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The Role of Sucrose in Regulation of Trunk Tissue Development in *Betula pendula* Roth

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Abstract

Comparative ultrastructural analysis of the conducting and non-conducting phloem cells in the common straight-grained silver birch (*Betula pendula* var. *pendula*) and the Karelian birch (*B. pendula* var. *carelica*) with abnormal patterned wood was carried out, leading to the conclusion that there is an elevated sucrose content in the conducting phloem of the Karelian birch. A connection between sucrose levels and formation of abnormalities in the development of conducting tissues in the Karelian birch trunk was surmised. Experiments in which exogenous sucrose was applied to the silver birch trunk tissues have demonstrated the effects of different sucrose concentrations (0 g L^{-1}, 10 g L^{-1}, 25 g L^{-1}, 50 g L^{-1}, 100 g L^{-1}) on the formation of xylem and phloem structural elements, and they yielded the types of tissue development that correspond to the abnormal tissue development in the Karelian birch trunk.

Key words: *Betula pendula* Roth; Influence of sucrose; Trunk tissue development.

Introduction

The silver birch (*Betula pendula* Roth) is an intriguing study object through which the mechanisms of trunk tissue formation can be investigated, because the species comprises forms differing notably in wood texture, the common, straight-grained, silver birch (*B. pendula* var. *pendula*) and the Karelian birch (*B. pendula* var. *carelica*), which has patterned wood. The peculiarity of Karelian birch wood lies in its wavy annual ring contours and dark-colored V-shaped inclusions forming radial rows (Figure 1). The wavy contours are due to local inhibition of wood growth and intensified bark growth. The V-shaped inclusions are related to the formation of parenchymal tissue layers in the zones of wood growth inhibition. An important distinguishing feature of the Karelian birch trunk is its bark, which is normally 4–6 or more times as thick as that of the common birch (Figure 2). The bark of Karelian birch is mainly composed of parenchyma cells and more or less even inclusions of sclereid.

Wood and bark formation is the result of cambial activity, the heterotrophic nature of which makes it totally dependent on the influx of photoassimilates. In most woody plants of the boreal zone, birch in-

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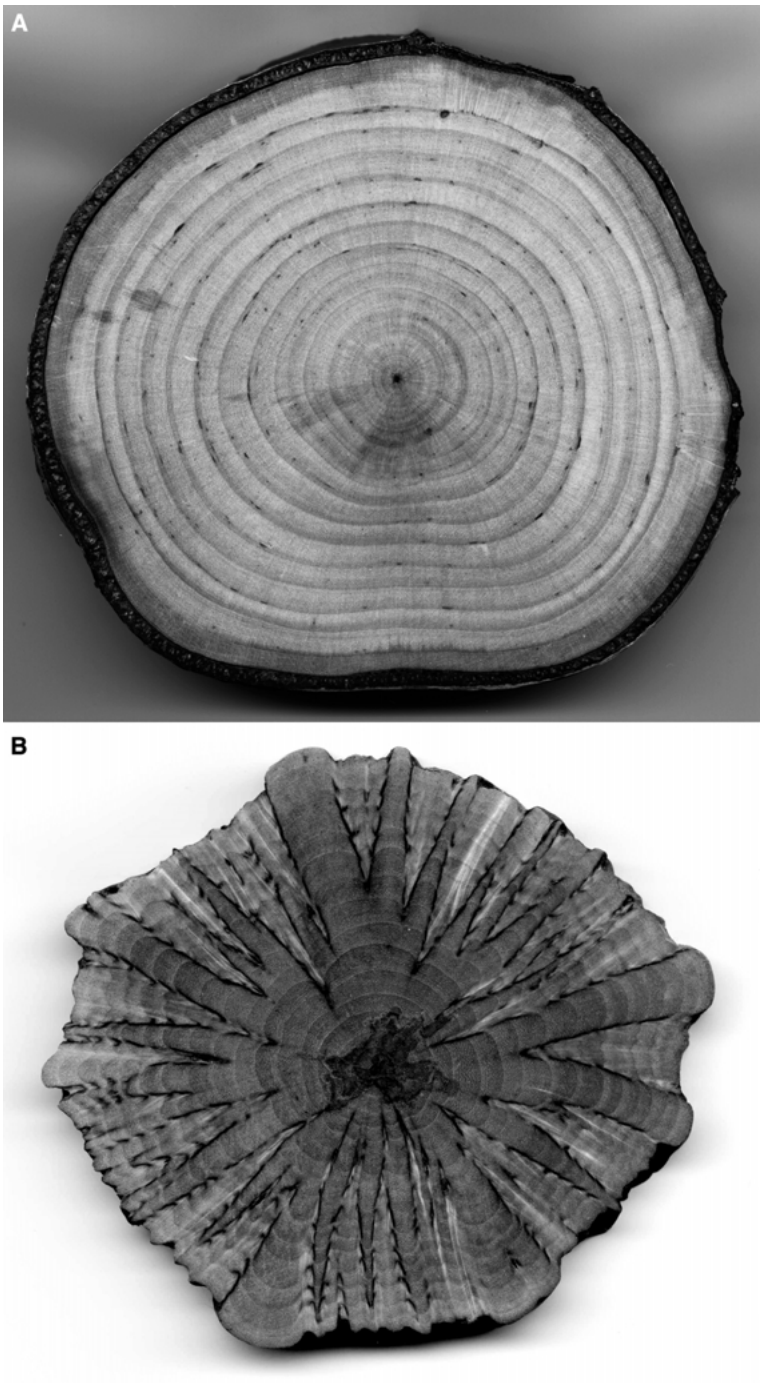

