Seasonal patterns of reserve and soluble carbohydrates in mature sugar maple (*Acer saccharum*)

B.L. Wong, K.L. Baggett, and A.H. Rye

Abstract: Sugar maple (*Acer saccharum* Marsh.) trees exhibit seasonal patterns of production, accumulation, and utilization of nonstructural carbohydrates that are closely correlated with phenological events and (or) physiological processes. The simultaneous seasonal patterns of both reserve and soluble carbohydrates in the leaves, twigs, branches, and trunks of healthy mature sugar maple trees were characterized. The concentrations of starch and soluble sugars (sucrose, glucose, fructose, xylose, raffinose, and stachyose) were determined. Starch, the major reserve carbohydrate in sugar maple, is low during the active photosynthetic growth season. Starch is accumulated in the xylem ray tissues in late summer and early fall. During the cold season, there is a close relationship between starch hydrolysis–accumulation and temperature. Soluble sugars increase when starch concentrations decrease during the cold months, and these sugars may play a role in cold tolerance. Patterns of change in the stem tissues are similar to those in the root tissues but with slight differences in the timing.

Key words: starch, sucrose, glucose, fructose, raffinose, stachyose.

Résumé : L'érable à sucre (*Acer saccharum* Marsh.) affiche des patrons saisonniers de production, d'accumulation et d'utilisation des glucides non-structuraux, étroitement corrélés avec des évènements phénologiques et (ou) physiologiques. Les auteurs ont caractérisé les patrons saisonniers simultanés des glucide solubles et de réserve dans les feuilles, les rameaux, les branches et les troncs d'érables à sucre matures et en santé. Ils ont déterminé les teneurs en amidon et en sucres solubles (saccharose, glucose, fructose, xylose, raffinose et stachyose). La teneur en amidon, principale réserve en glucides de l'érable à sucre, est faible au cours de la période photosynthétique active. L'amidon s'accumule dans les tissus des rayons du xylème vers la fin de l'été début de l'automne. Au cours de la saison froide, on note une étroite relation entre hydrolyse et accumulation, selon la température. Les sucres solubles augmentent lorsque les teneurs en amidon diminuent, pendant les mois froids, et les sucres peuvent jouer un rôle dans la tolérance au froid. Les patrons de changements dans les tissus de la tige sont semblables à ceux qui surviennent dans les tissus des racines, mais avec un léger décalage dans le temps.

Mots clés : amidon, saccharose, glucose, fructose, raffinose, stachyose.

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Introduction

The carbohydrate status of healthy trees reflects their vitality and photosynthetic capacity (Kramer and Kozlowski 1979). The amount of starch stored in deciduous trees has been shown to be negatively impacted by biotic (Parker and Houston 1971; Gregory and Wargo 1986; Gregory et al. 1986), abiotic (Wilmot et al. 1995; Long et al. 1997), and anthropogenic (Pitelka and Raynal 1989; McLaughlin et al. 1996) stressors. High levels of starch stored in the fall following a stress event are indicative of trees with high vitality, while low starch levels after a similar stress event reflect low tree vitality (Gregory and Wargo 1986; Gregory et al. 1986; Rasmussen and Henry 1990; Renaud and Mauffette 1991). Low levels of stored starch have been implicated as a contributing factor in the dieback and mortality of deciduous trees (Gregory and Wargo 1986; Gregory et al. 1986; Rasmussen and Henry 1990; Renaud and Mauffette 1991).

Most of the organic carbon produced during the photosynthetic process is utilized by numerous sinks during plant development in the growing period. Following the growing period with reduced sink strength, starch is the major reserve carbohydrate stored during the leafless period and is the main source of carbon and energy substrate for metabolic processes and for primary growth in the spring the following year. The patterns of starch and soluble sugars in the tissues from healthy trees reflect normal physiological performance and tree vitality. Variations from such patterns could reflect changes in physiological performance and could be useful in understanding the physiology of trees affected by environmental stress (Parker and Houston 1971; Parker 1974; Wargo 1981; Carroll et al. 1983; Gregory et al. 1986; Rasmussen and Henry 1990; Renaud and Mauffette 1991; Wong et al. 2001).

To better understand the effect of stressors on tree health and carbon allocation, it is important to understand seasonal

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changes in carbohydrate levels in relation to phenological events and physiological processes during the photosynthetic period (active growth to carbohydrate storage) and nonphotosynthetic period (dormancy to vernal growth). Jones and Bradlee (1933) determined that the starch content at breast height of mature trees fell each month from November to October. Only the outer four annual rings were analyzed for sucrose and reducing sugars. Gregory et al. (1986) quantified starch, sucrose, glucose, and fructose in the main stem of sugar maple (*Acer saccharum* Marsh.) saplings. Changes in seasonal sugar complements (stachyose and raffinose) present in the wood during the cold season have not been determined.

This study provides information with respect to seasonal changes in carbohydrate levels in leaves, twigs, branches, and trunks of healthy mature sugar maple trees in relation to phenological events and physiological processes.

Sampling of twigs and branches permitted evaluation of carbohydrate levels throughout the photosynthetic period and nonphotosynthetic period. This information will be useful as a baseline to better understand the effect of stressors on tree health and carbon allocation.

Materials and methods

Field sites

Sugar maple trees were selected randomly from several hundred phenotypically healthy trees growing in central Vermont and in two private sugarbushes of older trees located in northeastern New York. The site in Vermont is dominated by 25- to 30-year-old sugar maple trees (about 90%) along with white birch (*Betula papyrifera* Marsh.), red maple (*Acer rubrum* L.), white ash (*Fraxinus americana* L.), yellow birch (*Betula alleghaniensis* Britt.), and black cherry (*Prunus serotina* Ehrh.). One site in New York is a mature sugarbush with trees approximately 200 years old that have been tapped for syrup production for decades. The sugarbush is a monoculture of sugar maple and has no understory woody plants. The other New York site is a younger sugarbush with trees 50–100 years old that have been tapped for syrup production for several years. This sugarbush is a mix of sugar and red maple with some eastern white pine (*Pinus strobus* L.).

