolism in stems and roots of northern red oak

In lower stems and roots, the patterns of current photosynthate partitioning into different chemical fractions showed both storage during lag and bud growth stages and utilization during expansion of the next flush. Both patterns of ^{14}C incorporation into different chemical fractions (Figs. 2 and 3) and changes in concentration of these fractions in stem and root tissue were similar (Fig. 6). These changes in carbon metabolism and in total pool size are related to changing sink strength of the whole plant. During the lag and bud phases of the flush cycle, no expanding leaves were present, and sink demand on source leaves decreased. Although photosynthetic carbon fixation (Hanson et al. 1998*a*) and export from source leaves decreased (Dickson

Fig. 5. Distribution of ^{14}C among chemical fractions of taproots at different QMI growth stages 48 h after exposure of second-flush leaves to $^{14}CO_2$. (A) TSC and starch in taproot tissue. (B) Sugars and protein in taproot tissue.



et al. 2000), excess carbon was still available that could not be utilized by stem and root growth. This excess carbon was stored as starch and sugar in the stem and root tissue. These storage patterns are similar to those found in plants under mild environmental stress (Chapin et al. 1990; Chapin 1991; Geiger and Servaites 1991) or in the early stages of dormancy induction (Nelson and Dickson 1981; Dickson and Nelson 1982; Nguyen et al. 1990; Dickson 1991). Both of these environmental constraints (mild stress, e.g., nutrient or water stress, and early dormancy induction) decrease plant sink strength, usually by decreasing the rate of leaf growth before photosynthesis is severely constrained. In addition, changes in hormone production and transport are involved. In episodic-flushing species, these changes in sink strength and in carbohydrate storage or utilization are related to the different ontogenetic growth stages. The variety of potential mechanisms and their interactions that control these metabolic changes may differ widely among different plant species, but the integrated response of the whole plant may be basically the same in all plants (Chapin 1991). It is impossible to tell from our data whether the increase in root starch is simply a way to sequester excess carbon (carbon accumulation as defined by Chapin et al. (1990)) or is directed reserve formation. Directed carbon reserve formation is the metabolically regulated or programmed synthesis of storage compounds that directly compete for carbon that might be used for growth (Chapin et al. 1990). More carbon is allocated to the root system during lag and bud growth stages, and root relative growth rate increases at these stages (Dickson et al. 2000). It is clear from the changes in the percentage of ^{14}C found in different chemical fractions at different QMI growth stages that considerable metabolic regulation is present. For example, at the 3 Bud growth stage, current photosynthate from second-flush leaves is largely allocated to lower stem and roots (Dickson et al. 2000). At this growth stage, 1912 dpm ^{14}C/mg dry mass was found in the taproot TSC plus starch fractions, and TSC contained 59% while starch contained 41% of this ^{14}C (Fig. 5A). At 3SL, most current photosynthate from second-flush leaves is allocated upward to the third flush (Dickson et al. 2000) and 214 dpm ^{14}C/mg dry mass was found in the taproot TSC plus starch; 89% in TSC and 11% in starch (Fig. 5A). In this root tissue, when little current photosynthate was available (214 dpm vs. 1912 dpm) relatively more ^{14}C was incorporated into TSC. In contrast, when first-flush leaves were treated and second-flush stems were analyzed at 3 Bud (Figs. 2C and 2D), the TSC plus starch fractions contained 134 dpm/mg dry mass with 79% of the total ^{14}C in TSC and 21% in starch. At 3SL, when more current photosynthate was allocated upward, these fractions contained 1876 dpm/mg dry mass with 95% of the total ^{14}C in TSC and 5% in starch. In second-flush stem tissue at 3 Bud, when little current photosynthate was available from first-flush leaves, less ^{14}C was incorporated into TSC and more was incorporated into starch. Thus, the pattern of incorporation into different chemical fractions was not related to the total amount of current photosynthate present in the tissue but was related to the QMI growth stage. In all stem and root tissue analyzed (Figs. 2–5), the maximum proportion of the available ^{14}C was incorporated into TSC (minimum proportion into starch) at the SL and LL growth stages when the new flush was expanding, and the minimum proportion of available ^{14}C was incorporated into TSC (maximum proportion into starch) at the lag and bud stages when there was no expanding new leaf tissue. Although the above information highlights some interesting changes in carbon partitioning to TSC versus storage associated with different QMI growth stages, total carbon allocated to root systems was two to four times greater at lag than at LL (Dickson et al. 2000, Table 3). Therefore, the cycling of shoot/root ratios associated with different QMI growth stages may still be consistent with the partitioning patterns observed.

