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ORIGINAL ARTICLE

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Variation of non-structural carbohydrates in silver birch (*Betula pendula* Roth) wood

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Abstract Non-structural carbohydrates in silver birch (*Betula pendula* Roth) wood were analysed in a 7-year-old clone and in five mature stems. The analysis was conducted to obtain more detailed information on seasonal fluctuation of these components and of the tree-to-tree variation and within stem variation. The sugars were analysed by GLC-MS. The smallest total soluble sugar amounts (consisting of sucrose, fructose, glucose, raffinose and *myo*-inositol) in young trees were measured during mid-summer (ca. 0.3%) and the largest while in dormancy (ca. 1.6% on wood dry weight basis). Raffinose was detected in autumn as a minor component. The proportion of monosaccharides and the amount of *myo*-inositol were largest during growth. Compared to other studies silver birch showed more evident seasonal fluctuation in soluble sugars than evergreen tree species. The sugar amount in mature stems was approximately at the same level as in young trees that had the same felling time. Tree-to-tree variation in the non-structural carbohydrates in the mature wood was fairly large. However, the amount of total soluble sugars, sucrose and glucose showed significant variation within the stem. The amount of these sugars was largest in samples that were taken close to the cambium. Starch was also detected close to pith. According to the heartwood definition and starch measurement results in this paper, it could be stated that silver birch does not form heartwood.

Keywords *Betula pendula* · Soluble sugars · Starch · Secondary xylem

Introduction

The most obvious use of carbohydrate reserves in the secondary xylem of trees is in the maintenance of respiration and growth at times when they are not driven by photosynthesis (Hansen and Grauslund 1973; Glerum and Balatinecz 1979; Kramer and Kozlowski 1979; Höll 1997). However, the increase of soluble sugars in the secondary xylem during late fall has also been connected with frost hardening (Höll 1981; Bonicel et al. 1987; Sauter 1988), although the entire mechanism causing increases in soluble sugars during early winter (primarily sucrose and its galactosides) is not fully understood (Sauter et al. 1996). Lastly, the storage and mobilisation of non-structural carbohydrates may also have an immediate effect on biomass development in trees (Chapin et al. 1990; Abod et al. 1991).

Seasonal cycles in the accumulation of storage components tend to be more pronounced in deciduous than in evergreen species (Kramer and Kozlowski 1979). Due to the lack of 2nd and 3rd year needles, which can function as storage tissues in softwoods, the storage components in the secondary xylem are more important for deciduous than for evergreen tree species.

Reserve materials are mainly stored in living cells. In the wood of silver birch soluble sugars and starch are mostly located in axial and ray parenchyma cells. Seasonal fluctuation and partitioning of storage carbohydrates in the secondary xylem has been particularly well defined in many deciduous trees of the temperate zone. However, most studies in the fluctuation of low-molecular-weight carbohydrates have concentrated on branches (e.g. in *Betula populifera* Marsh., Gibbs 1940; and in *Betula pendula* Roth, Sauter and Ambrosius 1986) or on young 1- to 4-year-old stems (e.g. in *Populus* × *canadensis* Moench '*robusta*', Sauter and van Cleve 1994; and in *Tilia* and *Betula*, Abod and Webster 1991) whilst only a few studies have concentrated on mature stemwood (Lindberg and Selleby 1958; Höll 1981).

The purpose of this investigation was to study the seasonal variation and longitudinal and radial distribution of non-structural carbohydrates in silver birch wood in order to obtain basic information about sugar fluctuations that could be utilised in the processing of wood. For example, by selecting correct felling time it might be possible to avoid high sugar concentrations that could

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cause discoloration in wood during timber drying. The present study concentrates on the seasonal variation in storage carbohydrates (e.g. soluble sugars and starch) in the secondary xylem of young silver birch stems. The reason for using a 7-year-old clone instead of mature stems was to avoid taking either increment borings or branches repeatedly from the same stems. Instead, the young trees were sampled throughout one growing season, while the partitioning of storage carbohydrates in the secondary xylem was studied in mature silver birch stems. The role of these reserve components in wood ageing (and in possible heartwood formation) is discussed.

Materials and methods

Plant material

The material for this study was collected during 1996 and 1997 from silver birch (*Betula pendula* Roth) stems and young trees growing in Punkaharju, Laukansaari, Finland. Two stems were felled from a 35-year-old stand and three from a 70-year-old silver birch stand (61°47′N, 29°17′E). The young trees were felled from a 7-year-old silver birch clone (61°48′N, 29°20′E). The number of degree days (d.d.) in 1997 in Punkaharju was 1,326, which was slightly more than the average of the previous 30 years (1,241).

