Cold-season patterns of reserve and soluble carbohydrates in sugar maple and ice-damaged trees of two age classes following drought

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

Abstract: This study examines the effects of summer drought on the composition and profiles of cold-season reserve and soluble carbohydrates in sugar maple (*Acer saccharum* Marsh.) trees (50–100 years old or ~200 years old) in which the crowns were nondamaged or damaged by the 1998 ice storm. The overall cold season reserve carbohydrate profiles in twig wood tissue of drought-stressed (DS) trees and non-drought-stressed (NDS) trees were generally similar, although differences were observed in the amount of reserve carbohydrates in DS and NDS trees. The cold-season level of starch stored in DS trees in early autumn in the wood tissue was about one-third to one-fifth that in NDS trees. The cold season sugar content in the DS trees was significantly greater than can be attributed to degradation of stored starch, only. The level of sucrose in DS trees remained high throughout the winter until termination of dormancy and dehardening. The concentrations of winter glucose and fructose in DS trees attained peak levels at the time of dormancy termination and declined during dehardening. The profiles of glucose and fructose in DS and damaged DS trees were generally different from that of sucrose throughout the leafless phase. In contrast, profiles of glucose and fructose in NDS trees closely paralleled that of sucrose. Elevated levels of sucrose, glucose, and fructose in DS sugar maple trees during the cold season may function as osmoregulators for freeze protection. Low sugar level or lack of increase in sugar level following dehardening in DS trees may suggest limited change in cellular constituents in adapting to low temperatures.

Key words: starch, sucrose, glucose, fructose.

Résumé : Les auteurs ont examiné les effets de la sécheresse estivale sur la composition ainsi que les profils des glucides solubles et de réserve en saison froide chez l'érable à sucre (*Acer saccharum* Marsh.) (âgés de 50–100 ans ou ~200 ans) dont les couronnes avaient été endommagées par le verglas de 1998. Les profils de l'ensemble des glucides en saison froide, dans les tissus des ramilles ligneuses des arbres stressés par sécheresse (DS) ou non stressés par la sécheresse (NDS) sont apparus généralement semblables, bien qu'on observe des différences dans la quantité de glucides de réserve chez les arbres DS et NDS. En saison froide, la teneur hivernale en amidon, accumulée dans les tissus ligneux des arbres DS en début de l'automne, atteint environ un tiers ou un cinquième de celle des arbres NDS. On observe une teneur significativement plus forte des sucres en saison froide chez les arbres DS, qu'on peut attribuer uniquement à la dégradation de l'amidon. La teneur en saccharose chez les arbres DS demeure élevée tout au long de l'hiver jusqu'à la fin de la dormance et du débourrement. Les teneurs hivernales en glucose et fructose chez les arbres DS atteignent des degrés maximums au moment de la fin de la dormance et diminuent au cours du débourrement. Les profils du glucose et du fructose chez les arbres DS et NDS diffèrent généralement de celui du saccharose tout au long de la phase sans feuille. Au contraire, les profils du glucose et du fructose, chez les arbres NDS, suivent étroitement celui du saccharose. Les teneurs élevées en saccharose, glucose et fructose chez les érables DS au cours de la saison froide peuvent agir comme osmorégulateurs pour prévenir le gel. De faibles teneurs en sucres, ou le manque d'augmentation de la teneur en sucre suite au débourrement chez les arbres DS pourrait suggérer des modifications limitées dans les constituants cellulaires au cours de l'adaptation aux basses températures.

Mots-clés : amidon, saccharose, glucose, fructose.

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Introduction

The reserve carbohydrate stored in early autumn is the main source of carbon utilized during the leafless cold season for cold acclimation, development and maintenance of cold tolerance, and cellular respiration (Sakai 1960, 1966; Raese et al. 1978; Levitt 1980; Siminovitch 1981; Carroll et al. 1983; Gregory et al. 1986). The amount of starch stored in early autumn has been used as a predictive indicator of sugar maple tree health and as a measure of tree vitality and productivity, with high levels indicating high vitality (Wargo 1981; Gregory et al. 1986, Rasmussen and Henry 1990; Renaud and Mauffette 1991). Low levels of stored starch have been reported to be associated with tree dieback and mortality (Wargo 1979; Gregory et al. 1986; Rasmussen and Henry 1990; Renaud and Mauffette 1991; McLaughlin et al. 1996; Wong et al. 2001; Wargo et al. 2002). Cold-

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season survival during the leafless period depends on adequate reserves, and in sugar maple, starch is the main reserve carbohydrate. In early spring, the stored starch is important for bud break and early season growth (Gregory 1980; Wargo 1981; Gregory and Wargo 1986; Godman et al. 1990; Wong et al. 2003).

Summer drought has been reported to reduce photosynthetic carbon fixation and thus decrease the supply of carbohydrate for growth (Kramer 1964). Decreases in rates of production and accumulation of nonstructural carbohydrates for utilization during the leafless period (Levitt 1980) could affect the cold-season physiology of deciduous trees. The trees of the northeast United States experienced drought conditions during the 1999 growing season. Precipitation during the 1999 growing season, April through September, was below normal, while temperatures were above normal during this period (Northeast Regional Climate Center). In 1999, northern hardwood forest trees showed evidence of drought symptoms, such as dieback and thinner crowns (Vermont Department of Forests, Parks and Recreation Report 2000).

The main focus of this study was to investigate the impact of drought on the cold season nonstructural carbohydrate dynamics of trees that were undamaged or slightly impacted versus those severely damaged by ice glazing in the previous year (1998). Two sugar-maple stands (sugarbush) aged 50–100 years old and ~200 years old, were utilized, and the effect of drought on cold season carbohydrate metabolism was examined by comparing: (*i*) the cold-season profiles of starch, sucrose, glucose, and fructose of drought-stressed (DS) versus non-drought-stressed (NDS) sugar-maple trees; and, (*ii*) the dynamics of soluble sugars in the autumn, winter, and spring transition periods in DS compared with NDS trees.

Materials and methods

Site descriptions

The study was conducted in two private maple stands (sugarbush) located in northeast New York. In the older of the two stands, trees were approximately 200 years old, and tapped for syrup production for about the last 50 years. This stand is a monoculture of sugar maples and has no understory woody plants. In the other stand, trees were younger (50 to 100 years old), and tapped for syrup production for about 5 years. This stand is predominately a mix of sugar and red maple (*Acer rubrum* L.) with some white pine (*Pinus strobus* L.). The two sugar-maple stands were previously used to determine the effect of the January 1998 ice storm damage on cold-season carbohydrate profiles and composition (Wong et al. 2005). Following the 1999 summer drought, samples were collected from trees with less than 25% crown damage (designated as "non-damaged," DS or NDS) or greater than 50% crown loss (designated as "damaged," DS-d or NDS-d). The criteria for tree selection for each collection date for drought-stressed (DS) trees were similar to those for non-drought-stressed (NDS) trees the previous year (1998). Drought-stressed trees with no large wounds, cankers or other obvious disease signs or symptoms were identified and flagged in August at each of the stands.