Figure 1. Cross-sections of the trunks of *Betula pendula* var. *pendula,* silver birch (**A**), and *Betula pendula* var. *carelica,* Karelian birch (**B**).

cluded, photoassimilates move from the leaves to the cambial zone, mostly as sucrose (Gamalei 1990, 2004). There have been a significant number of publications in the past decade indicating that transport sugars—first of all sucrose and glucose—influence the regulation of the genes responsible for photosynthesis, sink metabolism, and plant protective responses (Graham 1996; Koch 1996; Smeekens 1998; Roitsch 1999; Sheen and others 1999; Wobus and Weber 1999; Yu 1999; Gibson 2000, 2004; Pego and others 2000; Rolland and others 2002; Sinha and others 2002; Roitsch and others 2003; Rook and Bevan 2003). A high potential for change in the plant development program in response to sugar supply has been demonstrated for herbaceous species. In the process, the role of glucose in inducing and maintaining cell division was identified (Borisjuk and others 1998; Meijer and

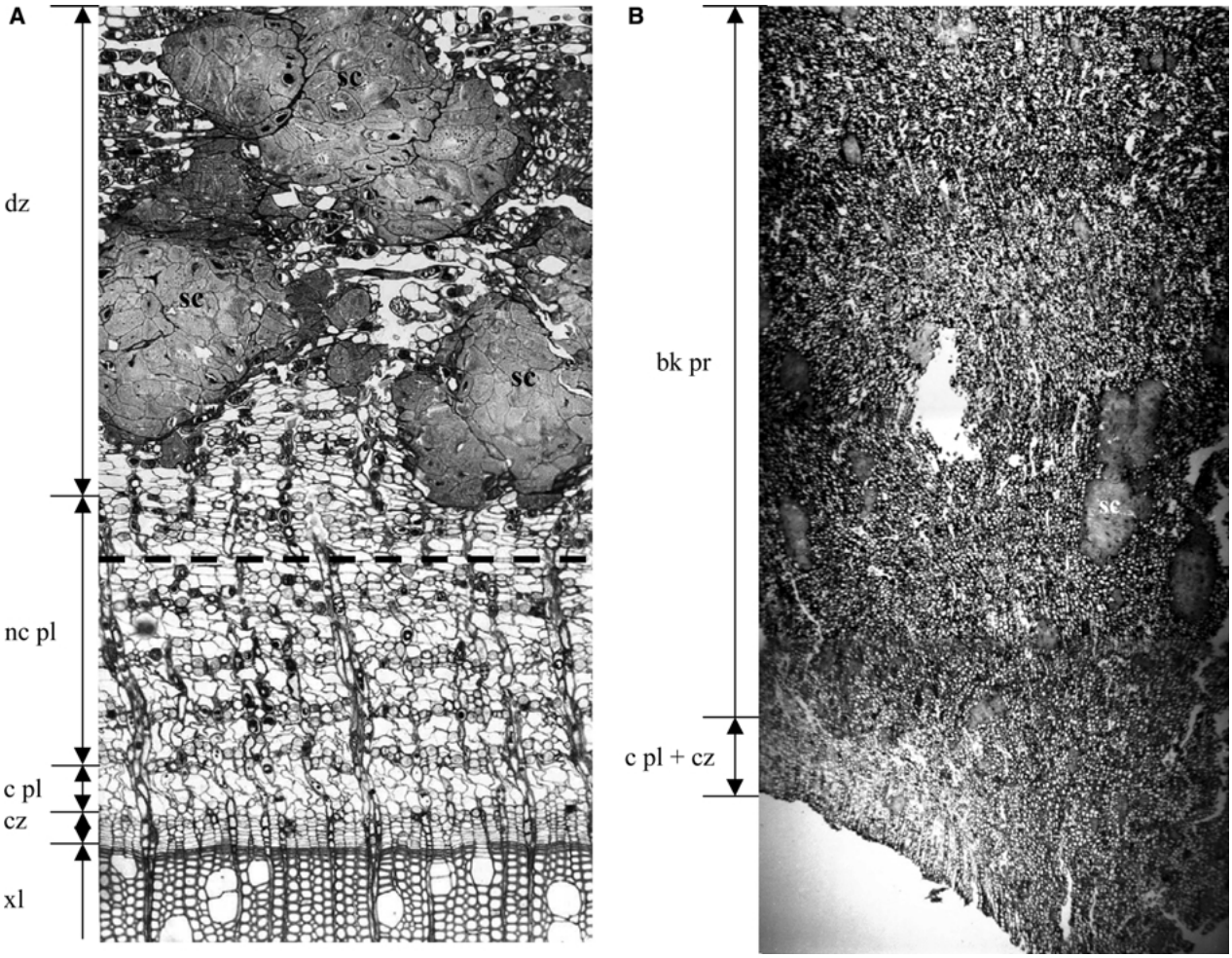

Figure 2. Cross-sections of the bark of silver birch (**A**) and Karelian birch (**B**). (**A**) The cross-section doesn't contain only the narrow outside zone of the bark, the periderm; dotted line indicates the zone of the bark cut in an experiment with injection of sucrose solutions. Magnification ×100. (**B**) The area of small bark protrusion into the wood; the wood had separated at the moment the sample was taken; the cross-section contains about half of the bark thickness. Magnification ×32. bk pr, bark parenchyma; c pl, conducting phloem; cz, cambial zone; dz, dilatation zone; nc pl, non-conducting phloem; sc, sclereids, xl, xylem.