To study the seasonal variation in the amount of non-structural carbohydrates, a silver birch clone (V5952, micropropagated by Stora Enso Ltd in 1990) was used (Table 1). The studied clone was growing in fertile, formerly agricultural and land where the spacing of the trees was 1×1 m. Five young trees were sampled six times: (1) while dormant (7 March 1997), (2) before bud burst (6 May 1997), (3) 2 weeks after bud burst (21 May 1997), (4) during growth (4 July 1997), (5) at the beginning of leaf senescence (15 September 1997) and (6) at the beginning of dormancy (4 November 1997). On each occasion, five saplings were chosen randomly, felled and a 50-cm-long section of the stem was sawn at the height of 1 m, which was above the crown limit. The samples were immediately transported to a freezer in containers (+4°C) and stored at –20°C. Two 1-cm-thick sample disks were also sawn at a 10-cm distance from both ends of the section and combined to form a single sample. The disks were debarked and the cambium was removed carefully.

To study the horizontal variation in the amount of non-structural carbohydrates in the stem, two silver birch stems were felled on 28 June 1996 (trees 1 and 2) and three additional stems (trees 3, 4 and 5) on 4 July 1997 (Table 2). Samples from the stems were sawn at heights of 0 m, 1 m, 6 m and 12 m. In addition, a narrow strip was sawn through the pith immediately after the trees were felled. These 5-cm narrow strips were taken in an east to west direction in trees felled in the summer of 1997. The samples were immediately transported to a freezer in containers (+4°C) and stored at –20°C. The samples were recovered from three zones: (1) 1–3 cm from the pith, (2) 3–6 cm from the pith and (3) outermost sapwood; all samples were separated from both sides of the pith. The two samples taken from different sides of the pith were mixed and combined to form a single sample.

The wood samples were cut into pieces, lyophilised (–60°C, 4 days) and homogenised into a fine powder with a Polymix (Kinematica) mill (–30°C). For enzymatic hydrolysis and gas-chromatographic determination of starch, the homogenisation efficiency was tested. The samples were first homogenised with the Polymix (Kinematica) mill (–30°C) for 5 min and then with a mortar and pestle in liquid nitrogen. No significant differences were detected between starch amounts in samples that were ground only in a Polymix (Kinematica) mill or by both methods.

Table 1 The variation of stem height and growth in the 7-year-old silver birch clone. (*DIAM* diameter at 1 m height in the stem, *GRW* growth ring width at 1 m height in the stem, *SE* standard error, based on rings at 1 m height in the stem excluding the forming ring, *CV* coefficient of variation as percentage, based on rings at 1 m height in the stem excluding the forming ring)

	Average	SE	CV
Height (m)	5.39	0.28	5.15
DIAM (cm)	3.30	0.02	0.55
GRW (mm)	3.16	0.08	2.67
n	30	30	30

Table 2 Tree description of the mature stems. (*DBH* diameter at breast height, *GRW* average growth ring width at 1 m height in the stem, *SE* standard error, based on growth rings measured at 1 m height in the stem)

Tree	Height (m)	Age	DBH (cm)	GRW (mm)	SE (mm)	Crown height (m)
1	20.9	27	22.9	4.36	0.26	4.5
2	22.6	34	19.3	2.96	0.24	9.8
3	26.3	73	27.0	1.88	0.09	11.9
4	22.5	69	22.0	1.56	0.09	8.5
5	23.6	69	21.0	1.56	0.05	10.8

Extraction of soluble sugars

Wood powder (100 mg) was extracted with 2 ml of 80% ethanol first in an ultrasonication bath for 45 min and then for 18 h at +23°C (Mason and Slover 1971). Three parallel extractions were made per wood sample. Phenyl-β-D-glucopyranoside was added to the extraction solution as an internal standard (Marcy and Carroll 1982). After the extraction process, the samples were centrifuged in a Jouan B4 centrifuge (3,500 rpm, 6 min). One millilitre of the sample solution was evaporated dry under a nitrogen gas stream at +40°C. Recovery of the soluble sugars in the extraction process was tested in one sample by adding phenyl-β-D-glucopyranoside, D-glucose, fructose, *myo*-inositol, sucrose and raffinose as internal standards. The recovery of all other sugars tested was over 90%, except for raffinose, which had a recovery of 55%.