Almost all the work designed to elucidate plant response to changing sink demand has focused on leaf response. Changes in photosynthetic rates, sucrose/starch ratios, photosynthetic enzyme systems, cofactors, membrane transport compounds, and hormonal balance have all been studied in detail in leaves (Chapin 1991; Geiger and Servaites 1991). There is good reason to assume, however, that many aspects of growth and storage in nonphotosynthetic tissue also respond to changing sink demand. There is ample evidence that many plant species store reserves concomitant with active growth of the storage tissue (Chapin et al. 1990; Dickson 1991). Wargo (1979) found starch storage in older root tissue of sugar maple (*Acer saccharum* Marsh.) at the same time new xylem tissue was forming. Such partitioning of current photosynthate between new growth and reserve formation requires genetic and metabolic control, and the integrated response of the whole plant. In our oak seedlings, partitioning of ^{14}C to TSC (presumably xylem tissue) in both stems and roots was favored over storage during SL and LL growth stages, when rapidly expanding stem and leaf tissue were present. Cambium activation and new xylem production in trees is associated with indole acetic acid production and basipetal transport from expanding buds and leaves

Fig. 6. Quantitative changes of sugars, starch, and amino acids in northern red oak tissues at different QMI growth stages. Quantitative estimates of these chemical fractions were based on residue dry mass (given as total structural carbohydrates, TSC, in the text and figures). (A) Leaf tissue of the first flush. (B) Stem tissue of the first flush. (C) Taproot tissue. (D) Lateral root tissue. Because of relatively low concentration in all tissues, amino acids are presented at 10× the actual concentration to emphasize changes with QMI.

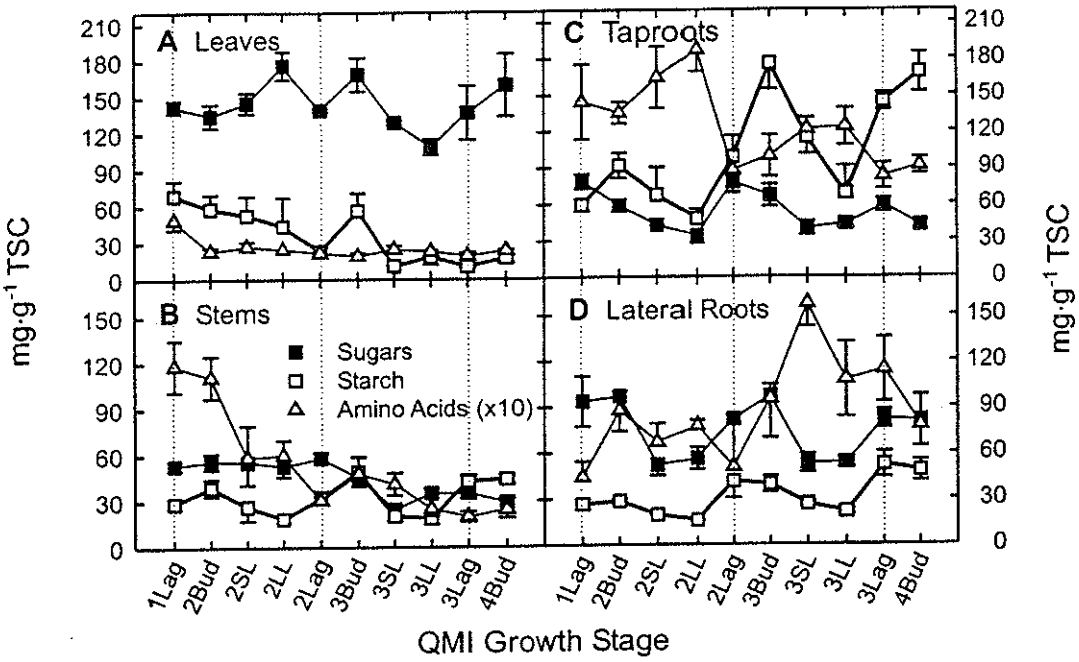
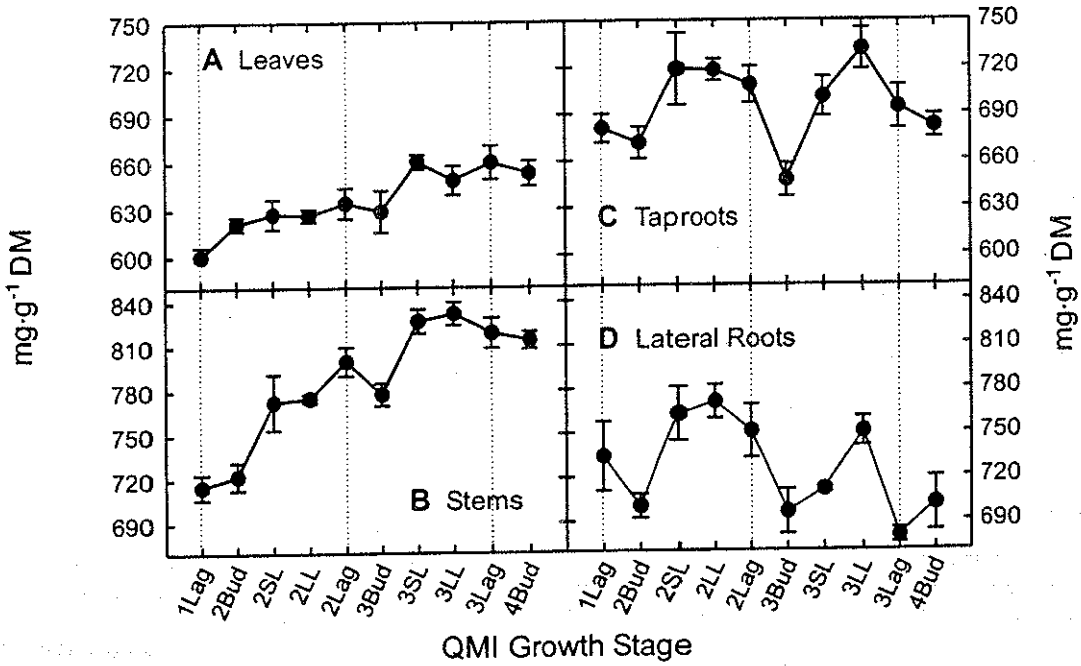