Murray 2000; Riou-Khamlichi and others 2000; Halford and Dickinson 2001; Newcomb and others 2003), and the effect of rising sucrose concentrations on transition from growth through cell division to growth through cell enlargement and storage tissue formation was described (Perl and others 1991; Simko 1994; Weber and others 1997a, 1997b, 1998; Xu and others 1998; Wobus and Weber 1999; Borisjuk and others 2002, 2003; Viola and others 2001).

Our studies have shown that Karelian birch bark tissues contain significantly more total sucrose, glucose, and fructose (sucrose breaks up into glucose and fructose outside transport channels), than bark tissues of the common silver birch (Novitskaya 2000; Korovin and others 2003). We have assumed that deviations from normal growth and development of the Karelian birch trunk tissues, seen as local disturbances in cambial activity and formation of layers of storage parenchyma cells, may be due to excessive sucrose influx to the trunk. To check that assumption, we carried out comparative electron microscopy of the conducting phloem of the common silver birch and Karelian birch, and we studied the effect of different exogenous sucrose concentrations on cambial zone morphogenesis in the common silver birch.

Materials and Methods

Electron Microscopy

The objects of our research were 25-year-old silver birch trees (*Betula pendula* Roth var. *pendula*) with

normal straight grained wood and Karelian birch trees (*B. pendula* var. *carelica* [Merckl.] Hämet Ahti) with arbnormal patterned trunk wood (three of each type). All trees were grown under identical conditions in the agrobiological station of the Karelian Research Centre of RAS, 2 km from Petrozavodsk (61°45′N, 34°20′E).

Samples were taken from deep excisions made in the trunk 1.3 m above the ground during intensive cambial activity (early July). Samples of Karelian birch were excised from globular swellings of the trunk, where the wood pattern was the richest. Three pieces from different sides of the trunk were excised from each tree. Cubes with a side of 1 mm, including the ''conducting phloem–cambial zone'' complex, were cut out of the excisions for electron microscopy.

Samples were fixed following a standard technique in 30 g L^{-1} glutaraldehyde −25 g L^{-1} sucrose −0.1 M phosphate buffer (pH 7.4) at room temperature for 6 h. After samples were rinsed in buffer, post-fixation was carried out in 20 g L^{-1} OsO_4 −0.1 M phosphate buffer (pH 8.0) at 6°C for 13 h. After the material was washed in distilled water, it was dehydrated in an ethanol series and embedded in epoxy resin. Sections were cut on an LKB-Ultratome IV using glass knives, contrasted by lead citrate and uranyl acetate. Photographs were made on the electron microscope ПЭМ-100 (Ukraine).

Biochemical Research

The biochemical study was carried out over three seasons (1996, 2001, 2002) on 25–30-year-old silver and Karelian birch trees, growing at the agrobiological station of the Karelian Research Centre of RAS. Bark samples were collected from seven silver birch and seven Karelian birch trees during the period of high cambial activity (second half of June and July). The average level of sampling was 1.3 m. For extracting the pieces of bark from the birch trunks, a grafting knife was used. Dead outer bark was removed before cutting the samples. Bark was fixed immediately upon sampling. Bark for carbohydrate and tannin determination was fixed in a flowing steam—the technique proved to be convenient in the field and showed high reproducibility of the results in quantification of sugars and other carbohydrate fractions (Sofronova and others 1978), for lipids—in isopropanol containing antioxidant (0.001% ionol solution).

Inner layers of bark were cut from the samples after fixation. Finally, the samples cut were about 0.5 mm thick and contained the cambial zone, the conducting phloem, and part of the non-conducting phloem. Air-dried samples were homogenized to a fine powder.

Determinations of Sucrose and Starch. A 0.1-g sample was placed in a volumetric flask with 20–30 ml of distilled water and heated in a water bath for 1 h at 45°C. After cooling, proteins were precipitated by adding 2 ml of 5% $ZnSO_4$ and 0.5 ml of 5% NaOH, and the sample was passed through a glass filter. Mono- and disaccharide content was determined in the filtrate; starch, in the sediment (Sofronova and others 1978). Quantitative analysis was done using a CFC 3 (concentrating photoelectric colorimeter, Russia 1990) with a red color filter and a wavelength of 691 nm.

Sucrose concentration was determined by the difference between mono- and disaccharide content. Monosaccharide sum total: 0.5 ml of the filtrate, 1.5 ml of H_2O, 2 ml of potassium ferricyanide were placed in a 10-ml test tube, heated for 15 min in a boiling water bath, and cooled; 2 ml of iron sulfate was added, the contents were diluted to the mark, and colorimetric analysis was performed. Disaccharide content: 0.5 ml of the filtrate, 1 ml of H_2O, 1 ml of 5% HCl were placed in a 20-ml test tube, heated for 5 min in a boiling water bath at 70°C; the test tube contents were neutralized with 5% NaOH until the methyl red test (1 drop of the indicator per test tube) turned from pink to yellow; the rest of the procedure was the same as for monosaccharide determination.

To measure the starch content, the sediment was washed first with 1% HCl, then with H_2O, after which it was transferred to a 50-ml flask using 0.25% salicylic acid (20 ml). The flask was heated in a boiling water bath for 45 min, and the contents were diluted to the mark and reheated. The hot solution was screened. Then 8 ml of the cooled filtrate was supplemented with 1.5 ml of 1 N HCl, 0.5 ml of H_2O, and 3 drops of J solution in KJ. The stained solution was subjected to colorimetric analysis.

Lipid Determination. Total lipids were extracted by a mixture of chloroform and methanol with a volume ratio of 2:1 (Folch and others 1957), whereupon the solvent mixture was removed on a rotary evaporator at 40°C, and the residue was dried to a constant weight in an exsiccator over P_2O_5.