Extraction and enzymatic hydrolysis of starch

The wood powder was first heated at 100°C for 10 min to halt enzyme activity. Fifty milligrams of wood powder was extracted with 450 µl of 0.05 M citrate buffer, pH 4.6, and 50 µl of amyloglucosidase (ca. 7 units, EC 3.2.1.3, 102 857, Boehringer Mannheim) and incubated at 37°C for 24 h. Three parallel extractions were made for each wood sample. During incubation the samples were stirred occasionally (Saranpää and Höll 1989). After enzymatic hydrolysis of starch, the samples were centrifuged in an Eppendorf Centrifuge 5417 C (14,000 rpm, 10 min). The hydrolysis efficiency of starch was tested by staining the pellets with potassium iodide-iodine solution (2%, w/v, KI and 1%, w/v, I_2 in distilled water, Wargo 1975) and viewing under a light microscope (Olympus BH-2). Some starch grains remained non-hydrolysed, but longer incubation time did not affect the amount of residual starch. To deactivate amyloglucosidase, the clear supernatant solution was kept at 100°C for 10 min and centrifuged as mentioned above. Forty microlitres of sample solution was used for gas chromatographic determination of glucose. As an internal standard 0.5 ml of 1,000 ppm phenyl-β-D-glucoside solution in 80% ethanol was added to the sample (Marcy and Carroll 1982). The samples were evaporated dry under a stream of nitrogen (+40°C).

Derivatisation and gas chromatography of non-structural carbohydrates

Trimethylsilyl derivatives of non-structural carbohydrates were formed. Samples were evaporated dry, and 400 µl of *N*-trimethylsilylimidazole(T-7510, Sigma Chemical)/pyridine (21:100, v/v) was added. After half an hour of incubation at 80°C, the TMS-derivatised non-structural carbohydrates were subjected to GC-MS (Brittain et al. 1971). A Hewlett Packard gas-chromatograph 5890 series II with a mass spectrometer 5988A was used for the quantitative measurement of soluble sugars and starch. The TMS derivatives of carbohydrates were determined on a 25 m HP-5 (5% phenyl methyl siloxane) column (Hewlett Packard) with an internal diameter of 0.2 mm and a film thickness of 0.33 µm.

The column-temperature program started at 110°C. The temperature increased at a rate of 10°C/min reaching a final temperature of 300°C, which was held for 36 min. Helium with an inlet pressure of 100 kPa served as the carrier gas. A split-injection mode was used. The injection volume was 1 µl and the split flow was 15 ml/min (split ratio 1:15), whilst the septum purge was 3 ml/min. Total ion chromatograms (TIC) of TMS derivatives (carbohydrates) were used for analysis with the mass range being 50–600 ion mass units. TMS carbohydrates were identified by co-chromatography of authentic TMS derivatives by GC-MS (Hewlett Packard, HP 6890, with a mass selective detector, temperature program and gas flow adjustments as above).

Phenyl-β-D-glucopyranoside was used as an internal standard (Marcy and Carroll 1982), whilst fructose, D-glucose, *myo*-inositol, sucrose and raffinose were used as external standards in the quantitative analysis of TMS-derivatives of the non-structural carbohydrates. In addition an external standard solution, which contained silylated standard sugars, was injected after every 15 injected samples.

Protein determination

The wood samples of five mature trees taken at the height of 1 m (1–3 cm from the pith, 3–6 cm from the pith and outermost sapwood) were selected for protein content determination. Three parallel extractions were made for each wood sample. One and a half grams of lyophilised wood powder was extracted twice with 10 ml of 0.05 M TRIS-maleate buffer, pH 7.7 containing 5 mM Na_2EDTA (Titriplex III), 1 mM $CaCl_2$, 10 mM $Na_2B_4O_7$, 1 M NaCl, 1.5% Polyclar AT and protease inhibitor (Complete, Roche Diagnostics, No. 1 697498, 1 tablet/500 ml). The samples were occasionally shaken during extraction. After two extractions for 30 min at +4°C the samples were filtered through Miracloth (Calbiochem) and the supernatants were further filtered through Gelman Acrodisc filters (pore size 0.45 µm) for ion chromatography (Fagerstedt et al. 1998). Protein contents of the filtered extracts were determined with Bio-Rad microassay procedure (Bradford 1976).

Statistics

Statistical analyses were made using SPSS for Windows (Version 8.0.1). Gauss distribution of populations was checked using the Kolmogorov-Smirnov test with Lilliefors significance correction. Homogeneity of variances was studied using Levene's test.

The amount of non-structural carbohydrates in the seasonal fluctuation study followed Gauss distribution, but variance was not equal in the case of *myo*-inositol, total soluble sugars, sucrose or starch. Therefore, both non-parametric and parametric tests were used, and their results were compared. Kruskal-Wallis analysis and one-way ANOVA gave similar results for all dependent variables tested (also, when variances were not equal: *myo*-inositol, total soluble sugars, sucrose and starch). The data were finally analysed with a one-way ANOVA followed by the Tukey test.