Sample collection

To determine the cold season carbohydrate profiles for DS and drought-stressed damaged, (DS-d) trees, twig sample collections in 1999 were processed similarly to NDS twig samples collected in 1998. Four twigs per tree were collected at each collection date from three trees of each crown damage condition (n = 12 twigs) at each site (young and older maple stands). Samples were collected biweekly from October to mid-May and transported at ambient temperature to the Northeastern Forest Research Station in South Burlington, Vermont. To avoid diurnal influences and for consistency, all collections were made between 0900 and 1100 h at mid-crown level.

Sample preparation

All samples were immediately processed when returned to the laboratory. Twig samples removed from the basal internode area containing 3–5 growth rings were used. After removal of the bark, phloem, cambium, and pith, samples of the xylem tissue (two pieces approximately 5 mm in length) were each separately submerged in 5 mL of 80% ethanol, placed in a boiling water bath for 15 min, and then evacuated to –52 kPa for 15 min. Each wood sample was homogenized with a Brinkman Instruments (Westbury, Mass.) Polytron™ in 80% ethanol, centrifuged for 15 min at 1000*g* and the macerated samples were extracted twice more with 5 mL of 80% ethanol. For each sample, the supernatants were combined, filtered through a 0.45 µm syringe filter, and used for soluble sugar analysis. The ethanol insoluble pellets were used to determine the starch content.

Sugar analysis

Ethanol-soluble fractions were analyzed as described by Wong et al. (2001, 2003, 2005) using an HPLC system with a Sugar-pak™ column (Millipore Corp., Milford, Mass.) and solvent (0.1 mmol·L^{-1} Ca EDTA) flow rate of 0.6 mL·min^{-1} at 90 °C for sucrose, glucose, fructose, stachyose and raffinose. Sugars were detected with a Waters model 410 Refractive index detector connected to a personal computer equipped with Waters-Millenium software. The separated soluble sugars were identified and quantified with known standards and converted to milligrams of sugar per gram residue of dry mass of tissue. Total soluble sugar (TSS) concentrations were calculated by summing the individual sugar concentrations (sucrose, glucose, fructose, stachyose, and raffinose).

Starch analysis

Starch was quantified by the method of Hendrix (1993) with some modification. The starch was gelatinized in each pellet with 0.2 N KOH and hydrolyzed to glucose with amyloglucosidase (No. 10115, Fluka Chemical Co.). Glucose was quantified colorimetrically using the INT assay (glucose assay kit No. 115-A, Sigma Chemical Co.) with the microplate sugar analysis method as described by Hendrix (1993). The concentration of starch was calculated from glucose standard curves and expressed as milligrams per gram residue of dry mass.

Net photosynthetic capacity (P_{max})

Photosynthesis measurements were made in late July 1999 using leaves from mid-crown level and leaves from epicor-

mic shoots developed following the 1998 ice storm. Field measurements of leaf photosynthesis were not obtained, owing to the drought condition and high temperature at the two sugar-maple sites. Branches containing leaves from mid-crown and epicormic shoots were collected and transported to a laboratory at the University of Vermont, School of Natural Resources to determine photosynthetic capacity of the leaves. The maximum photosynthetic rates of rehydrated (in water in a cold room at 4 °C) DS shoots under near optimal growing seasonal temperature and light condition were measured using a Li-6262 CO_2·H_2O IRGA (LI-COR Inc., Lincoln, Nebr.) as described by Ellsworth and Reich (1993). Stomatal conductance was calculated as by von Caemmerer and Farqubar (1981).

Statistical analyses

After testing for normality using the Shapiro–Wilk test, data were analyzed using the SAS Statistical package (SAS Institute Inc. 1985). Analysis of variance and Duncan's multiple range test were used to test for differences in means of starch, sucrose, glucose, fructose, stachyose, and raffinose concentrations for each collection date between stress conditions (DS and NDS) of damaged and undamaged trees of two age classes at $p < 0.05$.

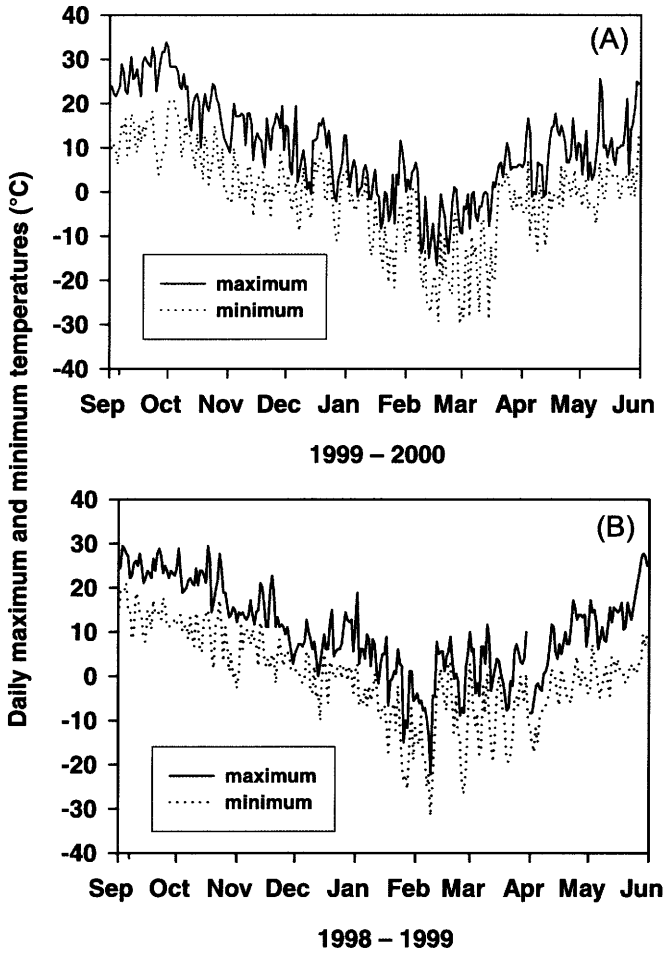
Results

The nonstructural carbohydrate patterns of sugar maple generally followed a seasonal cycle of accumulation and use during the leafless phase which coincided with cold-season changes in temperature. The temperature pattern during the leafless phase of 1999 to 2000 (Fig. 1A) was similar to that of the previous year (Fig. 1B). Figure 2 shows the relation between the cold-season patterns of starch and total soluble sugar in DS and NDS sugar-maple trees. As there was no significant difference in starch and total sugar between nondamaged and damaged trees, results from only nondamaged trees are presented in Fig. 2. The cold-season profile of starch concentration (Fig. 2) closely paralleled changes in temperature (see Fig. 1) and was inversely correlated with the total soluble sugar concentration profile (Fig. 2). In this paper, as a point of easy reference, the leafless phase is divided into three distinct activity periods which correspond with cold season physiological activity: Autumn Transition I (October–December): development of dormancy, acclimation, and cold tolerance; Winter II (January – mid-March): maintenance of cold tolerance, dormant cellular respiration, late winter cessation of dormancy, and increased cellular respiration with dehardening; Early Spring III (late March–May): starch resynthesis and vernal growth activity.