Tannin Determination

A 2-g sample was covered by 50 ml of boiling H_2O and heated in a water bath for 30 min. The liquid was left to settle and was then filtered into

a 250-ml flask. Extraction was repeated until the sample did not react to tannins (test with iron ammonium alum solution). The liquid in the flask was cooled and diluted to the mark, after which 25 ml of the liquid was transferred to a beaker flask, supplemented with 750 ml of H_2O, 25 ml of indigo-disulfonic acid, and titrated with 0.1 N $KMnO_4$ solution. One ml of 0.1 N $KMnO_4$ solution corresponds to 4.157–3 g of tannin substances recalculated to tannin (USSR State Pharmacopoeia 1986).

Experiments with Injection of Sucrose Solutions

Sucrose injection experiments were made on 25-year-old silver birch trees. Sample trees grew in a well-lit site in the Mashezero forestry district, 30 km from Petrozavodsk.

Rectangular incisions sized 10 cm (vertically) by 1.5 cm (horizontally) were made with a grafting knife on the birch trunk 1.3 m above the ground at five equidistant points around the trunk. The knife was driven into the bark to about one half the bark depth. The surface layers of bark were carefully removed, leaving the conducting phloem zone and part of the non-conducting phloem on the trunk (Figure 2a). This manipulation resulted in five rectangular wound ''apertures'' around the trunk.

The wounded stem area was tightly wrapped with adhesive waterproof tape immediately after removal of the bark layers, so as to form chambers for injection of sucrose solutions. The solution was injected with a syringe. Two needle punctures were made in the top corners of each chamber, one for injection of solution, the other for air diversion. The concentrations of sucrose solutions were 0 (distilled water), 10, 25, 50, and 100 g L^{-1}.

When choosing the solution concentrations, we took into account the range of fluctuations of sucrose concentrations in the phloem exudate of woody plants over one year: 0.01–0.2 M in summer and 0.3–0.9 M at the end of the growing season and during dormancy (Zimmermann 1958; Peel and Weatherley 1959; Hill 1962; Zimmermann and Ziegler 1975; Sudachkova 1977). Sucrose concentrations in the phloem exudate of five birch species were as follows: *Betula fruticosa* Pall., 20%–30% (0.6–0.9 M); *B. papyrifera* Marsh. and *B. populifolia* Marsh., 10%–20% (0.3–0.6 M); *B. lenta* L. and *B. lutea* Michx., 2%–10% (0.06–0.3 M) (Zimmermann and Ziegler 1975). The sucrose concentration in *B. pendula* Roth in August, prior to growth cessation was 0.35 M (Kolesnichenko 1985).

Thus the sucrose concentrations used in our experiments are physiological relative to possible sucrose concentrations in the phloem exudate of woody plants, namely *B. pendula*. The highest injected sucrose concentration in our study—100 g L^{-1} (0.3 M)—corresponds to the sucrose concentration in the phloem exudate of *B. pendula* at the end of the secondary growth period and, hence, is one of the highest physiological concentrations of the phloem exudate sucrose for this tree species.

Active transpiration of the leaves during the experiment ensured uptake of the solutions into the stem. Sucrose solutions were replenished as they were consumed. Owing to the notably elongated shape of the regeneration chambers, solutions were constantly present in the lower half of the chambers. Each experiment was made in three replications: sucrose solutions were injected in five wound ''apertures'' (chambers) around each of three silver birch trees. A chamber without any solution near 30 cm above the experimental impact site on each tree served as control. The experiment began on July 13, and the material for microscopic investigation was fixed on August 27, 2003.

Three tissue samples for examination were taken from the lower half of each chamber. Fixation of samples was made as for electron microscopy. Sections (2.0 μm) were stained with safranin and examined and photographed under an Amplival light microscope.

Tabulated measurements are presented as mean values and confidence intervals. The indicated confidence intervals for all values meet 95% reliability according to Student's *t*-distribution.

Results

Electron Microscopy

Sieve elements contain primitive structure plastids, which accumulate starch. It normally appears as aggregations of small grains. One can see in Figure 3a, b that few starch grains formed in sieve tube amyloplasts in the common birch trunk during the period of active formation of new xylem and phloem layers. The sections of all common birch bark samples taken from different parts of the conducting phloem in this period represented a very similar pattern.

There were some sieve tubes in the Karelian birch phloem that contained about the same number of amyloplasts as the sieve tubes in the common birch. At the same time there were areas where sieve tubes were virtually filled with starch (Figure 3c, d).