The data in the study of the partitioning of non-structural carbohydrates within mature stems also followed Gauss distribution and variance as equal except in the cases of sucrose and total soluble sugars. The data were analysed by multivariate analysis of variance with the General Linear Model. This is composed of repeated measures of analysis including Huynh-Feldt's test and the *t*-test. Note that the *t*-test was only used when there were significant differences in the samples taken from different heights. In the analysis model two internal subject factors and their interaction were tested. Internal subject factors were the height of the samples (4 levels: 0 m, 1 m, 6 m and 12 m) and the distance of the sample from the pith (2 levels: 1–3 cm from the pith and 1–3 cm from the cambium). Only two levels of the distance factor could be tested, because the diameter at 6 m and 12 m heights was much less than at 0 m and 1 m heights and only two samples were taken at the heights of 6 m and 12 m.

The protein analysis data followed Gauss distribution and the variance was equal. The data were analysed by one-way ANOVA.

Results

Silver birch wood contained non-structural carbohydrates, which were identified as starch, sucrose, glucose, fructose, *myo*-inositol, raffinose, galactose, maltose and stachyose. Only trace amounts of galactose, maltose and stachyose (under 0.01 µg/mg of wood on dry weight basis) were detected. Total soluble sugar content was given as the sum of the five dominant sugars measured in the wood (sucrose, glucose, fructose, *myo*-inositol and raffinose). Verbascose, which has been detected in *B. verrucosa* by Lindberg and Selleby (1958), could not be detected by GC-MS with the HP-5 column. *Myo*-inositol was included in the quantitative analysis although it is not a sugar according to its chemical structure.

Seasonal variation

The major non-structural carbohydrates in the secondary xylem of the 7-year-old silver birch clone throughout the year were sucrose, starch, fructose and glucose (Fig. 2 A–C). The amount of total non-structural carbohydrates (soluble sugars + starch) was smallest in July (4.3± 0.2 µg in 1 mg of wood on a dry weight basis ±SE) and largest in November (35.4±0.8 µg of sugar in 1 mg of wood on a dry weight basis ±SE; Figs. 1, 2A, Table 3). The total amount of soluble sugars (sucrose, glucose, fructose, raffinose + *myo*-inositol) followed the trend of

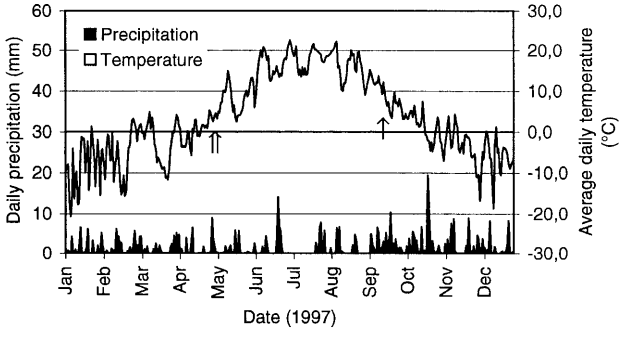

Fig. 1 Average daily air temperatures and daily precipitation during the year 1997 at Punkaharju. The Finnish Meteorological Institute provided data. The arrows indicate the time of bud break (⇑) and leaf yellowing (↑)