Sample collections

Trees with relatively little crown loss (less than 10%) and no large wounds, cankers, or other obvious disease symptoms were selected. To determine the seasonal carbohydrate content in branch and twig wood, collections were made at monthly intervals starting in late spring and continuing through late spring of the following year. At each sampling date, three branches at midcrown level from each of three trees were removed and transported to the laboratory at ambient temperature. Two cross-sectional samples were taken from the lower third, middle third, and upper third of each branch and from the base of each twig (three from each branch). To ameliorate diurnal influences and for consistency, all collections were made between 9 and 11 a.m.

To determine the carbohydrate patterns of the foliage, 10 fully expanded leaves were selected randomly from each of three branches from three trees at biweekly intervals from late May to leaf drop in October. Ten discs (each approximately 6.5 mm in diameter) were removed from along the outer margin of each leaf (avoiding the major veins), immediately placed into test tubes containing cold 80% ethanol, and stored in an ice chest for transport.

To characterize the profile of reserve carbohydrate (starch) distribution and mobilization in the trunk, three trees (approximately 20 cm in diameter at 1.2 m height) were felled on collection dates in June, July, and October. Discs, approximately 1–2 cm thick, were obtained from five levels along the tree axis: at breast height (about 1.2 m above ground level), half the distance to the base of the crown, the base of the crown, midcrown, and upper crown. The discs were placed in plastic bags for transport to the laboratory at ambient temperature. Upon returning to the laboratory, a 1.25-cm radial strip of wood from each disc was removed from the southeast aspect of the tree and immediately processed as for the other wood samples.

Sample preparation

All samples were processed immediately when returned to the laboratory. After removal of the bark, phloem, cambium, and pith, the branch and twig wood samples and individual annual rings from the trunk were submerged into test tubes containing 5 mL of 80% ethanol, placed into a boiling water bath for 15 min, and evacuated in a vacuum oven at –52 kPa for 15 min. Wood samples were homogenized with a Brinkman Instruments (Westbury, N.Y.) Polytron™ in 80% ethanol and centrifuged for 15 min at 3000 rpm and the macerated samples were extracted twice more with 5 mL of 80% ethanol. For each sample, the ethanol fractions were pooled, filtered through a 0.45-μm syringe filter, and used for sugar analysis. The ethanol-insoluble pellets were assayed to determine the starch content.

Immediately upon arrival at the laboratory, the tubes containing the leaf discs were placed into a boiling water bath for 15 min and then evacuated at –52 kPa for 15 min. This extraction procedure was repeated four times with 5 mL of 80% ethanol. The supernatants were combined and used in determining soluble sugars. The extracted leaf discs were assayed for starch content.

Soluble sugar determination

Two analytical methods were used to quantify the soluble sugar fractions. The ethanol-soluble sugar fractions of the leaves, twigs, and branches from the photosynthetic period were analyzed enzymatically for glucose, sucrose, and fructose as described by Hendrix (1993). For glucose determination, the INT assay reagent (glucose assay 115-A; Sigma Chemical Co., St. Louis, Mo.) was added to each sample and glucose standards and the reaction terminated by the addition of 0.1 N HCl. For fructose determination, phosphoglucose isomerase (Sigma P9544 or P9010) in HEPES buffer (pH 7.8) was added to each well and fructose determined after correcting for glucose present in the sample. For determination of sucrose, sucrose was converted to glucose by adding invertase (Sigma I-4504) and phosphoglucose isomerase to the unknown and sucrose standards before assaying for glucose. The concentration of sucrose was determined after correcting for glucose and fructose present in the sample. The concentrations of glucose, fructose, and su-

crose were calculated from glucose standard curves and expressed as milligrams per gram dry mass of twig and branch samples and as micrograms per square millimetre of leaf disc samples. Dry mass was determined after starch extraction.

The ethanol-soluble sugar fractions of the twigs and branches collected during the cold season were analyzed using an HPLC system with a Waters (Milford, Mass.) Sugar-pak™ column and solvent (0.1 mM Ca EDTA) at a flow rate of 0.6 mL·min^{-1} at 90 °C for sucrose, glucose, fructose, stachyose, raffinose, and xylose. Sugars were detected with a Waters model 410 refractive index detector connected to a Digital Equipment Corp. (Maynard, Mass.) personal computer equipped with Waters-Millennium™ software. The separated soluble sugars were identified and quantified with known standards and converted to milligrams per gram dry mass of tissue.

Starch determination

Starch was quantified by the method of Hendrix (1993) with some modifications. The starch was gelatinized in each pellet and in each tube containing leaf discs with 0.2 N KOH and was hydrolyzed to glucose with amyloglucosidase (Sigma 10115). Glucose was quantified colorimetrically using the INT assay as described by Hendrix (1993). The concentration of starch was calculated from glucose standard curves and expressed as milligrams per gram dry mass of twigs and branch samples, as micrograms per square millimetre of leaf disc diameter, and as milligrams per cubic centimetre of each annual ring sample.

Statistical analyses

After testing for normality using the Shapiro–Wilk test, data were analyzed using the SAS statistical package for personal computers (SAS Institute Inc. 1985). Analysis of variance and Duncan's multiple range test were used to test for differences in means of collection dates at the $p < 0.05$ level of significance.