Autumn transition (period I)

At the time of leaf drop (October) the concentration of reserve carbohydrate present in twig samples from DS and DS-d trees was different from that in NDS and NDS-d trees. The early autumn (October) starch concentration in DS trees was approximately 1/3 to 1/6 of that in NDS trees (Table 1, period I). In addition, the concentration of October starch (Table 1, period I) in DS and DS-d wood samples in early autumn constituted 25%–28% of the total nonstructural carbohydrate (starch + total soluble sugar), whereas in NDS and NDS-d trees, starch constituted 68%–70% of the total nonstructural carbohydrate stored in trees from both the older and younger maple stands.

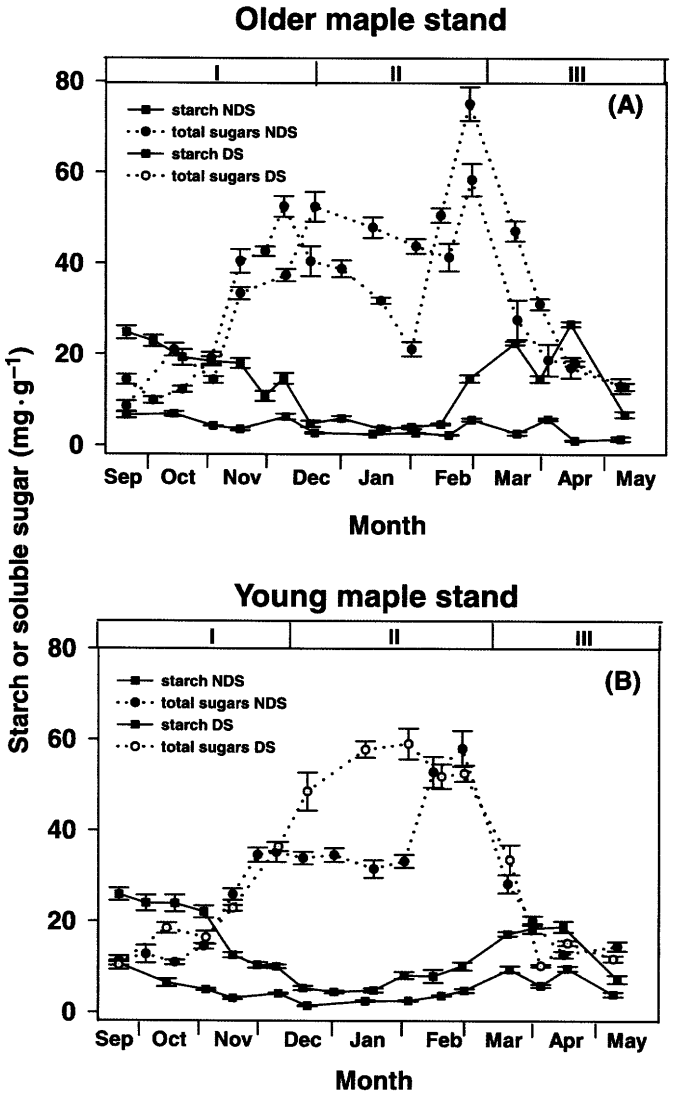
Fig. 1. Daily maximum and minimum temperatures of leafless phase following (A) 1999 summer drought and (B) 1998 growing season. Temperature data compiled from the Northeast Regional Climate Center at Cornell University, Cornell, N.Y.



To assess whether the low level of reserve starch in DS trees could be attributed to impact of drought on photosynthetic activity, it was decided to measure photosynthesis. In this study however, field measurements of leaf photosynthesis from DS and DS-d trees during the 1999 summer in northeast New York were not consistently obtainable or detectable. To assess whether the net photosynthetic capacity (P_{max}) of DS and DS-d trees was impaired by drought, measurements of net photosynthetic capacity were taken from leaves of rehydrated branches from DS trees (Table 2). The concentration of total soluble sugar was higher than the amount of reserve starch in the DS October wood samples, the ratio of total soluble sugar to starch was higher in DS (3.2:1) and DS-d (2.9:1) compared with NDS (0.48:1) and NDS-d (0.40:1).

With decreasing temperatures, the starch concentration (October) fell to low concentration by mid-December in both NDS and DS trees (Fig. 2). The concentration of early October starch depletion by late December in the NDS and

Fig. 2. Starch and total soluble sugar profiles in twigs from drought-stressed (DS) and non-drought-stressed (NDS) sugar-maple trees from (A) the older (~200 years old trees) stand and (B) the young (50–100 year old trees) stand. Mean values and standard error bars are shown at each collection date (n = 12). Roman numerals refer to periods which correspond to cold season physiological activity (see text).



DS trees was significantly different, i.e., a drop of 18–19 mg·g^{-1} of dry mass in NDS and only 5–6 mg·g^{-1} of dry mass in DS trees (Table 1, period I). In concert with starch disappearance, the concentration of total soluble sugars increased to winter (January) high level (Fig. 2, period II; Table 3, period II).

Sucrose was the most abundant sugar during the cold season in sugar maples; glucose and fructose were also present. The concentration of sucrose in early October in DS and DS-d trees was generally higher than that in NDS and NDS-d trees (Table 4). Early autumn glucose and fructose concentrations in DS and DS-d trees were significantly higher than in NDS trees (Table 4). In fact, glucose and fructose were not detectable in early October (Table 4) in NDS and NDS-d trees from the younger and older maple stands.