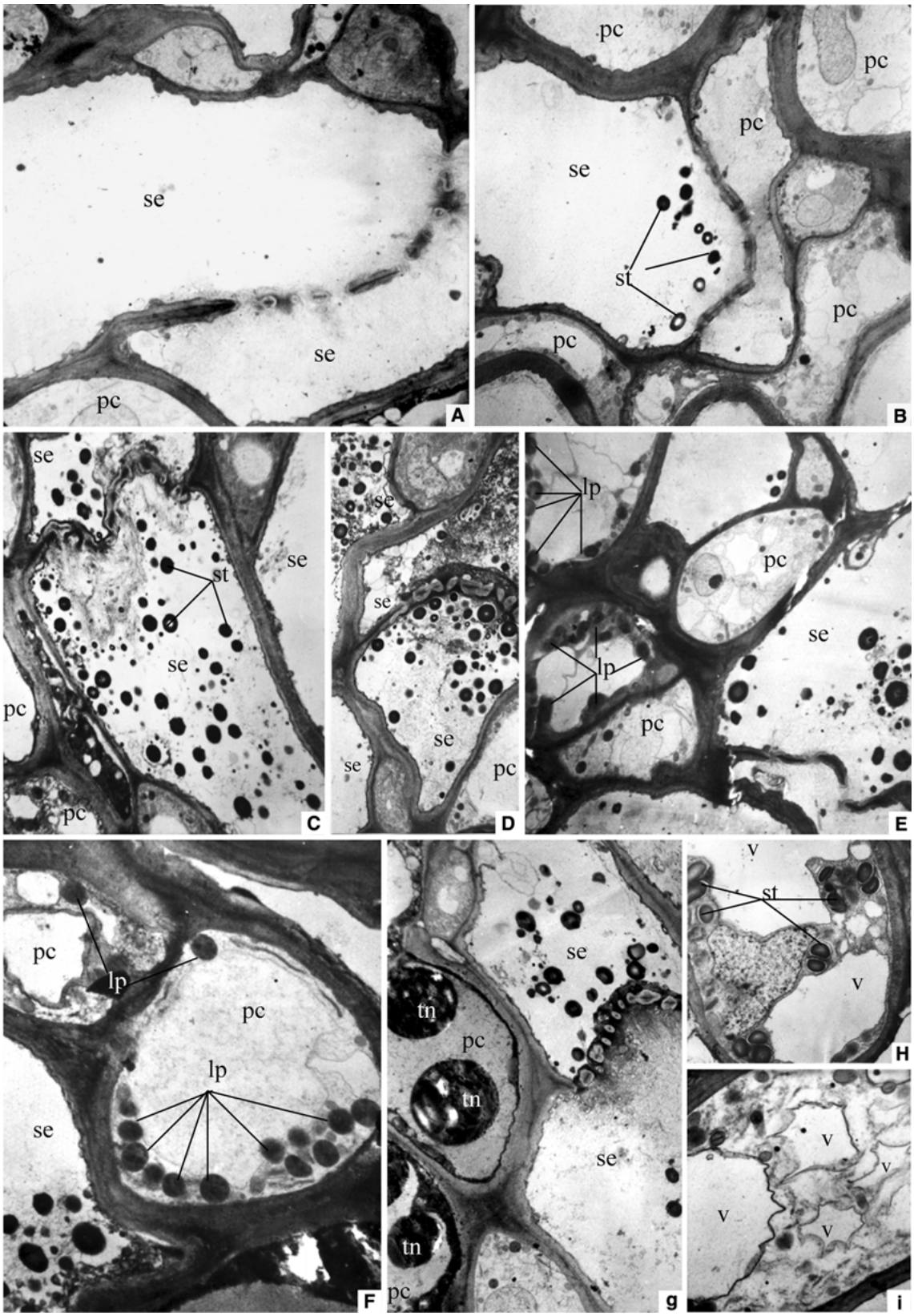

Figure 3. Fragments of conducting and non-conducting phloem of common birch (**A, B, I**) and Karelian birch (**C–H**). Conducting phloem (**E–G**), nonconducting phloem (**H, I**). Magnification: (**A–E, G, I**) ×1500, (**F**) ×3000, (**H**) ×4000. lp, lipid drop; n, nucleus; pc, parenchymal cell; se, sieve element; st, starch; tn, tannin; v, vacuole.

Table 1. Sucrose, Starch, Total Lipids, and Tannin Contents in the Phloem Tissues of *Betula pendula* var. *pendula* (silver birch) and *B. pendula* var. *carelica* (Karelian birch)

		Date				
		1996			2001	2002
	Name of *Betula pendula* trees	18.06	15.07	29.07	26.06	18.06
Sucrose	Silver birch	11.8 ± 0.5	25.2 ± 1.0	15.6 ± 0.7	20.0 ± 1.5	27.6 ± 0.4
	Karelian birch	25.1 ± 2.3	39.1 ± 2.7	21.6 ± 1.0	43.1 ± 3.3	42.3 ± 1.2
Starch	Silver birch	2.8 ± 0.1	3.1 ± 0.1	4.3 ± 0.2	6.9 ± 0.1	—
	Karelian birch	4.2 ± 0.4	4.7 ± 0.5	6.3 ± 0.3	6.9 ± 0.4	—
Total lipids	Silver birch	45.2 ± 2.3	—	68.5 ± 2.1	53.6 ± 1.4	—
	Karelian birch	63.8 ± 3.8	—	83.9 ± 2.2	96.8 ± 4.6	—
Tannins	Silver birch	5.6 ± 0.2	6.1 ± 0.4	7.0 ± 0.5	—	—
	Karelian birch	6.9 ± 0.3	6.4 ± 0.6	7.0 ± 0.9	—	—

Sucrose, starch, total lipids: mg (g dry weight)$^{-1}$; tannins: % dry weight.
Tabulated measurements are presented as mean values and confidence intervals. A dash indicates that the measurement was not taken. The indicated confidence intervals for all values meet 95% reliability according to Student's t-distribution.

During the active growth period, parenchyma cells of the common birch conducting phloem contain almost no storage nutrients (Figure 3a, b), whereas the same cells of the Karelian birch store considerable amounts of lipids and tannins (Figure 3e–g).

Non-conducting phloem of the Karelian birch was predominantly composed of starch-bearing cells with large vacuoles (Figure 3h), often occupying nearly the whole volume of the cell, as well as cells with lipid inclusions and tannin-bearing cells. Most parenchyma cells in the non-conducting phloem of the common birch had small, irregularly shaped vacuoles (Figure 3i).