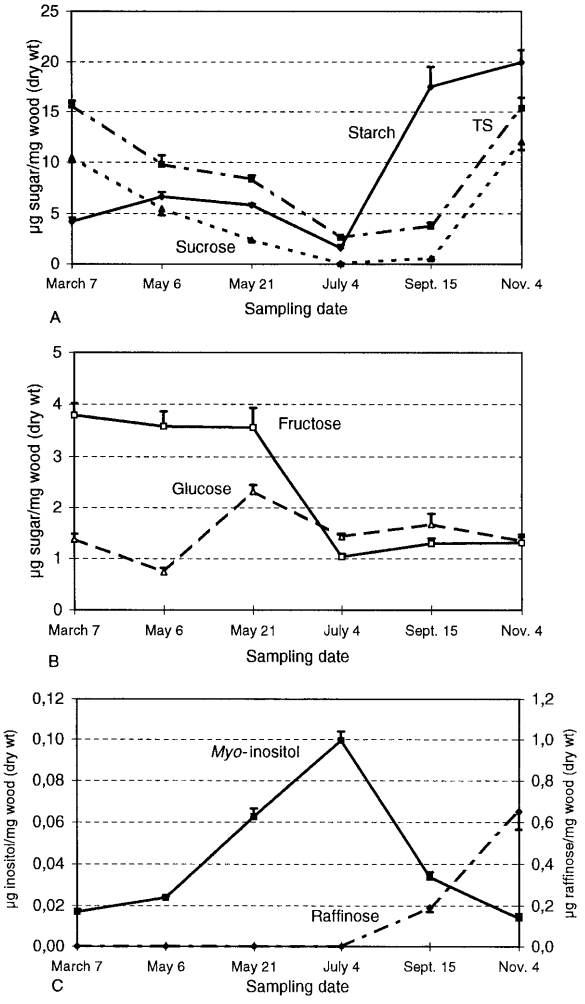

Fig. 2 Seasonal changes in **A** total soluble sugars (– · –), sucrose (···) and starch (—), **B** fructose (—) and D-glucose (– – –) and **C** *myo*-inositol (—) and raffinose (– · –). Each value connected with a line represents the average sugar amount of five independent trees expressed as micrograms of sugar per milligram of wood (on a dry weight basis). Starch is measured as glucose units. Bud break occurred at the beginning of May and yellowing of leaves in the middle of September. *Error bars* indicate tree-to-tree variation (standard error, n=5). For significance of differences, see Table 3

sucrose on all sampling occasions through the year. The amount of sucrose decreased gradually during the spring until July, stayed near the minimum level in September and increased dramatically in November (Figs. 1, 2A, Table 3).

The amount of starch increased slightly during the bud break in May and stayed at the same level for 2 weeks. The amount of starch was smallest (1.6±0.1 µg of glucose in 1 mg of wood on dry weight basis ±SE) in July, and during the autumn it increased gradually until November (Fig. 2A, Table 3).

The amount of monosaccharides was largest 2 weeks after the bud break in May (5.9±0.3 µg in 1 mg of wood on a dry weight basis ±SE) and smallest in mid-summer in July (2.5±0.1 µg in 1 mg of wood on a dry weight basis ±SE). However, the proportion of monosaccharides was about 57% of the total amount of non-structural carbohydrates (soluble sugars + starch) in July (Fig. 2B, Table 3). The amount of fructose in March and May was approximately 3.6±0.2 µg in 1 mg of wood on a dry weight basis ±SE. Before mid-summer the amount of fructose decreased and stayed relatively small in September and November. The amount of glucose decreased slightly before the bud break and increased significantly 2 weeks afterwards. In July the amount of glucose decreased once again (Fig. 2B, Table 3).

In Fig. 2C the seasonal variation in *myo*-inositol and raffinose is presented. In comparison with the amounts of soluble sugars and starch, the amount of *myo*-inositol had a different fluctuation profile. The amount of *myo*-inositol was about three times larger in July during growth than in March, early May, September and November. Raffinose was not detected in March, May or July. The amount of raffinose increased 3-fold from leaf yellowing in September to leaf fall in November. However, raffinose was a minor component of non-structural carbohydrates in November (approximately 2% of total non-structural carbohydrates = soluble sugars + starch; Figs. 1, 2C, Table 3).

Variation within the stem

The sugar composition of mature stemwood was similar to that which was measured on 4 July 1997 in the stemwood from the young trees in the 7-year-old clone. The predominant non-structural carbohydrates were starch, sucrose, glucose and fructose (Fig. 3A–F). The amounts of sucrose, glucose and total soluble sugars (sucrose, glucose, fructose + *myo*-inositol) showed similar distribution patterns and were larger in samples taken from closer to the cambium than in samples taken close to the pith (Fig. 3A–C, Table 4). The average amounts of su-

Table 3 Statistics of seasonal variation data (The same letters on the same row are not significantly different at P<0.001 by Tukey's test)

Sugar						
Starch	a	ab	ab	a	bc	c
Sucrose	a	b	bc	c	c	a
TS	a	b	b	c	c	a
Fructose	a	a	a	b	b	b
Glucose	ab	a	c	ab	bc	ab
Raffinose	a	a	a	a	a	b
Myo-inositol	ab	ab	c	d	b	a
Sampling date	March 7	May 6	May 21	July 4	September 15	November 4