Results and discussion

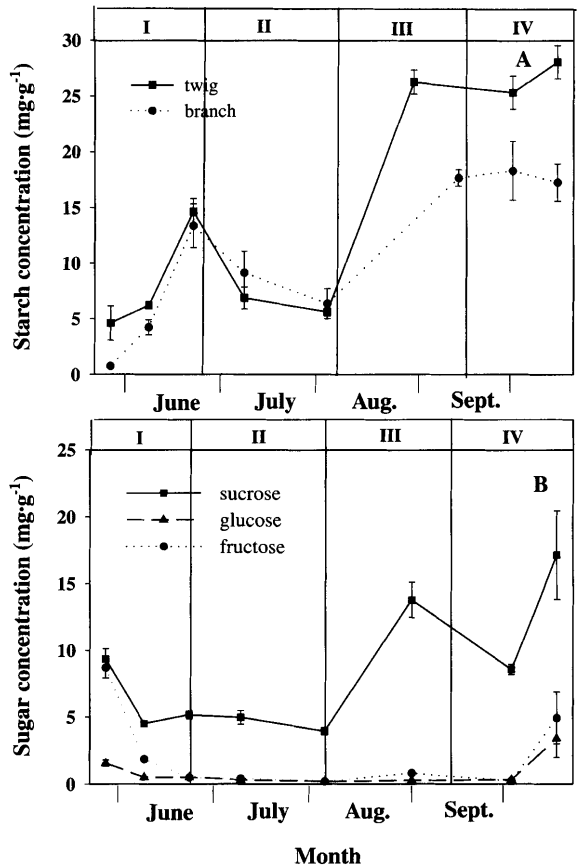
Variations in seasonal carbohydrate patterns occurred simultaneously in various tissues of healthy mature sugar maple trees, and such variations coincided with observed and (or) reported phenological events and physiological processes (Table 1). Roman numerals in the figures and tables refer to the timing (period) of these events and processes.

Carbohydrates in the photosynthetic period (late spring through early fall)

Stored starch is the main source of carbon for growth and metabolism of developing buds and cambia in early spring. Starch content in the branches and twigs was low in late May due to an increase in sink strength (Fig. 1A). The brief elevation in starch concentration in leaves (Fig. 2A) and in branches and twigs (Fig. 1A) in early June may reflect a high rate of photosynthesis with reduced sink strength in developing areas, as reported by Quick and Neuhaus (1997) and Pollock (1997).

Starch concentrations in the leaves (Fig. 2A), twigs, and branches (Fig. 1A) remained low during the period of high

Fig. 1. (A) Starch and (B) sucrose, glucose, and fructose concentrations in foliage of sugar maples (*Acer saccharum*) (25–30 years old) during the growing season. Mean values and standard errors are shown with $n = 90$ for each collection date. Roman numerals refer to the phenological and physiological events discussed in Table 1.



photosynthetic activity (July–August, periods II and III). The level of sucrose in the twig and branch tissues was higher than those of glucose and fructose during the active growing period (Fig. 1B). During this period, the photosynthate produced by leaves is not stored but is mobilized and transported as sucrose to various sinks for growth and metabolism (Chatterton and Silvius 1980; Quick and Neuhaus 1997). During the active growing season (mid-June to the end of August, periods I–III), a significant amount of carbon is allocated for secondary growth processes (Goodman et al. 1990). R.A. Gregory (USDA Forest Service, personal communication) found that secondary xylem production was about 80% completed by mid-July. Root growth occurs from spring to fall and fine root production and turnover occur simultaneously throughout the growing season (Brundett and Kendrick 1988; Hendrick and Pregitzer 1992, 1993). Wargo (1979) observed that in lateral roots, wood production began in early July and continued through August and that primary fine root production occurred simultaneously.

By mid-August to late September (periods III and IV), the concentrations of starch (Fig. 1A) and sucrose (Fig. 1B) in the twig wood were at high levels corresponding to the reduction in sink strength and to allocation to storage. By

Table 1. Phenological and physiological processes in sugar maple (*Acer saccharum*) throughout the year.

Period	Season	Month	Phenological and physiological processes
I	Late spring	Early June	Completion of primary growth; leaf expansion; photosynthesis
II	Summer	Mid-June to July	Active period of secondary growth
III	Late summer	August to mid-September	Near completion of secondary growth; reduced photosynthate to sinks; accumulation of reserve carbohydrates
IV	Fall	Late September to mid-December	Leaves senesce; beginning of dormancy; leaf drop; development of cold tolerance
V	Early winter	Mid-December to mid-January	Period of low cellular activity
VI	Winter	Mid-January to mid-February	Cessation of dormancy; dehardening
VII	Late winter	Mid-February to April	Increase in cellular activity
VIII	Spring	Mid-April to early June	Vernal growth; active period of primary growth

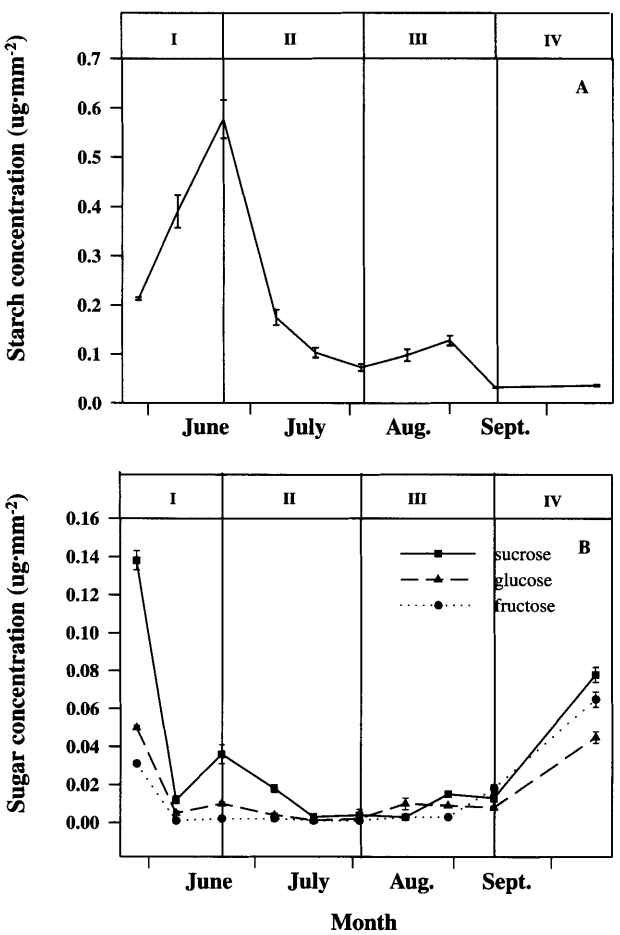


Fig. 2. (A) Starch in branches and twigs and (B) sucrose, glucose, and fructose concentrations in twigs of sugar maples (*Acer saccharum*) (25–30 years old) during the growing season. Mean values and standard errors are shown with $n = 27$ for each collection date. Roman numerals refer the to phenological and physiological events discussed in Table 1.

the end of September, the concentration of starch remained at constant high levels, while the level of sucrose dropped slightly. Similar patterns were observed for starch and soluble sugars in branch wood (data not shown for sugars in branch wood).