Results of Biochemical Analysis

Throughout the period of determinations, sucrose and lipid concentrations in the Karelian birch phloem were much higher than in the silver birch phloem (Table 1). The same can be said about starch concentrations, except for measurements taken on 26 June 2001, when no difference was recorded. Differences in tannin content between the birches were apparent at the first stages of intensive secondary growth, but they smoothed out afterwards.

Experiment with Injection of Sucrose Solutions

Xylem and Phloem Increment. A comparison would reveal a clear trend: the higher the sucrose solution concentration, the lower the number of xylem layers and the higher the number of phloem layers formed at the beginning of the experiment (Figure 4b–f, Table 2).

Xylem Parenchymatization. At the very beginning of the experiment, some wood layers with heavily radially compressed xylem elements formed. They looked like a false annual ring. Because these layers were present in all variants of the experiment, we have attributed them to the effect of wounding on the cambium. At the same time, significant differences could be seen between variants in the zone under consideration, namely as the concentration of the sucrose solution rises, the share of the xylem parenchyma among structural elements gradually increases. This rise was most pronounced in the variant with 100 g L^{-1} sucrose solution (Figure 4f). The parenchyma cell layers there grew to look like typical parenchyma inclusions in Karelian birch wood. Noteworthy is the emergence of new radial rays, which commence from the xylem parenchyma band and continue into the phloem.

Phloem Parenchymatization. Active phloem parenchymatization occurs in the 10 g L^{-1} sucrose variant (Figure 4f). A factor promoting this process is notable widening of the rays as a result of the increase both in the size and the number of cells in them. The main reason for the widening, however, is a conspicuous increase in the proportion of the axial parenchyma in the phloem.

Sclerification of Phloem Cambial Derivatives. In the microphotographs showing the results of applying 25 g L^{-1} and 50 g L^{-1} sucrose solutions, it is

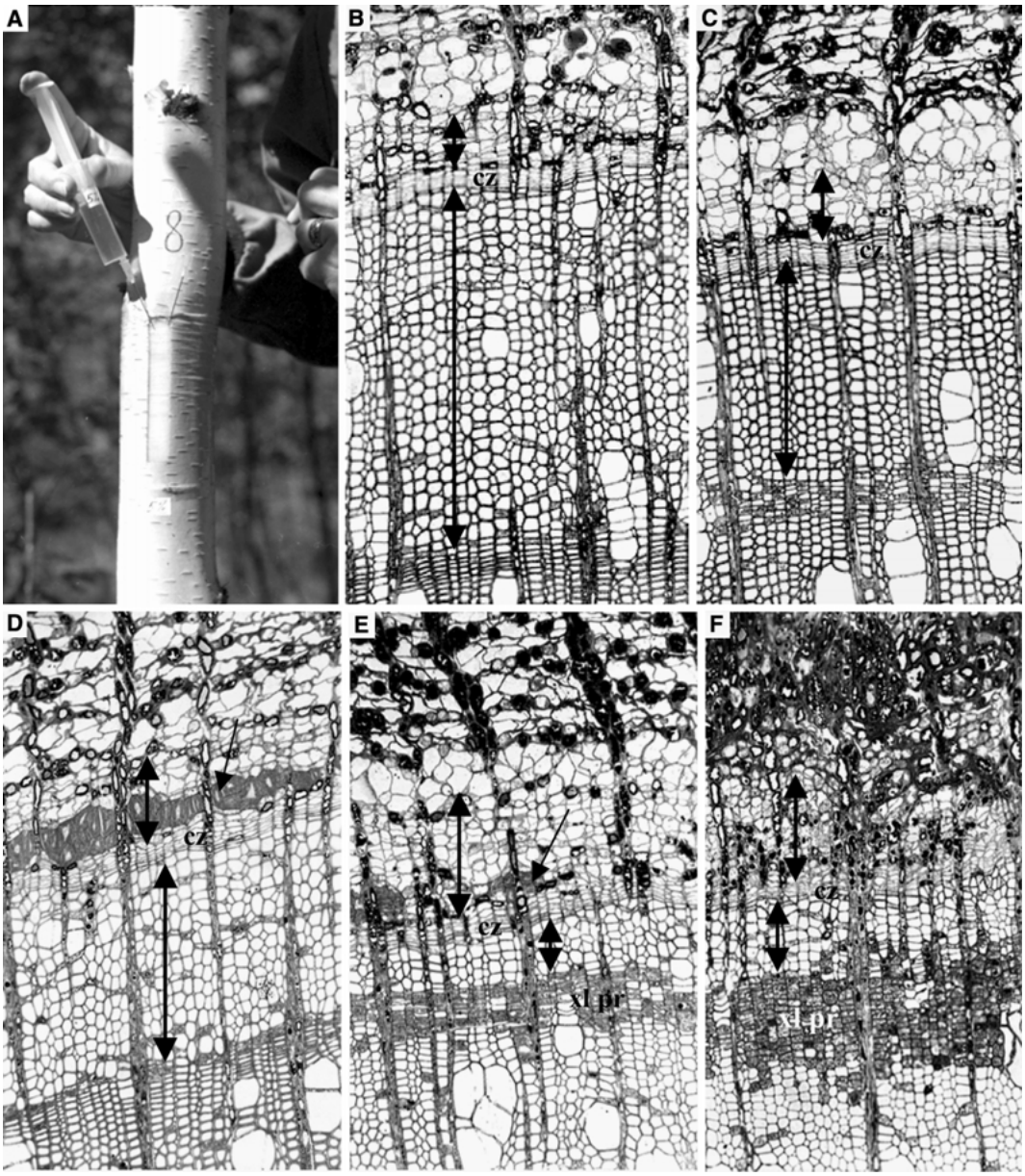

Figure 4. Experiment with injection of sucrose solutions. (**A**) Injection of solutions with a syringe. (**B–F**) Cross sections through tissues formed after injection of sucrose solutions: (**B**) 0 g L^{-1}, (**C**) 10 g L^{-1}, (**D**) 25 g L^{-1}, (**E**) 50 g L^{-1}, (**F**) 100 g L^{-1}. Magnification ×100. Double arrows, xylem and phloem increases formed after injection of sucrose. Single arrow, layer of sclereids formed from phloem cambial derivatives. cz, cambial zone; xl pr, xylem parenchyma.