Winter transition (period II)

As indicated above, the starch stored in the wood tissue of NDS and DS trees during the winter was not totally depleted but remained at a constant low concentration before increasing in late February – early March, presumably, with starch resynthesis (Fig. 2; Table 1) and increasing temperature (Fig. 1). In contrast to the winter starch profile (Fig. 2), the winter profiles of sucrose, glucose, and fructose were at elevated levels in DS and DS-d (Fig. 3) and in NDS and NDS-d (Fig. 4) trees. The ratios of winter total soluble sugar concentration (Table 1, period II) to early autumn (October) starch concentration (Table 2, period I) were 7.8:1 in DS and 7.0:1 in DS-d trees while in NDS and NDS-d trees the ratios were 1.5:1 and 1.6:1, respectively.

The mean winter concentration of sucrose (January) was higher by 31%–34% in DS and 32%–45% in DS-d trees than in respective NDS and NDS-d trees (Table 5). The concenetration of glucose in the younger and older trees (Table 5) was higher, by 18% and 29%, respectively, in DS, and by 25% and 44% in DS-d trees (early February) than at peak levels (January) in NDS and NDS-d trees (January). Fructose concentrations were 40% and 52% higher in younger and older DS trees, respectively, and by 48% and 56% in DS-d trees compared with NDS trees (Table 5).

The drop from the winter high concentration (January) of sucrose in DS (Fig. 3) and NDS (Fig. 4) trees to winter low concentration (February) coincided presumably with increased utilization in the dehardening process. In NDS trees sucrose declined 32% from January high level to February low (Table 5). Although the mean winter sucrose concentration was higher in DS trees, the sucrose concentration declined by only 16% (Table 5). At the same time, glucose and fructose in NDS trees declined by 50% in early February (Table 5). In contrast, the concentrations of glucose and fructose in DS and DS-d trees remained high during dehardening (early February), and did not decline until late March, when these concentrations dropped about 95% from the mid-February high.

With warming temperatures and following dehardening in late winter, the concentration of soluble sugars increased to levels either equal to or exceeding the mean winter level (Fig. 2, period II). In DS trees, the concentration of late winter sucrose (early March) was 1.6-times higher than the mean winter sucrose concentration (January) in older trees but was not significantly greater in younger trees (Table 5). In NDS trees (Table 5), the concentration of late winter sucrose (early March) was higher by 2.6-times and 4.4-times in younger and older trees, respectively, than the mean winter sucrose level (January). A significant difference was observed, however, in the patterns of glucose and fructose in DS and NDS trees. The concentrations of late winter glucose and fructose in DS trees continued to decline from early February to early spring (Fig. 3, period II). In NDS trees, the concentrations of late winter glucose and fructose following dehardening increased to concentrations similar or slightly higher than the mean winter concentration (Fig. 4, period II; Table 5).

Two other sugars present in sugar maple wood tissue during the cold season are stachyose and raffinose (data not shown). Stachyose and raffinose in DS and NDS trees were both at elevated concentrations at the time of leaf drop and

Table 1. Cold-season concentrations of starch [mg·(g residue dry mass)$^{-1}$] in twigs of drought-stressed (DS) and non-drought-stressed (NDS) sugar-maple trees.

Transition period	Collection dates (1998–1999)	Cold-season starch				Collection dates (1999–2000)	Cold-season starch			
		Non-drought-stressed sugar maple					Drought-stressed sugar maple			
		Y	O	Y-d	O-d		Y	O	Y-d	O-d	Pr > F
I	Oct. 1	24.0±1.8c	23.0±1.3c	30.2±3.1b	38.7±4.1a	Oct. 7	6.5±0.9d	6.9±0.5d	11.1±1.0d	9.1±0.8d	0.0001
	Oct. 15	23.9±1.9ab	19.3±1.7b	23.2±2.1ab	26.2±1.9a	Oct. 26	5.0±0.3b	4.3±0.1c	4.9±0.6c	4.5±0.6c	0.0001
	Nov. 12	12.6±0.6b	18.0±1.1a	13.2±1.2b	14.2±1.4b	Nov. 8	3.4±0.4c	3.5±0.3c	4.1±0.5c	3.7±0.4c	0.0001
	Nov. 24	10.4±0.6a	10.8±1.1a	7.6±0.7b	7.2±0.2b	Nov. 30	4.1±0.1c	6.3±0.6b	3.3±0.2c	4.4±0.4c	0.0001
	Dec. 16	5.3±0.5ab	4.7±0.7b	5.8±0.5ab	6.7±0.9a	Dec. 14	1.4±0.1c	2.7±0.2c	1.3±0.1c	1.5±0.2c	0.0001
II	Jan. 19	4.8±0.4a	3.6±0.4b	5.1±0.3a	4.5±0.2a	Jan. 11	2.4±0.2c	2.4±0.1c	2.7±0.1c	2.7±0.2c	0.0001
	Feb. 3	8.1±0.8a	4.0±0.4c	6.5±0.6b	4.9±0.4c	Feb. 1	2.5±0.1d	2.7±0.1d	2.6±0.1d	2.7±0.3d	0.0001
	Feb. 17	8.0±1.4a	4.6±0.2b	7.1±0.6a	7.0±0.6a	Feb. 17	3.6±0.1bc	2.3±0.1c	3.6±0.3bc	2.5±0.1c	0.0001
	Mar. 3	10.1±0.9b	14.6±0.8a	11.8±1.3b	7.0±0.7c	Feb. 28	4.8±0.4c	5.3±0.4c	5.6±0.3c	5.2±0.5c	0.0001
III	Mar. 25	17.3±0.5b	22.4±0.4a	22.1±1.1a	14.0±1.2c	Mar. 22	9.5±0.7d	2.6±0.5e	7.6±0.9d	7.3±0.5d	0.0001
	Apr. 6	18.5±1.1a	14.6±0.7b	18.3±1.2a	16.4±0.8ab	Apr. 6	5.9±0.3c	5.7±0.3c	7.3±0.4c	6.0±0.3c	0.0001
	Apr. 21	18.9±1.2b	26.7±0.6a	19.4±1.7b	16.9±0.9b	Apr. 19	9.6±0.5c	1.0±0.2e	4.2±0.6d	6.7±1.0-cd	0.0001
	May 17	7.3±0.9bc	6.8±0.7cd	10.0±1.4b	15.7±1.8a	May. 11	4.0±0.5de	1.4±0.5e	3.5±0.4e	1.8±0.6e	0.0001

Note: I, autumn transition; II, winter transition; III, spring transition. Y, young stand; O, older stand; -d, crown >50% damaged. Data are mean ± SE (n = 12); numbers within a row that do not share a letter are significantly different.

Table 2. Leaf photosynthesis measurements of sugar-maple trees from rehydrated braches of drought-stressed (DS) and drought-stressed trees with severe crown damage (DS-d).