Foliage senescence occurs in September into early autumn with leaf drop by mid-October (period IV). At the time of leaf drop, there was very little starch present in the leaves (Fig. 2A, period IV). In contrast, the levels of leaf soluble sugars were elevated (Fig. 2B, period IV). The high levels of soluble sugars in senescing leaves may be the result of degradation of glycosidic compounds (e.g., anthocyanins and aromatic amino acids) and numerous other compounds (e.g., lipids and proteins) to sugars during autumn (Kramer and Kozlowski 1979).

A seasonal pattern of starch storage also occurred in the trunk tissue of sugar maple trees (Table 2). There were also differences in distribution of starch along the trunk for all three sampling dates. The concentration of starch along the main stem was higher within the crown compared with the lower portion (breast height) of the trunk (Table 2).

The starch concentration in the annual rings was inversely related to the age of the ring in trees felled in June and October (Figs. 3A and 3C). The correlation coefficients (r) for ring age and the pooled starch concentration were $r = -0.42$ ($p < 0.0005$) for June trees and $r = -0.56$ ($p < 0.0001$) for October trees. In trees felled in late July, the starch content was uniformly higher across all annual rings, and the correlation coefficient for starch content and ring age was not significant ($r = -0.012$) (Fig. 3B). In this study, the ring data indicate that starch stored in the ray parenchyma cells is depleted first or at a faster rate in the inner rings than in the outer rings. The ring data also demonstrate that ray tissues in the inner rings (+25 years) are still vital and active metabolically as indicated by the changes in starch storage (see Sauter et al. 1973; Kramer and Kozlowski 1979). The xylem rays consist of living cells that function as storage sites for reserve materials, mostly starch in sugar maple stems (Gregory 1982). New secondary rays are constantly formed so that the ray density is approximately maintained. According to Gregory (1977), the relative volume of xylem ray tissue represents approximately 9% of total xylem volume in annual ring widths of 1 mm.

Carbohydrates in the nonphotosynthetic period

During the nonphotosynthetic period (periods IV–VIII), stored carbohydrates are critically important for maintenance of trees during the cold season and for vernal growth. The seasonal profiles of starch in the twigs were similar in healthy mature sugar maple trees from three different age classes, i.e., 25–30, 50–100, and approximately 200 years old (Fig. 4). The seasonal carbohydrate profiles of twig woody tissues from healthy mature sugar maple trees in the

Table 2. Mean wood starch concentrations of all rings at different heights along the trunk of sugar maples (*Acer saccharum*) (25–30 years old).

		Starch concentration ($mg \cdot cm^{-3}$)				
Physiological function (season)	Month	Breast height	Midtrunk	Base of crown	Midcrown	Upper crown
Depletion (early summer)	Early June	2.12 (0.11)c	1.98 (0.18)c	3.41 (0.25)ab	3.92 (0.33)a	2.91 (0.34)b
Accumulation (summer)	Mid-July	5.40 (0.19)c	4.30 (0.24)d	5.23 (0.18)cd	7.34 (0.32)b	11.36 (1.43)a
Dissolution (fall)	Mid-October	3.00 (0.25)d	3.87 (0.35)b	3.59 (0.33)b	5.74 (0.54)a	6.61 (0.47)a

Note: In each row, mean values and standard errors in parentheses followed by the same letter are not significantly different at $p < 0.05$ with $n = 3$.

Fig. 3. Starch concentration of individual annual rings at breast height of sugar maples (*Acer saccharum*) (25–30 years old) in (A) June, (B) July, and (C) October. Mean values and standard errors are shown with $n = 3$ for each collection date.