Fig. 7. Quantitative changes of total structural carbohydrates (TSC) in northern red oak tissues at different QMI growth stages. Quantitative estimates of this chemical fraction were based on tissue total dry mass. (A) Leaf tissue of the first flush. (B) Stem tissue of the first flush. (C) Taproot tissue. (D) Lateral root tissue.



(Aloni 1987). Such hormonal control, or more likely a combination of hormones and other metabolic products (Chapin 1991), may be involved in partitioning of current ^{14}C-photosynthate to different chemical fractions in stems and roots of oak. Additional evidence for hormonal involvement in control of root growth comes from studies on the periodicity of root growth and flushing of both oak and rubber trees (Harmer 1990; Thaler and Pages 1996). While root growth, particularly lateral root growth, decreased during a flush when current photosynthate was limited, fine root initiation, the number of fine root tips, and root branching increased during leaf expansion, perhaps indicating a response

to hormones produced in expanding leaves and translocated to the root system.

Conclusions

The partitioning of current ^{14}C-photosynthate into different chemical fractions of northern red oak leaves, stems, and roots changed during a flush cycle. These changes reflected leaf maturation, leaf senescence, and changing sink demand during a flush. Leaf response was somewhat different from stem and roots indicating different metabolic responses with changing sink strength and with leaf aging. Both ^{14}C incorporation into and total concentration of starch and sugar in stem and roots increased during lag and bud stages, indicating storage of both of these carbohydrate fractions. Cyclic changes were greater in taproot tissue than in leaves or stems and were probably related to the greater storage capacity of the root tissue. Carbon fixation and utilization in oak seedlings grown under our favorable controlled environmental conditions is apparently sink limited except during active growth of a new flush. Sink limitation of carbon utilization probably would be even greater in seedlings exposed to environmental stresses that limit flushing. Plant response to sink-limited utilization of carbon (e.g., decreased photosynthetic rates, retention of photosynthate in source leaves) might confound response to imposed environmental stresses.

Most studies on plant response to changing sink demand require plant manipulation, such as leaf or fruit removal, or top clipping. Such manipulation may change normal allocation patterns and disrupt hormonal signals that control or initiate many adaptive metabolic changes. Northern red oak or other flushing species with episodic shoot growth patterns provide an experimental system with large changes in sink strength that occur naturally and require no plant manipulation. Such plant systems should be useful for studying photosynthetic and metabolic changes in source leaves and in stem and root storage systems.

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