Crown loss	Leaf type	P_{max}
Young-maple stand		
DS	Canopy	1.40b
DS-d		2.90ab
DS	Epicormic	1.95b
DS-d		2.68ab
Older-maple stand		
DS	Canopy	4.67a
DS-d		3.16ab
DS	Epicormic	3.00ab
DS-d		1.76b

Note: DS and DS-d, respectively, refer to drought-stressed trees and drought-stressed trees with crown > 50% damaged. Numbers within a column that do not share a letter are significantly different.

remained at relatively constant concentration through the winter. In DS trees stachyose and raffinose were not observed following dehardening. In contrast, these sugars were detectable in NDS trees. The concentrations of stachyose and raffinose during dehardening (early February) increased in NDS trees and declined with starch resynthesis (Fig. 2, period II).

Spring transition (period III)

The concentrations of late winter sucrose in DS (Fig. 3, period III) and NDS trees (Fig. 4, period III) declined by late March with the appearance of spring starch (Fig. 2, period III). The concentrations of glucose and fructose in DS trees (Fig. 3, period III; Table 6) and in NDS trees (Fig. 4, period III; Table 6) remained low with increase in spring starch concentration, perhaps owing to utilization with vernal growth and (or) conversion to sucrose.

The concentration of spring starch in DS trees and NDS trees (Fig. 2, period III) prior to vernal growth was similar to or lower than the concentration of starch stored in early autumn (Fig. 2, period I; Table 2). In DS trees (Table 2, period III), the concentration of starch did not significantly increase in spring despite the large concentration of sucrose present during the winter period (Fig. 3, period II). It appears that a large portion of winter sugar (Fig. 3, period II) of the DS trees was not utilized in starch resynthesis. By mid-April (late spring), the starch concentration declined with the advent of vernal growth (Fig. 2, period III; Table 2).

Discussion

The differences in cold season carbohydrate metabolism between DS and NDS trees suggest drought-associated changes in cellular biochemical and physiological processes in adaptation to low temperature. During the leafless cold-season period, adequate storage of reserves is important for maintenance at low temperatures. In sugar maples, starch is the main source of carbon stored and utilized for (*i*) the development and maintenance of cold tolerance (Raese et al. 1978; Siminovitch 1981; Gregory et al. 1986); (*ii*) cellular maintenance and respiration (Kramer and Kozlowski 1979);

Table 3. Cold-season concentrations of total soluble sugar [mg·(g residue dry mass)$^{-1}$] in twigs of drought-stressed (DS) and non-drought-stressed (NDS) sugar-maple trees.

Collection dates (1998–1999)	Cold season total soluble sugar: Non-drought-stressed sugar maple				Collection dates (1999–2000)	Cold season total soluble sugar: Drought-stressed sugar maple				
	Y	O	Y-d	O-d		Y	O	Y-d	O-d	Pr > F
Transition period I										
Oct. 1	10.8±1.6c	10.2±0.7c	18.0±1.9bc	13.8±2.1bc	Oct. 7	19.1±1.0a	21.0±1.4a	27.1±2.8a	26.7±2.7a	0.0001
Oct 15	10.9±0.5c	12.3±0.7bc	13.9±0.9ab	12.4±0.5b	Oct. 26	16.4±1.4a	14.4±0.8ab	16.0±0.2a	13.3±1.5ab	0.0079
Nov. 12	25.9±1.3de	40.5±2.6a	29.2±0.6bcd	31.9±1.5bc	Nov. 8	22.9±0.6c	33.4±1.4b	27.6±2.4cde	28.1±1.4cde	0.0001
Dec. 16	39.8±1.8c	40.5±3.3c	40.6±3.3c	39.9±2.8c	Dec. 14	48.5±4.2ab	52.5±3.3a	34.3±2.0bc	55.0±3.3a	0.0013
Transition period II										
Jan. 19	43.0±1.0d	31.8±0.7e	40.7±2.3d	42.1±1.9d	Jan. 11	57.8±1.8b	47.9±2.3d	69.5±2.6a	52.1±3.0bc	0.0001
Feb. 3	33.3±1.5c	21.1±1.6c	—	—	Feb. 1	59.1±3.4a	43.8±1.6b	—	—	—
Feb. 17	73.8±4.5a	54.3±2.0bc	65.3±4.4ab	65.6±3.8ab	Feb. 17	51.9±2.7cd	41.4±3.1d	48.5±4.6cd	54.1±2.7bc	0.0001
Mar. 3	70.0±4.6b	96.8±3.0a	50.5±4.8cd	58.0±2.9c	Feb. 28	52.5±1.8cd	58.4±3.6c	44.2±3.9d	49.0±1.5cd	0.0001
Transition period III										
Mar. 25	28.2±2.0bc	47.1±2.2a	31.0±2.4b	25.7±1.6bc	Mar. 22	33.6±3.3b	22.4±3.0c	25.8±1.8bc	22.4±1.9c	0.0001
Apr. 6	20.2±1.0b	31.0±1.2a	23.9±1.8b	20.1±1.2b	Apr. 6	10.3±0.3d	15.8±2.7c	20.6±1.1b	12.2±0.8cd	0.0001
Apr. 21	12.7±0.7bc	17.0±2.2a	15.2±0.7ab	11.1±0.8c	Apr. 19	15.3±0.6ab	18.0±0.6a	17.8±0.8a	16.8±1.5a	0.0001
May 17	14.6±1.0bc	11.5±0.7c	22.0±2.0a	21.3±0.9a	May 11	11.8±0.7c	13.1±1.7bc	11.8±0.7c	15.7±0.6b	0.0001

Note: I, autumn transition; II, winter transition; III, spring transition. Y, young stand; O, older stand; -d, crown > 50% damaged. Data are mean ± SE (n = 12); numbers within a row that do not share a letter are significantly different.

Table 4. Autumn transition (period I) concentrations of soluble sugars [mg·(g residue dry mass)$^{-1}$] in twigs of drought-stressed (DS) and non-drought-stressed (NDS) sugar-maple trees.