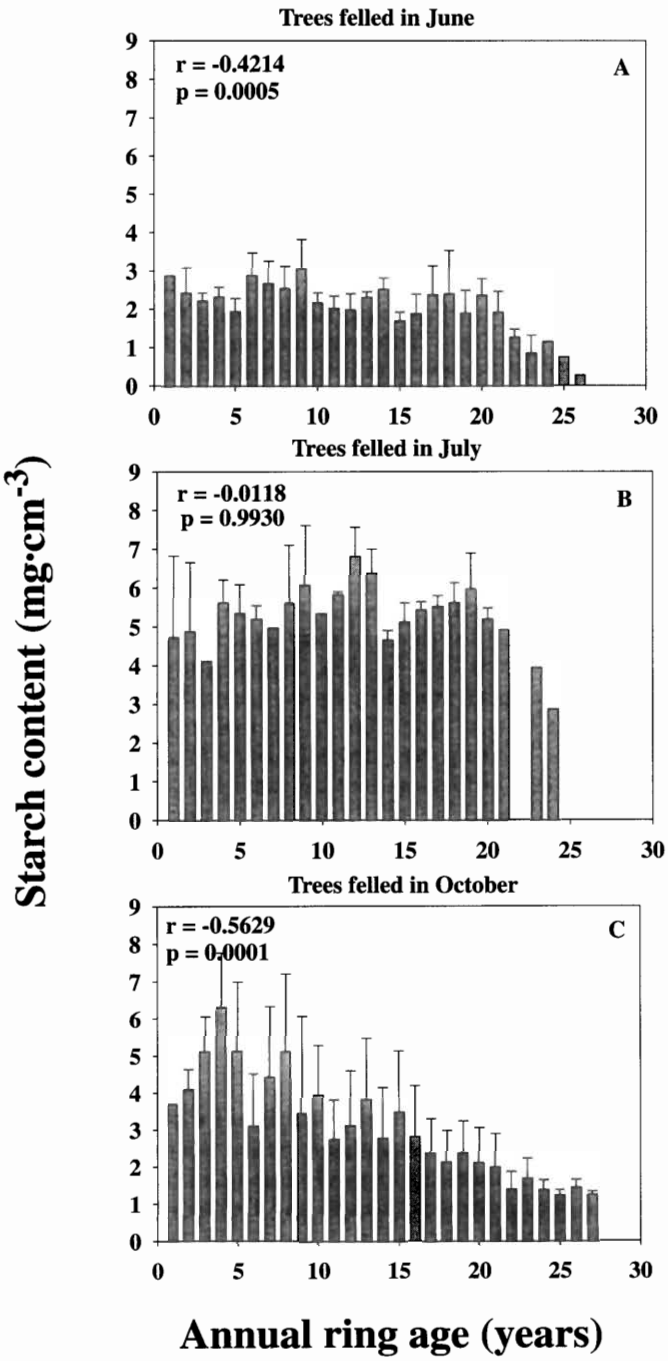
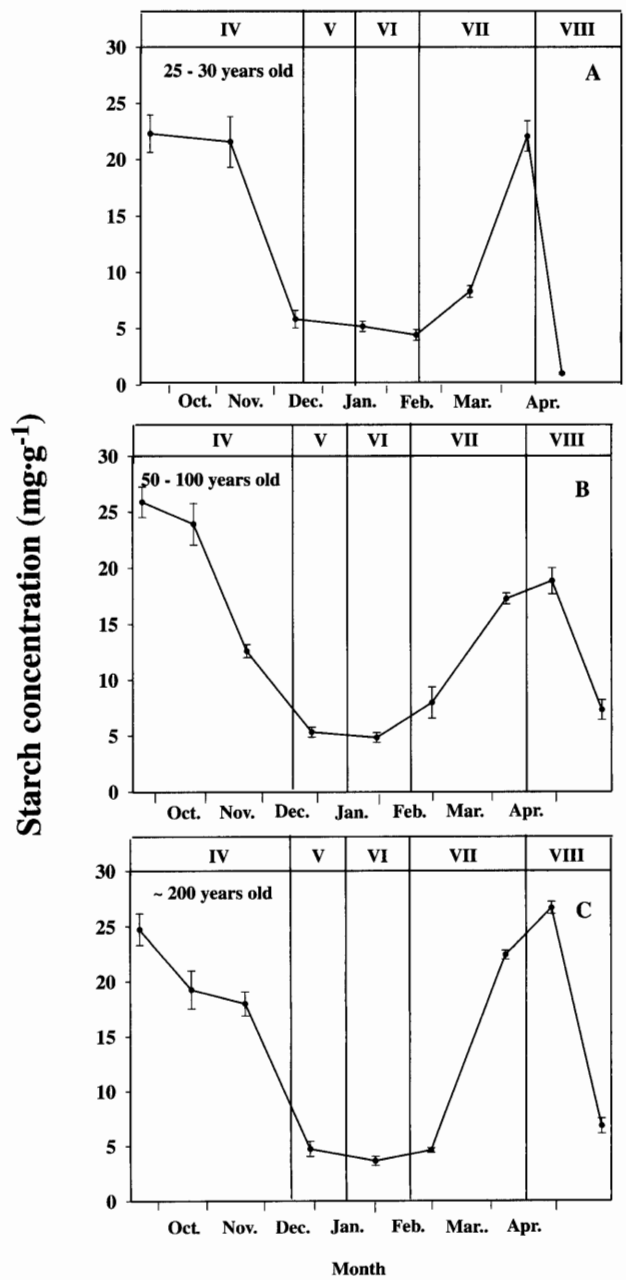


Fig. 4. Starch concentrations in twigs of sugar maples (*Acer saccharum*) from different age classes, (A) 25–30, (B) 50–100, and (C) approximately 200 years old, during the leafless period. Mean values and standard errors are shown with $n = 54$ for twigs for each collection date. Roman numerals refer to the phenological and physiological events discussed in Table 1.



present study were similar to those observed in earlier studies in the main stem and lateral roots of sugar maples (Wargo 1971; Gregory et al. 1986).

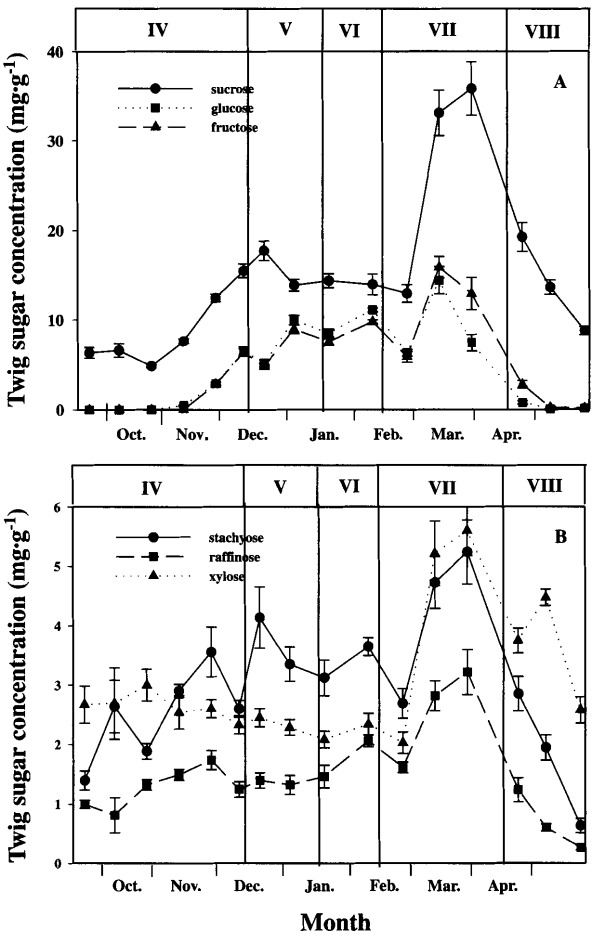
In the present study, a sharp decline in the level of starch in the twigs through the autumn from a high in mid-September to a low in mid-December (Fig. 4) coincided with physiological processes associated with adapting or avoiding freezing temperatures. This drop in starch level with decreasing temperatures has been observed in other studies on sugar maple stems (Marvin et al. 1971), cottonwood stems (Nelson and Dickson 1981), sugar maple trunks and roots (Gregory et al. 1986), and sugar maple twigs (Wong et al. 2001). Between December and February, there was little change in starch level, which remained low until late winter when starch increased presumably by resynthesis with the termination of dormancy (Fig. 4). From April to the end of May, the starch level rapidly declined in the twigs (Fig. 4). This change coincides with the resumption of spring growth, such as flowering, budbreak, shoot growth, leaf emergence and enlargement, primary root growth, etc. (Gregory 1980; Wargo 1981; Gregory and Wargo 1986; Goodman et al. 1990).