possible to see either a solid layer (25 g L^{-1}) or groupings (5 g L^{-1}) of sclereids formed on the phloem side, close to the cambial zone (Figure 4d,i). The interesting thing about this finding is that Karelian birch, in contrast to common birch, also shows sclerification of the phloem cambial derivatives in the zones where structural abnormalities of conducting tissues form. The problem of phloem cell sclerification will be discussed in detail in a separate publication.

Discussion

Assimilates move from the crown to the trunk via sieve tubes. The actively dividing cells of the cambial zone and all other bark and wood cells and tissues are supplied with photosynthesis products through lateral exchange between sieve elements and other cells of the conducting phloem. For the phloem to function normally, the sucrose concentration in sieve tubes must be maintained at a cer-

Table 2. Effects of Different Sucrose Concentrations on Xylem and Phloem Growth in *Betula pendula* var. *pendula* (silver birch)

	Xylem and phloem increase after beginning of the experiment, μm			
Sucrose concentration	Xylem	Phloem	Xylem/phloem	Width of the parenchyma layer in xylem
Control	328 ± 8.8	101 ± 5.7	3.3	–
0 g L^{-1}	473 ± 9.2	99 ± 2.9	4.8	–
10 g L^{-1}	296 ± 23.0	105 ± 2.8	2.8	–
25 g L^{-1}	267 ± 25.3	147 ± 6.3	1.8	–
50 g L^{-1}	68 ± 9.7	169 ± 5.2	0.4	70 ± 4.2
100 g L^{-1}	95 ± 11.0	170 ± 10.0	0.6	122 ± 12.0

Tabulated measurements are presented as mean values and confidence intervals. The indicated confidence intervals for all values meet 95% reliability according to Student's t-distribution.

tain level. The simplest mechanism for controlling sucrose concentration in the phloem transport channel is starch deposition and mobilization by sieve tube amyloplasts (Kursanov 1976; Evert 1990; Gamalei 2004). Hence, it is quite common that some starch would be found in the conducting phloem sieve tubes, as happens in the common silver birch (Figure 3a, b).

In contrast, emergence of abundant starch-bearing amyloplasts in the sieve tubes of the Karelian birch (Figure 3c, d) would appear to indicate the presence of excess transport of sucrose. If, however, the sucrose concentration in the sieve tubes exceeds a certain threshold, the stabilizing function of amyloplasts proves inadequate and is complemented with the activity of the conducting phloem parenchyma cells (Kursanov 1976; Kholodova and others 1980). The role of storage reservoirs is then played by vacuoles and plastids of the phloem parenchyma cells. The stores are in exchange equilibrium with the sucrose moving along the sieve tubes. There exists, however, a biological limit to sugar accumulation—that is, a maximum level of sucrose concentration in the storage space of cells. It was found, for example, that starch formation in the cells of sugar beet roots ceases during active sugar accumulation, and substances of a lipid nature form. In that case lipids are stored in the form of plastoglobulars in plastids and lipid drops in the cytoplasm (Kholodova and others 1980). Lipids, which may form from sucrose decomposition products, are known to have one of the highest energy storage capacities among storage substances. Tannin synthesis is also related to sucrose through systems of metabolic reactions (Zaprometov 1993). Thus, we consider the formation of significant stores of lipid and tannin inclusions in the axial parenchyma cells of the Karelian birch conducting phloem (Figure 3e–g) to be evidence for the need to remove excess sucrose from metabolism. It cannot be stored in vacuoles any more, and radial ray cells fail to withdraw it to be utilized in growth and maintenance of the function of other trunk tissues.

Results of the quantitative analysis of the sucrose, starch, lipid, and tannin concentrations in the silver and Karelian birch bark tissues are in conformity with the above hypotheses (Table 1).

Experiments with callus cultures have shown that a certain sucrose/auxin ratio promotes differentiation of conducting elements. This was first demonstrated for the callus *Syringa vulgaris* L. (Wetmore and Sorokin 1955; Wetmore and Rier 1963; Wetmore and others 1964). The xylem/phloem ratio largely depended on sucrose concentration: when the disaccharide concentrations were low, xylem formation prevailed; high concentrations promoted phloem formation; a medium concentration caused the formation of both xylem and phloem, normally with cambium between them. Quantitatively, this appeared as follows: a combination of 0.1 mg L^{-1} IAA and 15–25 g L^{-1} sucrose primarily favored the establishment of tracheids; a rise in the sucrose concentration to 45–50 g L^{-1} (IAA concentration remained the same) resulted in the intensive formation of sieve elements; both elements of the conducting tissues formed at a sucrose concentration of 25–35 g L^{-1} (Wetmore and Rier 1963; Wetmore and others 1964).