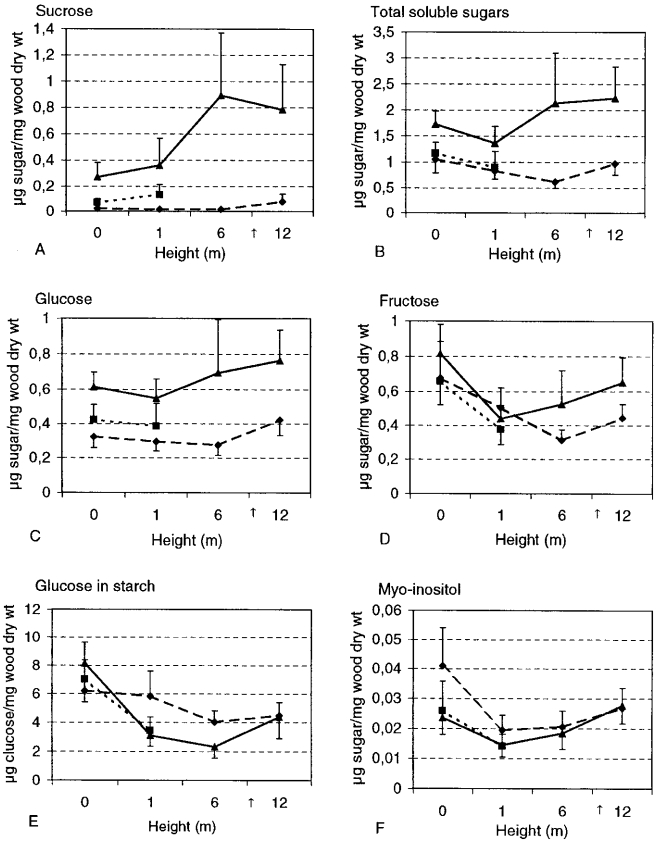

Fig. 3 Partitioning of **A** sucrose, **B** total soluble sugars (containing sucrose, glucose, fructose and *myo*-inositol), **C** glucose, **D** fructose, **E** starch in glucose units and **F** *myo*-inositol within the stem. Each value connected with a line represents the average sugar amount of five independent trees expressed as micrograms of sugar per milligram of wood (dry weight). Each *line* represents distance from the pith: 1–3 cm from the pith (- -), middle part of the wood 6–12 cm from the pith (···) and outermost part of the wood 1–3 cm from the cambium (—). The *arrow* (↑) indicates average crown height of five stems. *Error bars* indicate tree-to-tree variation (standard error, n=5). For statistics, see Results and Table 4

crose at a height of 1 m were 0.02 (±0.001) close to the pith, 0.13 (±0.03) in the middle part of the wood and 0.36 (±0.09) µg of sugar in 1 mg of wood (dry weight) ±SE close to the cambium (Fig. 3A). The amounts of soluble sugars, except fructose, seemed to be larger in the upper parts of the stem than near the stump (Fig. 3A–D, Table 4). However, the amounts of starch and *myo*-inositol seemed to be rather large at stump height (Fig. 3E, F, Table 4).

The amount of starch varied between 18.0 and 0.9 µg in 1 mg of wood dry weight with tree-to-tree variation being large (Fig. 3E, Table 4). In July the average amount of fructose in mature silver birch stems was approximately 0.54±0.04 µg in 1 mg of wood on a dry weight basis (Fig. 3D, Table 4). *Myo*-inositol was the only sugar measured that showed significant variation at different heights of the stem and hence pair-wise comparisons were made (Fig. 3F, Table 4). The amount of *myo*-inositol was larger at the heights of 0 m and 12 m than at the heights of 1 m and 6 m (at P<0.009, when 1 m and 12 m were compared, at P<0.021, when 6 m and 12 m were compared, at P<0.031, when 1 m and 0 m were compared and at P<0.047, when 6 m and 0 m were compared by t-test; for full results, see Fig. 3F, Table 4).

The average protein contents in the five mature trees at 1 m were 0.074 (±0.008), 0.068 (±0.006) and 0.076 (±0.006) µg/mg wood dry weight (±SE, n=5) close to the pith, in the middle and in the outermost sapwood, respectively. The differences in the amounts of proteins in the samples taken at different distances from the pith at a height of 1 m were non-significant.

Discussion

Seasonal variation

Seasonal changes in the amount of total soluble sugars and sucrose in silver birch wood are in agreement with other studies on deciduous trees. In the wood of deciduous trees, stored reserves decrease sharply during the bud break and with the onset of shoot expansion (Kozlowski and Keller 1966). In coniferous trees (e.g. *Pinus sylvestris* L., Fischer and Höll 1992) the changes in soluble sugars were less pronounced than in this study on silver birch. The amount of total soluble sugars in poplar (*Populus* × *canadensis* Moench '*robusta*'), 3-year-old branch wood was at its minimum (2–4 µg of sugar in 1 mg of wood on a dry weight basis) in late

Table 4 Manova results (Huynh-Feldt's test) for sucrose, total soluble sugars (*TS*, glucose, fructose, sucrose and *myo*-inositol), glucose, fructose, starch and *myo*-inositol in mature silver birch trees felled on 4 July. The F values are presented for within subject factors and their interaction with levels of significance indicated

Source of variation	Sucrose			TS			Glucose		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Height	2.29	1.47	0.282	1.60	0.75	0.481	2.11	1.28	0.329
Distance	1.00	5.69	0.076	1.00	7.16	0.055	1.00	7.14	0.056
Height×Distance	2.38	1.24	0.340	2.29	1.12	0.376	1.79	0.48	0.618
	Fructose			Starch			*Myo*-inositol		
Height	1.51	2.74	0.146	1.63	2.27	0.180	1.39	9.62	0.019
Distance	1.00	3.74	0.125	1.00	1.71	0.262	1.00	2.37	0.199
Height×Distance	3.00	2.10	0.154	1.39	4.04	0.089	2.22	2.67	0.121