The seasonal patterns of soluble sugars in the wood tissue showed an inverse relationship with the seasonal starch pattern during the cold season. The concentrations of sucrose, glucose, and fructose (Fig. 5A) attained high levels by mid-December and remained at elevated levels until mid-January at the end of dormancy. During this time of increasing cold temperature, several biochemical and physiological changes occur, such as de novo synthesis and (or) structural changes of substances, for freeze resistance. The reserve carbohydrate is important for metabolic activity during cold acclimation (Jung and Larson 1972; Levitt 1980) and for development and maintenance of cold tolerance (Raese et al. 1978; Siminovitch 1981; Parker 1959; Sakai 1960, 1962, Levitt 1980; Carroll et al. 1983; Gregory et al. 1986). The large pool of sugars in the wood tissues between mid-December and the end of January is important in maintaining respiration in living cells (Kramer and Kozlowski 1979) and has been implicated as an osmoregulating substance for freeze protection by lowering the freezing point of the tissues, thus reducing vulnerability to and damage from cold temperature (Hansen and Grauslund 1973; Sakai 1960; Levitt 1980).

By mid-January to mid-February (period VI), the concentrations of sucrose, glucose, and fructose declined, which coincided with the cessation of dormancy (Fig. 5A). After this decline, the levels of sucrose, glucose, and fructose increased significantly by mid-March (period VII). The high sugar concentration in storage tissues following dehardening (Fig. 5A, period VII) coincides approximately with the time when xylem sap is harvested from the sugarbush.

The increase in soluble sugars (Fig. 5A) in ray tissues observed in the present study was not attributable to an increase in starch hydrolysis because at this time, starch either remained at low levels or showed only a slight increase (Fig. 4). Such increases in sugar concentration could result from the dehardening process, which occurs during the termination of dormancy. For example, during dehardening, phospholipids, fats, and cytoplasmic water-soluble polymers formed in the development of frost resistance are converted

Fig. 5. (A) Sucrose, glucose, and fructose concentrations and (B) stachyose, raffinose, and xylose concentrations in twigs of sugar maples (*Acer saccharum*) (25–30 years old) during the leafless period. Roman numerals refer to the phenological and physiological events discussed in Table 1.



to sugars. There was a rapid decline in soluble sugars associated with warming temperatures during spring (Fig. 5A), and this decline was inversely related to the starch content in the wood tissues (Fig. 4, period VII).

The amount of the less abundant sugars, i.e., stachyose and raffinose, in healthy trees also increased gradually through the autumn and remained at high levels during the colder months before declining at the end of dormancy (late January to early February) (Fig. 5B). A positive relationship between stachyose, raffinose, and frost hardiness has been observed in other studies (Parker 1959; Sakai 1960; Levitt 1980). Wargo (1971) observed similar changes in these sugars in roots in sugar maples presumably related to frost hardiness changes. Another sugar detected in the wood tissue during autumn was xylose (Fig. 5B). High levels of xylose were detected in the wood during cold acclimation or cold hardening in the autumn and also during deacclimation or dehardening events at the end of dormancy and during vernal growth, which coincided with cell wall structural modification and deposition. The role that xylose plays in cold season biochemical and physiological processes is not well understood. Xylose has been reported to be associated with

Table 3. Dominant ethanol-soluble sugars in sugar maple (*Acer saccharum*) twigs during the leafless period.

Sugar	Tree age (years)	Sugar concentration (mg·g dry mass^{-1}) Sept. (IV)	Oct. (IV)	Nov. (IV)	Dec. (V)	Jan. (VI)	Feb. (VII)	Mar. (VII)	Apr. (VIII)
Sucrose	25–30	6.17 (0.05)	17.70 (0.94)		17.95 (0.78)	17.07 (0.85)	16.12 (0.88)	18.98 (0.59)	4.78 (0.19)
	~200	8.63 (0.77)	6.27 (0.29)	19.63 (1.17)	18.70 (2.25)	12.28 (0.27)	26.89 (1.52)	28.84 (1.23)	11.42 (1.51)
Glucose	25–30	0.12 (0.03)	1.93 (0.16)		9.55 (0.45)	13.41 (0.53)	11.08 (0.54)	2.34 (0.21)	0.47 (0.03)
	~200	0.00 (0.00)	0.28 (0.13)	5.37 (0.57)	6.55 (0.53)	6.61 (0.21)	7.40 (0.61)	2.53 (0.68)	0.13 (0.01)
Fructose	25–30	0.44 (0.04)	2.19 (0.12)		8.26 (0.40)	9.47 (0.48)	10.04 (0.45)	3.56 (0.29)	0.34 (0.04)
	~200	0.00 (0.00)	0.00 (0.00)	5.15 (0.54)	5.81 (0.45)	6.04 (0.13)	8.22 (1.18)	3.89 (0.64)	0.30 (0.05)

Note: Values are means with standard errors in parentheses with $n = 27$. Roman numerals refer to the phenological and physiological events discussed in Table 1.

Table 4. Concentrations of stachyose, raffinose, and xylose in sugar maple (*Acer saccharum*) twigs during the leafless period.