Collection dates (1998–1999)	Autumn soluble sugars: Non-drought-stressed sugar maple				Collection dates (1999–2000)	Autumn soluble sugars: Drought-stressed sugar maple				
	Y	O	Y-d	O-d		Y	O	Y-d	O-d	Pr > F
Sucrose										
Oct. 1	6.6±0.8c	5.6±0.7c	10.4±2.0c	6.5±1.7c	Oct. 7	11.2±0.7bc	12.8±0.9ab	13.9±1.3ab	17.6±2.6a	0.0001
Oct. 15	4.9±0.2bc	6.3±0.3ab	6.6±0.4ab	6.4±0.3ab	Oct. 26	7.3±1.1ab	8.7±0.5a	3.3±1.3c	6.0±1.9b	0.0052
Nov. 12	12.5±0.4e	19.6±1.2ab	14.5±0.3de	15.7±0.8cde	Nov. 8	12.5±0.4e	21.4±1.0a	16.7±4.8bcd	18.2±1.1abc	0.0001
Dec. 16	13.9±0.7d	17.3±1.9cd	18.6±1.8bcd	16.4±1.6cd	Dec. 14	23.1±1.9b	29.9±2.a	21.4±1.0bc	28.4±2.0a	0.0001
Glucose										
Oct. 1	0.0±0.0e	0.0±0.0e	0.6±0.6de	0.0±0.0e	Oct. 7	1.2±0.3cd	2.1±0.1ab	2.6±0.4a	1.7±0.2bc	0.0001
Oct. 15	0.0±0.0d	0.3±0.1cd	0.0±0.0d	0.4±0.1bc	Oct. 26	0.7±0.1a	0.1±0.0cd	0.6±0.1ab	0.1±0.0cd	0.0001
Nov. 12	2.9±0.2bc	5.4±0.6a	2.5±0.3c	3.6±0.3b	Nov. 8	2.4±0.2c	1.5±0.1e	2.3±0.3cd	1.6±0.1de	0.0001
Dec. 16	10.0±0.5a	6.6±0.5bc	7.2±0.7bc	7.9±0.6bc	Dec. 14	8.0±0.7b	6.2±0.2c	7.4±0.4bc	7.5±0.3bc	0.0005
Fructose										
Oct. 1	0.0±0.0b	0.0±0.0b	0.3±0.3b	0.0±0.0b	Oct. 7	0.9±0.2a	1.2±0.1a	0.9±0.3a	0.9±0.1a	0.0001
Oct. 15	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c	Oct. 26	2.4±0.8b	0.1±0.0c	4.1±0.5a	0.1±0.0c	0.0001
Nov. 12	2.8±0.2c	5.2±0.5a	2.5±0.3c	3.8±0.3b	Nov. 8	1.3±0.2de	0.6±0.1e	1.4±0.2d	0.9±0.2de	0.0001
Dec. 16	9.0±0.6a	5.8±0.5c	6.9±0.6bc	7.0±0.5bc	Dec. 14	7.3±0.7abc	7.4±0.4abc	8.2±0.4ab	7.8±0.4ab	0.0036

Note: Y, young maple stand; O, older maple stand; -d, crown >50% damaged. Data are mean ± SE (n = 12); numbers within a row that do not share a letter are significantly different.

Fig. 3. Profiles of sucrose, glucose, and fructose in twigs from drought-stressed (DS) trees: (A) nondamaged (DS) older trees; (B) older damaged (DS-d) trees; (C) damaged (DS) young trees; and, (D) damaged (DS-d) young trees. Mean values and standard error bars are shown on points at each collection date (n = 12). Roman numerals refer to periods that correspond to cold season physiological activity (see text).

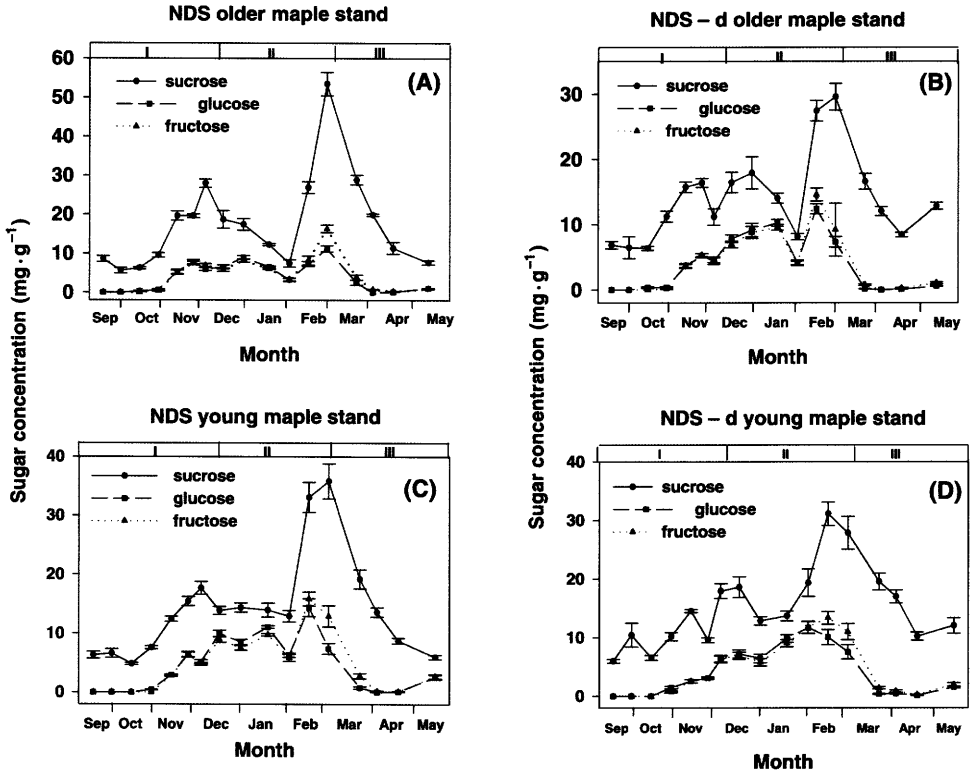


Fig. 4. Profiles of sucrose, glucose, and fructose in twigs from non-drought-stressed (NDS) trees: (A) nondamaged (NDS) older trees; (B) damaged (NDS-d) older trees, (C) nondamaged (NDS) young trees, and (D) damaged (NDS-d) young. Mean values and standard error bars are shown on points at each collection date (n = 12). Roman numerals refer to periods which correspond to cold season physiological activity (see text).