April/early May during the bud break (Sauter and van Cleve 1994). In the 7-year-old silver birch clone studied, the minimum amount of soluble sugars was detected much later (4 July) than in trees of the temperate zone examined in previous studies (e.g. Sauter and van Cleve 1994). Furthermore, the dramatic increase during autumn in the amount of sucrose was detected in this study earlier than in *B. verrucosa* Ehrh. mature stems (Höll 1981). The reason for these differences is probably the later onset of growth and shorter growth period in the more northern latitudes than in Central Europe. The thermal growing season displays a well-known gradual decrease from south to north (Koski and Sievänen 1985). The annual temperature rhythm is the main regulating factor of the environment (Koski and Sievänen 1985). Thus, seasonal changes in soluble sugars in the silver birch clone studied show a similar overall pattern to other deciduous trees in the temperate zone although the timing is somewhat different depending on the length of the growing season.

The gradual decrease in the amount of total soluble sugars in silver birch wood (on 6 May and 21 May) correlated with the onset of shoot growth (Fig. 2A, Table 3). There was more variation between individual trees in the amount of total soluble sugars (sucrose, glucose, fructose + *myo*-inositol) during the bud break (on 6 May, coefficient of variance, CV=19.7%) than in the samples that were taken 2 weeks later (on 21 May, CV=9.4%). The timing of bud break was probably not synchronised in the 7-year-old silver birch clone although the clone was growing on the same site. Therefore, tree-to-tree variation during this period may exist. Consequently, there can be more variation in the amounts of sugars in the samples that were taken during the bud break.

The slight and non-significant change in the amount of starch after the bud break in silver birch wood is in disagreement with some reports on woody plants. This "springtime starch increase" was not as marked as in other studies (e.g. Sauter and Ambrosius 1986; Fischer and Höll 1992). A prominent resynthesis of starch and a great decrease in sugars in the symplast of silver birch branches has been found to be parallel to the growth and blossoming of catkins (Sauter and Ambrosius 1986). These results were based on a more pronounced change in the amount of measured starch in silver birch branches than in silver birch stemwood during the spring. However, in this study the silver birch clone was only 7 years old and was not yet mature enough to form catkins. In addition, it has been found that in Scots pine sapwood a marked increase in starch occurred at the beginning of the growing season (Fischer and Höll 1992). Unlike deciduous trees, coniferous trees have a functioning photosynthetic system when annual shoot growth begins. Therefore, the "springtime starch increase" can be more pronounced than in deciduous tree species.

The amount of sucrose and raffinose in silver birch wood increased significantly in November, when defoliation had finished. In addition, the largest amount of starch was detected at this time (Fig. 2A, C, Table 3). However, the amount and proportion of raffinose was rather small (5.1% and 4.2% of soluble sugars in September and in early November, respectively). An increase in the amount of soluble sugars, particularly sucrose and its galactosides, has been reported during natural and artificial frost hardening (Nelson and Dickson 1981; Fischer and Höll 1992; Sauter and van Cleve 1994). In late autumn the total amount of soluble sugars in the 3-year-old branch-wood of poplar began to increase, reaching 17–32 µg of sugar in 1 mg of wood on a dry weight basis (Sauter and van Cleve 1994). The increase in sugars has been related to the disappearance of starch (Sauter and van Cleve 1994). The maximum amount of starch has been detected at the time of leaf fall, e.g. in poplar (Sauter and van Cleve 1994). The temperature in Punkaharju, Finland had remained below –2.0°C for the 2 weeks before sampling on 4 November; consequently the silver birches were completely defoliated and dormant (Fig. 1). In Punkaharju, Finland the average duration of the growing season was 162 days (heat sum, >+5°C, d.d. 1,250), which was much less than in Central Europe (202–194 days from latitudes 53° to 47°, heat sum, >+5°C, d.d. varied between 1,650 and 2,030; Koski and Sievänen 1985).

The maximum amount of *myo*-inositol was detected during growth in July (Fig. 2C, Table 3). This non-structural carbohydrate plays a central role in growth and development (Loewus and Loewus 1983) and is involved, for example, in the biosynthesis of cell wall polysaccharides (Roberts and Loewus 1966), in phospholipid metabolism (Dumville and Fry 2000) and in biosynthesis of raffinose series of oligosaccharides (Horbowicz and Obendorf 1994; Loewus and Murthy 2000). The maximal amount of *myo*-inositol in the secondary xylem of young silver birch trees coincided with the rapid growth phase, when the differentiating xylem needs this component. In cyclitol-storing trees like *Acer pseudoplatanus* (quebrachitol), *Quercus robur* (quercitol) and *Fraxinus excelsior* (mannitol) the amount of cyclitol increased in autumn (Popp et al. 1997). In cyclitol storing trees the amount of polyols was considerably larger than the amount of *myo*-inositol in silver birch, where sucrose and its galactosides had taken over the function as osmotica during dormancy.