Sugar	Tree age (years)	Sugar concentration (mg·g dry mass^{-1}) Sept. (IV)	Oct. (IV)	Nov. (IV)	Dec. (V)	Jan. (VI)	Feb. (VII)	Mar. (VII)	Apr. (VIII)
Stachyose	25–30	0.52 (0.04)	0.98 (0.06)		2.34 (0.18)	3.89 (0.19)	2.74 (0.15)	1.99 (0.11)	0.00 (0.00)
	~200	1.84 (0.12)	1.91 (0.12)	4.89 (0.34)	4.39 (0.37)	2.78 (0.23)	6.45 (0.81)	3.42 (0.14)	0.95 (0.18)
Raffinose	25–30	0.10 (0.02)	0.31 (0.04)		1.38 (0.10)	1.63 (0.10)	1.12 (0.63)	1.17 (0.06)	0.00 (0.00)
	~200	0.64 (0.11)	1.02 (0.07)	2.27 (0.23)	1.97 (0.26)	2.60 (0.19)	2.87 (0.25)	1.43 (0.11)	0.40 (0.06)
Xylose	25–30	0.30 (0.02)	0.71 (0.03)		3.36 (0.28)	3.23 (0.17)	2.63 (0.10)	2.41 (0.11)	2.51 (0.15)
	~200	3.20 (0.18)	3.25 (0.27)	3.20 (0.19)	3.05 (0.30)	1.49 (0.08)	3.73 (0.44)	7.00 (0.22)	3.97 (0.45)

Note: Values are means with standard errors in parentheses with $n = 27$. Roman numerals refer to the phenological and physiological events discussed in Table 1.

the hemicellulose component of the cell wall (Labavitch 1981; Fry 1989; Figuciredo-Ribeiro et al. 1992).

As was observed in starch profiles (Fig. 4), the profiles of soluble sugars of healthy sugar maple trees were not significantly different among trees of different ages (Tables 3 and 4). Changes in cold season reserve carbohydrate profiles may indicate alteration in carbohydrate metabolism and (or) physiology (Wong et al. 2001). Such changes in carbohydrate profiles can be used to assess tree health.

Conclusion

The seasonal carbohydrate profiles of the leaves and stemwood of healthy mature sugar maples show seasonal patterns that closely relate to seasonal changes in phenological and physiological activities. During the active photosynthetic period, the amount of starch and soluble sugars stored in the wood tissues remains low; most of the carbon used for growth is supplied by currently produced photosynthate. Allocation of carbohydrate to storage occurs with reduced sink strength and onset of dormancy by late summer to early autumn. The reserve carbohydrate stored in the wood tissue is the main energy and substrate source of carbon throughout the cold season and for vernal growth. Early in the nonphotosynthetic period, the reserve carbohydrate is used for various biochemical and physiological activities associated with cold acclimation (hardening), freeze tolerance, cellular maintenance, and respiration. At the end of dormancy and the dehardening process, the levels of soluble sugars decline with an increase in starch levels prior to carbon mobilization for primary growth activities, i.e., flowering and shoot and root growth.

In the present study, the seasonal patterns of reserve and soluble carbohydrates in the twigs from mature sugar maple trees were independent of age and similar to seasonal patterns observed using the main stem and roots in an earlier study. For research in cold season physiology, the use of twigs for carbohydrate analysis is easier and less destructive than sampling the trunk or lateral roots, and less damage occurs with multiple sampling from the same trees in subsequent years.

The amount of reserve carbohydrate stored in the tree by autumn is an important indicator of tree vitality and photosynthetic capacity. The changes in cold season profiles of reserve and soluble carbohydrates can provide information about tree health. Changes in carbohydrate profiles caused by abiotic or biotic stressors when compared with those from healthy trees can be used to assess the magnitude of disturbance to tree physiology. In a separate study, differences were observed in the reserve and soluble carbohydrate profiles in the twigs of healthy sugar maple trees (with less than 10% crown loss) when compared with those in declining trees with greater than 50% crown loss (Wong et al. 2001). The observed shifts in the cold season profiles of reserve and soluble carbohydrates from those of healthy trees could reflect adaptative or stress-induced changes in the physiology of trees. Studies of the effect of environmental stressors on the carbohydrate status of sugar maple trees have been initiated.

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References

Brundett, M.C., and Kendrick, B. 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Can. J. Bot. **66**: 1153–1173.

Carroll, J.E., Tattar, T.A., and Wargo, P.M. 1983. Relationship of root starch to decline of sugar maple. Plant Dis. **67**: 1347–1349.

Chatterton, N.J., and Silvius, J.E. 1980. Photosynthate partitioning into leaf starch as affected by daily photosynthetic period duration in six species. Physiol. Plant. **49**: 141–144.

Figuciredo-Ribeieo, R.C.L., Isejima, E.M., Dietrich, S.M.C., and Correa, J.B.C. 1992. Hemicellulosic polysaccharides from the xylopodium of *Ocimum nudicaule*: changes in composition in dormancy and sprouting. Ann. Bot. **70**: 405–408.

Fry, S.C. 1989. The structure and functions of xyloglucan. J. Exp. Bot. **43**: 1–11.

Goodman, R.M., Yawney, H.W., and Tubbs, C.H. 1990. Sugar maple (*Acer saccharum* Marsh.). *In* Silvics of North America. Vol. 2. Hardwoods. *Edited by* R.M. Burns and B.H. Honkala. U.S. Dep. Agric. Agric. Handb. 654. pp. 78–91.

Gregory, R.A. 1977. Cambial activity and ray cell abundance in *Acer saccharum*. Can. J. Bot. **55**: 2559–2564.

Gregory, R.A. 1980. Annual cycle of shoot development of sugar maple. Can. J. For. Res. **10**: 316–326.

Gregory, R.A. 1982. Release of sap sugar and control of sap pressure. U.S. For. Serv. Gen. Tech. Rep. NE-72. pp. 1–7.

Gregory, R.A., and Wargo, P.W. 1986. Timing of defoliation and its effect on bud development, starch reserves, and sap sugar concentration in sugar maple. Can. J. For. Res. **16**: 10–17.

Gregory, R.A., Williams, J.W., Jr., Wong, B.L., and Hawley, G.J. 1986. Proposed scenario for dieback and decline of *Acer saccharum* in northeastern U.S.A. and southeastern Canada. IAWA Bull. New Ser. **7**: 357–369.