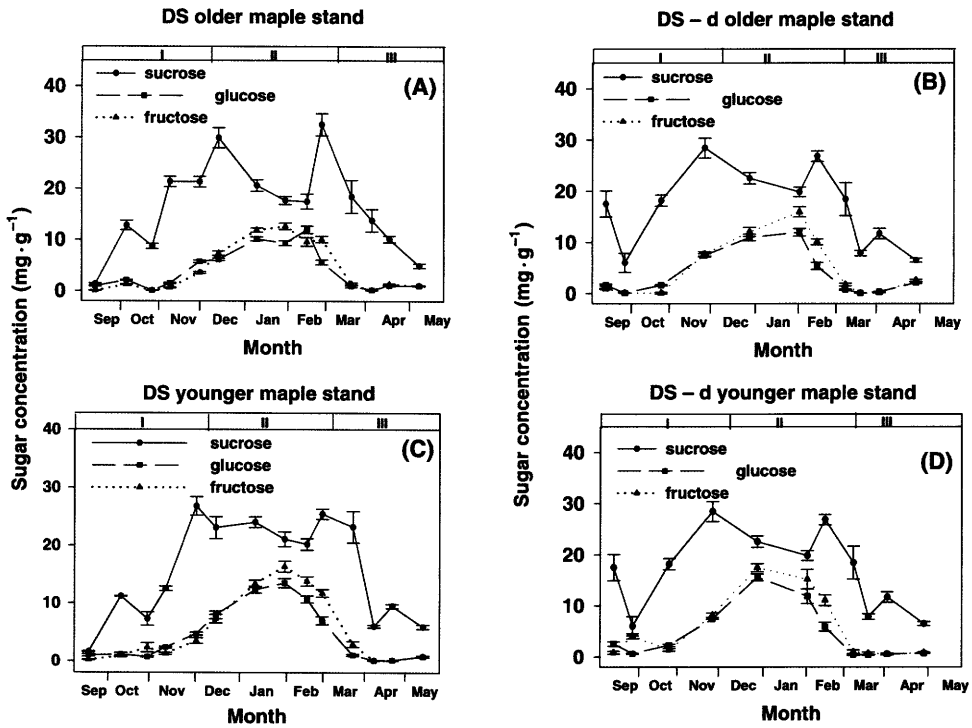


Table 5. Winter transition (period II) concentrations of soluble sugars [mg·(g residue dry mass)$^{-1}$] in twigs of drought-stressed (DS) and non-drought-stressed (NDS) sugar-maple tree.

Collection dates (1998–1999)	Winter soluble sugars Non-drought-stressed Sugar Maple				Collection dates (1999–2000)	Winter soluble sugars Drought-stressed Sugar Maple				
	Y	O	Y-d	O-d		Y	O	Y-d	O-d	Pr > F
Sucrose										
Jan. 19	14.0±1.2c	12.3±0.3c	13.7±0.8c	14.0±0.8c	Jan. 11	24.0±0.9ab	20.6±1.1b	27.2±1.3a	22.5±1.1b	0.0001
Feb. 3	12.9±1.0b	7.5±1.0c	—	—	Feb. 1	21.1±1.3a	17.7±0.8a	—	—	
Feb. 17	33.1±2.5a	26.9±1.5b	31.1±2.0ab	27.4±1.6b	Feb. 17	20.2±1.0c	17.5±1.5c	18.0±1.7c	19.8±0.9c	0.0001
Mar. 3	35.8±3.0b	53.4±3.0a	27.9±2.8cd	29.6±2.1cd	Feb. 28	25.4±0.9cd	32.5±2.2bc	22.9±2.6d	26.8±1.0cd	0.0001
Glucose										
Jan. 19	11.1±0.2	6.6±0.2	9.8±0.7	10.3±0.5	Jan. 11	12.3±0.6b	10.2±0.4c	15.6±0.7a	10.9±0.7bc	0.0001
Feb. 3	6.0±0.4c	3.2±0.4c	—	—	Feb. 1	13.5±0.8a	9.4±0.4b	—	—	
Feb. 17	14.4±1.5a	7.4±0.6c	10.1±1.3bc	12.4±1.6ab	Feb. 17	10.7±0.6bc	8.1±0.6c	11.9±1.5ab	11.9±0.7ab	0.0009
Mar. 3	7.4±0.9b	11.2±0.8a	7.6±1.2b	7.4±0.8b	Feb. 28	7.0±0.7b	5.6±0.5b	5.9±0.9b	5.4±0.7b	0.0004
Fructose										
Jan. 19	9.8±0.6c	6.0±0.1d	9.0±0.6cb	9.6±0.4cb	Jan. 11	13.4±0.6b	11.9±0.4b	17.5±0.8a	12.0±0.9b	0.0001
Feb. 3	5.9±0.6b	3.4±0.4b	—		Feb. 1	16.4±1.0a	12.6±0.6a	—	—	
Feb. 17	15.9±0.8a	8.2±1.2b	13.4±1.1a	14.6±1.0a	Feb. 17	13.8±0.8a	9.6±0.8b	15.2±2.0a	15.9±1.0a	0.0002
Mar. 3	12.9±1.8b	16.4±1.0a	11.0±1.4bc	9.2±0.c4	Feb. 28	11.8±0.6bc	10.1±0.6bc	11.1±1.1bc	10.1±0.6bc	0.0019

Note: Y, young maple stand; O, older maple stand; -d, crown >50% damaged. Data are mean ± SE (n = 12); numbers within a row that do not share a letter are significantly different.

Table 6. Spring transition (period III) concentrations of soluble sugars [mg·(g residue dry mass)$^{-1}$] in twigs of drought-stressed (DS) and non-drought-stressed (NDS) sugar-maple trees.

Collection dates (1998–1999)	Spring soluble sugars: Non-drought-stressed sugar maple				Collection dates (1999–2000)	Spring soluble sugars: Drought-stressed sugar maple				
	Y	O	Y-d	O-d		Y	O	Y-d	O-d	Pr > F
Sucrose										
Mar. 25	19.2±1.6bc	28.8±1.2a	19.6±1.4bc	16.6±1.2c	Mar. 22	23.2+2.7b	15.7+1.3c	15.9+1.4c	18.4+3.2bc	0.0001
Apr. 6	13.6±0.8b	19.9±0.3a	14.3±2.0b	12.1±0.7b	Apr. 6	6.0±0.2c	13.8±2.2b	13.2±0.9b	7.9±0.5c	0.0001
Apr. 21	8.8±0.5b	11.4±1.5a	10.2±0.7ab	7.8±0.8b	Apr. 19	9.5±0.4ab	10.1±0.6ab	12.1±0.6a	11.7±1.0a	0.0027
May 17	6.0+0.3bc	7.7+0.6b	12.1+1.3a	12.9+0.5a	May 11	5.9±0.4bc	3.3±0.4c	6.0±0.4c	6.5±0.4bc	0.0001
Glucose										
Mar. 25	1.2±0.4b	2.5±0.7a	0.5±0.1bc	0.2±0.0c	Mar. 22	1.1+0.2bc	1.0+0.3bc	0.4+0.1bc	0.7+0.1bc	0.0001
Apr. 6	0.1±0.0bc	0.0±0.0c	0.4±0.1a	0.0±0.0c	Apr. 6	0.1±0.0bc	0.2±0.0c	0.4±0.1	0.1±0.0	0.0001
Apr. 21	0.1±0.0d	0.1±0.0d	0.1±0.0d	0.1±0.0d	Apr. 19	0.2±0.0d	1.0±0.1a	0.6±0.2b	0.4±0.0c	0.0001
May 17	2.6+0.4a	1.1+0.1bc	1.5+0.1b	0.6+0.1c	May 11	0.8±0.2c	1.0±0.1bc	0.7±0.1a	2.1±0.2a	0.0001
Fructose										
Mar. 25	2.7±0.4bc	3.9±0.6a	1.6±0.3cd	0.8±0.1d	Mar. 22	3.0+0.5ab	1.4+0.3d	1.0+0.4d	1.7+0.3cd	0.0001
Apr. 6	0.2±0.1b	0.8±0.1a	0.9±0.2a	0.0±0.0c	Apr. 6	0.1±0.0b	0.1±0.0b	0.7±0.2a	0.1±0.0b	0.0001
Apr. 21	0.2±0.0c	0.3±0.1c	0.2±0.0c	0.3±0.0c	Apr. 19	0.1±0.0c	1.3±0.1a	0.5±0.2b	0.2±0.1c	0.0001
May 17	2.7+0.4a	1.0+0.1c	2.0+0.3b	1.0+0.1c	May 11	0.8±0.1c	1.0±0.0c	0.9±0.1c	2.6±0.2ab	0.0001