Further studies have proved that although tracheid elements may sometimes develop in the absence of sucrose in the medium, sucrose strongly enhances the quantities of formed tracheids up to a concentration of about 20 g L^{-1} (the figure varies notably depending on the species), whereupon sucrose begins to inhibit tracheid formation (Beslow

and Rier 1969—*Coleus*; Warren Wilson and others 1982—*Lettuce*). Wetmore and Rier (1963), in callusis from cambial regions of *Fraxinus, Salix, Syringa, Ligustrum*, and *Parthenocissus*, and tubers of *Helianthus*, found that 10–20 g L^{-1} sucrose induced strong xylem differentiation, 25–35 g L^{-1} sucrose favored phloem as well as xylem, and 40 g L^{-1} sucrose induced phloem but little or no xylem. *Cucumis* tissues cultured *in vitro* have provided confirmatory findings (Fadia and Mehta 1973).

It was found also that once a certain level is achieved, a further rise in sucrose concentration would result in overall inhibition of xylem and phloem differentiation (Sachs 1981). In addition to inhibiting the formation of secondary conducting tissues, high sucrose concentrations have an inhibitory effect on cambial activity (Sudachkova 1977; Sachs 1981; Warren Wilson 1984). In this connection some authors suppose that seasonal sucrose content fluctuations in phloem exudate lead to a periodicity of cambial activity and secondary growth (Sudachkova 1977; Gamalei 1990, 2004).

All the above facts taken together suggest that, in contrast to other sugars, sucrose is capable of a powerful morphogenetic effect. Therefore, it has lately been recognized as a hormone-like substance belonging to the morphogene group (Warren Wilson 1984). Over the past 10 years the problem has been widely discussed in the literature, and the amount of experimental evidence for the morphogenetic role of sucrose has been growing steadily (Gamalei 1990, 2004; Perl and others 1991; Sung and others 1993; Simko 1994; Koch 1996; Weber and others 1997a 1997b; Hauch and Magel 1998; Xu and others 1998; Borisjuk and others 2002, 2003; Gibson 2004).

The results of our experiments, as well as the above-mentioned data from the literature, indicate that sucrose strongly influences the morphogenesis of conducting tissues. Furthermore, the ranges of sucrose concentrations causing predominant formation of either xylem or phloem elements in adult birch trees (Figure 4b–f, Table 2) correspond to the results obtained *in vitro* for other species. A fact to be stressed specifically is the formation of parenchyma layers in the xylem and intensified phloem parenchymatization at elevated sucrose concentrations.

When the cambium functions normally, the phloem derivative of the cambium (phloem mother cell) undergoes yet another division prior to differentiation into a sieve element. That is, two phloem cells eventually form from one phloem mother cell. The xylem mother cell undergoes two consecutive divisions prior to differentiation into xylem elements, that is, four differentiated xylem elements form from one xylem mother cell (Larson 1994). Hence, a shift toward the phloem side in the divisions of the cambium initially would result both in a sharp decline in wood increment and in an overall reduction in radial growth (Figure 4b–f, Table 2).

Having analyzed the data obtained, we have concluded that abnormal wood formation in Karelian birch is caused by the emergence of areas with excess sucrose in the phloem. Local variations in the disaccharide content are responsible for the sinuosness of the annual ring, which develops when the formation of either xylem or phloem elements prevails. In addition to producing an overall inhibiting effect on the formation of xylem and phloem, high sucrose concentrations bring about changes in the development of the cambial zone cells, inducing the formation of storage parenchyma cells (Figure 4e, f). This appears to be the cause of the carinate bends in the annual ring of the Karelian birch (Figure 1b), in which the ''bottom'' is lined with parenchyma layers. In contrast, intensified parenchymatization of cells on the phloem side is also observed (Figure 4f).

The primary function of parenchyma cells is storing nutrients. It has recently been shown using the new method of high-resolution histographical mapping of sucrose and glucose that storage cell differentiation within *Vicia faba* cotyledons is induced by both high sucrose levels and a low hexose to sucrose ratio (Borisjuk and others 2002, 2003). The authors conclude that high sucrose levels provide the signal for induction of storage-associated gene expression. Much earlier studies have shown that sucrose influx stimulates the growth of storage tissues and, moreover, that sugar accumulation and growth processes are closely interrelated and lead to an increase in the organic mass of the tissue (Kursanov and Pavlinova 1967; Kursanov 1976). Research on the processes of sucrose metabolization in the sugar beet root revealed that up to 70% of the sucrose transported to the root from leaves accumulated in the storage parenchyma cells, whereas the rest—about 30%—was divided between biosynthesis and respiration (Kursanov 1976; Kursanov and others 1989).

It is important to remember that the sugar beet root is a highly specialized organ in which the metabolism of parenchyma cells is directed to sucrose accumulation. With regard to birch bark, its parenchyma cells feature a variety of stored substances, as can be seen in Figure 3e–h. These can be sugars, starch, lipids, or tannins. The pathway for sucrose metabolization would apparently depend on the enzymatic systems available (or active) in the cell. One can presume that the activation of one or

another enzymatic system would depend on the amount of the substrate supplied to the cell. It has already been mentioned, for instance, that when sugar accumulation is high, lipids form in cells instead of starch. It is also known that cells with high tannin content demonstrate minor starch formation, and vice versa (Wardrop and Cronshaw 1962; Esau 1969; Chafe and Durzan 1973).

Specific ultrastructural features of parenchyma cells in the conducting and non-conducting phloem of the Karelian and the common silver birch in the period of high cambial activity indicate that the bark of Karelian birch in this period acts as a storage tissue, in which some of the assimilates are deposited as storage substances and others are apparently consumed in building new storage cells. Thus, excessive assimilate storage in the trunk provides an explanation for the formation of both the abnormal patterned wood in Karelian birch (Figure 1b) and its very thick bark, composed predominantly of the storage parenchyma cells (Figure 2b).

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