Hansen, P., and Grausland, J. 1973. ^{14}C studies on apple trees. VIII. The seasonal variation and nature of reserves. Physiol. Plant. **28**: 24–32.

Hendrick, R.L., and Pregitzer, K.S. 1992. The demography of fine roots in a northern hardwood forest. Ecology, **73**: 1094–1104.

Hendrick, R.L., and Pregitzer, K.S. 1993. Patterns of fine root mortality in two sugar maple forests. Nature (Lond.), **361**: 59–61.

Hendrix, D.L. 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. Crop Sci. **33**: 1306–1311.

Jones, C.H., and Bradlee, J.L. 1933. The carbohydrate contents of the maple tree. Bull. 358. Vermont Experiment Station, Burlington, Vt.

Jung, G.A., and Larson, K.L. 1972. Cold, drought, and heat tolerance. *In* Alfalfa science and technology. *Edited by* C.H. Hanson. American Society of Agronomy, Madison, Wis. pp. 185–209.

Kramer, P.J., and Kozlowski, T.T. 1979. Physiology of woody plants. Academic Press, New York.

Labavitch, J.M. 1981. Cell wall turnover in plant development. Annu. Rev. Plant Physiol. **32**: 385–406.

Levitt, J. 1980. Response of plants to environmental stresses. Vol. I. Chilling, freezing, and high temperature stresses. Academic Press, New York.

Long, R.P., Horsley, S.B., and Liija, P.R. 1997. Impact of forest liming on growth and crown vigor of sugar maple and associated hardwoods. Can. J. For. Res. **27**: 1560–1573.

Marvin, J.W., Morselli, M., and Mathes, M.C. 1971. Rapid low temperature hydrolysis of starch to sugars in sugar maple stems and in maple tissue cultures. Cryobiology, **8**: 339–344.

McLaughlin, J.W., Reed, D.D., Jurgensen, M.F., Mroz, G.D., and Bagley, S.T. 1996. Relationships between soluble sugar concentrations in roots and ecosystem stress for first-year sugar maple seedlings. Water Air Soil Pollut. **88**: 1–19.

Nelson, E.A., and Dickson, R.E. 1981. Accumulation of food reserves in cottonwood stems during dormancy induction. Can. J. For. Res. **11**: 145–154.

Parker, J. 1959. Seasonal variations in sugars of conifers with some observations on cold resistance. For. Sci. **5**: 56–63.

Parker, J. 1974. Effects of defoliation, girdling, and severing of sugar maple trees on root starch sugar levels. U.S. For. Serv. Res. Pap. NE-306.

Parker, J., and Houston, D.R. 1971. Effects of repeated defoliation on root and root collar extractives of sugar maple trees. For. Sci. **17**: 91–95.

Pitelka, L.F., and Raynal, D.J. 1989. Forest decline and acidic deposition. Ecology, **70**: 2–10.

Pollock, C.J. 1997. Resource use efficiency and crop performance: what is the link? *In* A molecular approach to primary metabolism in higher plants. *Edited by* C.H. Foyer and W.P. Quick. Taylor and Frances, Bristol, Pa. pp. 327–336.

Quick, W.P., and Neuhaus, H.E. 1997. The regulation and control of photosynthetic carbon assimilation. *In* A molecular approach to primary metabolism in higher plants. *Edited by* C.H. Foyer and W.P. Quick. Taylor and Frances, Bristol, Pa. pp. 41–62.

Raese, J.T., Williams, M.W., and Billingsley, H.D. 1978. Cold hardiness, sorbitol, and sugar levels of apple shoots as influenced by controlled temperature and season. J. Am. Soc. Hortic. Sci. **103**: 796–801.

Rasmussen, T.S., and Henry, R.J. 1990. Starch determination in horticultural plant material by an enzymic–colorimetric procedure. J. Sci. Food Agric. **52**: 159–170.

Renaud, J.-P., and Mauffette, Y. 1991. The relationships of crown dieback with carbohydrate content and growth of sugar maple (*Acer saccharum*). Can. J. For. Res. **21**: 1111–1118.

Sakai, A. 1960. The frost-hardening process of woody plants. VII. Seasonal variations in sugars. Low Temp. Sci. Ser. B. Biol. Sci. **18**: 1–14.

Sakai, A. 1962. Studies on the frost-hardening of woody plants. I. The causal relation between sugar content and frost-hardiness. Contrib. Inst. Low Temp. Sci. Hokkaido Univ. Ser. B, **11**: 1–40.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5. SAS Institute Inc., Cary, N.C.

Sauter, J.J., Iten, W., and Zimmermann, M.H. 1973. Studies on the release of sugars into the vessels of sugar maple (*Acer saccharum* Marsh.). Can. J. Bot. **51**: 1–8.

Siminovitch, D. 1981. Common and disparate elements in the process of adaptation of herbaceous and woody plans to freezing: a perspective. Cryobiology, **18**: 166–185.

Wargo, P.M. 1971. Seasonal changes in carbohydrate levels in sugar maple. U.S. For. Serv. Res. Pap. NE-213.

Wargo, P.M. 1979. Starch storage and radial growth in woody roots of sugar maple. Can. J. For. Res. **9**: 49–56.

Wargo, P.M. 1981. Defoliation and tree growth. *In* The gypsy moth: research towards integrated pest management. *Edited by* C.C. Doane and M.L. McManus. U.S. For. Serv. Tech. Bull. 1384. pp. 225–240.

Wilmot, T.R., Ellsworth, D.S., and Tyree, M.T. 1995. Relationships among crown condition, growth, and stand nutrition in seven northern Vermont sugarbushes. Can. J. For. Res. **25**: 386–397.

Wong, B.L., Baggett, K.L., Burfeind, A.S., and Rye, A.H. 2001. Carbohydrate profiles in woody tissues of sugar maples with crown dieback symptoms during the leafless period. *In* L'Arbre 2000 – 2000 The Tree: Proceedings of the Fourth International Symposium on the Tree, Montreal, Que., 20–25 August 2000. *Edited by* M. Labrecque. IQ Collectif, Montreal, Que. pp. 314–319.