Note: Y, young maple stand; O, older maple stand; -d, crown >50% damaged. Data are mean ± SE (n = 12); numbers within a row that do not share a letter are significantly different.

and (*iii*) vernal growth, e.g., flower and shoot development (Wargo 1981; Gregory and Wargo 1986).

Low levels of stored carbohydrate reserves in autumn are implicated in tree dieback and mortality (Gregory et al. 1986; Rasmussen and Henry 1990; Renaud and Mauffette 1991). Gregory et al. (1986) showed that late season defoliation of sugar-maple trees results in low level of early autumn carbohydrate reserves and significantly reduces cold tolerance by about 6 °C when compared with nondefoliated trees. The low level of reserve carbohydrate in defoliated trees perhaps provides insufficient energy substrate to support metabolically active processes of cold acclimation.

Reduced reserve carbohydrate levels in drought-stressed trees have also been attributed to decrease in photosynthesis (Wample 1982; Abrams 1990; Epron and Dreyer 1993). In this study, the results obtained from leaves of DS and DS-d rehydrated branches were comparable to the P_{max} of leaves from rehydrated branches of photosynthetic active non-drought-stressed sugar-maple trees as determined by Ellsworth and Lui (1994). Thus, drought affected photosynthesis activity but not the photosynthetic mechanism.

Differences in cold-season profiles and concentrations of starch and sucrose, glucose, and fructose between DS and NDS trees could reflect stress-induced changes in cold season tree physiology (see Gregory et al. 1986 Wong et al. 2001; Wong et al. 2003 for comparison).

During the autumn transition (Period I) the level of autumn starch stored in DS trees (early October) was 20%–30% of that in NDS trees, whereas the level of soluble sugar was higher than that in NDS trees. Similar results were observed in earlier studies: a higher autumn soluble sugar to starch ratio was observed in trees with declining health (Wong et al. 2001) and lower autumn soluble sugar to starch ratios were observed in healthy sugar maples (Wong et al. 2001, Wong et al. 2003). The presence of a high ratio of soluble sugars to starch in DS trees in early autumn suggests some alteration in cold hardening capacity due to drought-stress effect. Freeze resistance in DS trees can be attributed to chemical changes (osmoregulation) rather than physiological changes of cell properties in the development of cold tolerance. The high level of soluble sugars in DS and DS-d in early autumn may play an important role in protecting cytoplasm macromolecules and membranes (Levitt 1980) and serve an osmoregulatory function in protecting against freezing by lowering the freezing point of the tissues (see Sakai 1966; Levitt 1980; Lineberger and Steponkus 1980). Accumulated low molecular weight organic solutes and higher molecular weight disaccharides and glucosides have been reported to serve as osmotica (Yancey et al. 1982; Clifford et al. 1998). Grierson et al. (1982) found osmotic adjustments with active solute accumulation in the cell sap of fruit trees in response to drought stress and this enhanced the development of cold hardiness.

The high level of sugars in the DS wood tissue during the winter transition (Period II) could not be accounted for exclusively by degradation of stored autumn starch. The ratio of the winter concentration of soluble sugars to autumn starch concentration was less than 1:1 in NDS trees and greater than 3:1 in DS trees. The high level of sucrose may be attributed to the influx of sucrose from the xylem sap and (or) apoplastic free spaces. It is not known whether influx is by a passive or active mechanism or a combination of both mechanisms. This influx of extracellular sucrose may compensate for insufficient amounts of starch stored and (or) inadequate development of cold hardening attributed to drought-stress effects. High levels of sucrose in drought stressed wood tissue may function to increase frost hardiness (see Hansen and Grausland 1973; Sakai 1960; Levitt 1980; Lineberger and Steponkus 1980).

Elevated levels of cellular glucose and fructose have been observed by others in stressed trees (Renaud and Mauffette 1991; McLaughlin et al. 1996). The higher concentrations of winter glucose and fructose in DS trees may function as cryoprotectant substances for cytoplasm and contribute to membrane stability (preserving membrane fluidity, membrane function and cell compartmentation), as well as increase osmotic potential and provide sources of carbon and energy substrate for increased cellular respiration (see Lyons 1973; Sakai and Larcher 1987; Guy 1990; Alberdi and Corcuera 1991).

The decline of sucrose in late winter (February) is presumably attributed to increased cellular respiration and metabolic activities to facilitate cellular changes with dehardening. As shown above (Table 5), although the level of sucrose was higher in DS trees, it declined significantly less than in NDS trees during dehardening in February. This difference suggests a lower degree of conversion of cellular components with hardening under drought stress. Examples of such cellular components include membranes, phospholipids, fats, water soluble polymers, and cytoplasmic material (Sakai and Larcher 1987; Guy 1990; Alberdi and Corcuera 1991). This is consistent with the significantly higher concentration of late winter soluble sugars in the wood tissue of NDS trees after dehardening, presumably associated with the conversion of cellular components back to sugar.

During the spring transition (Period III) the amount of spring starch at the end of the leafless season was equal to or less than the level of starch stored in the wood tissue at the time of leaf drop (early autumn). In DS trees, the level of starch did not significantly increase in spring despite the large amount of sucrose present during the winter period. It appears that a large portion of winter sugar of the DS trees was most likely reverted back to the xylem sap and apoplastic free space.

Differences in the carbohydrate status between DS and NDS sugar-maple trees during the cold season indicate changes in the mode of adaptation in freeze resistance and cold tolerance in trees following summer drought. Differences observed in trees stressed by drought were more significant than differences associated with age of trees or severity of crown damage.

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