Variation within the stem

Compared to the amount of non-structural carbohydrates in the young trees on 4 July 1997, the amounts were at the same level in the mature stems, except in the case of *myo*-inositol. In addition, tree-to-tree variation was larger in mature stems than in the 7-year-old clone.

The amounts of sucrose, glucose and total soluble sugars (sucrose, glucose, fructose + *myo*-inositol) in mature stems were largest in samples close to the cambium (Fig. 3A–C, Table 4). Sucrose is the principal form in which fixed carbon and energy are translocated in plants. Therefore, it seems evident that the sucrose gradient in-

creased towards the cambium and phloem in the mature silver birch stems. The mature trees were felled at the moment of rapid growth in mid-summer. Thus, the ray parenchyma close to the cambium was in an active metabolic state. The amounts of sucrose and glucose seemed to be more abundant in the upper parts of the crown, e.g. those parts of the stem that are closer to photosynthesising leaves, where the assimilation of CO_2 takes place.

Heartwood is defined as "the inner layers of wood which, in the growing tree, have ceased to contain living cells and in which the reserve materials (e.g. starch) have been removed or converted into heartwood substances" (Anonymous 1957). In our study starch was detected in all samples of mature silver birch trees (Fig. 3E). According to the seasonal variation study the amount of starch was at its minimum in the mid-summer (Fig. 2A). In spite of its small amount at the time of rapid growth, starch was also detected in samples which were taken close to the pith in the mature wood of silver birch. Furthermore, the amount of starch seemed to be larger close to the pith than close to the cambium at the heights of 1 m and 6 m (Fig. 3E, Table 4).

The differences between the total protein contents in the samples taken at three separate positions form the cambium were very small. Furthermore, we observed 4,6-diamidino-2-phenylindole-stained ray parenchyma cells of a 79-year-old silver birch stem under a fluorescence microscope (Olympus BX-60, excitation cube U-MWU). The observed ray parenchyma cells close to pith contained DNA in compact sickle-like structures. According to our study and based on the definition of heartwood (Anonymous 1957), it can be claimed that silver birch does not form heartwood. Thus, the ray parenchyma cells close to the pith were living, capable of starch biosynthesis and contained approximately the same amount of total proteins as cells close to cambium.

Significant differences in sugar amounts between samples taken at different heights were detected only in the case of *myo*-inositol (Table 4). *Myo*-inositol was present in all the samples of silver birch wood studied (Fig. 3F, Table 4). This cyclitol has been detected in *B. verrucosa* and *B. pubescens* stem-wood (Lindberg and Selleby 1958) and also in the heartwood of some other tree species (e.g. *Sequoia sempervirens*, Anderson et al. 1968; *Planchonella vitiensis*, Cambie et al. 1997). There may be several reasons why the highest amount of *myo*-inositol was detected in samples taken at stump height and in samples close to pith. *Myo*-inositol could in the inner and lower parts of the stem probably serve as a metabolic reserve pool for the more active parts of the stem (wood cells close to cambium).

The absolute values of *myo*-inositol were different when the young and mature trees were compared (Figs. 2C, 3F). This may be related to tree-to-tree variation, which was larger between mature trees than within the clone, or to maturation processes. *Myo*-inositol and starch seem to have almost similar within tree variation profiles, which supports the idea that these non-structural carbohydrates could serve as metabolic reserve components in the mature stems. The smallest amount of *myo*-inositol was detected in the middle of the stem (Fig. 2F). Popp et al. (1997) detected a positive correlation between the amount of quercitol and stem height in *Q. robur*, although they also detected seasonal variation in the quercitol gradients. The biological functions of cyclitols differ from each other. *Myo*-inositol in silver birch could probably serve as a reserve for metabolic intermediates of raffinose family oligosaccharides, which are needed during cold acclimation in autumn.

Conclusions

Large seasonal variation in the amounts of soluble sugar and starch was detected in the secondary xylem of young silver birch trees. Considerable variation in the amounts of non-structural carbohydrates within the tree and between the trees was also detected in 30-year-old stems of the same species. However, no pattern in the radial distribution of non-structural carbohydrates that could be explained by heartwood formation was observed. The enzyme activities of sugar metabolism in ray parenchyma of mature and young silver birch wood and the biochemistry behind the seasonal changes especially in the amounts of sucrose and starch are subjects for further studies.

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