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Structure and Function
Trees

Food reserves of Scots pine (*Pinus sylvestris* L.)

I. Seasonal changes in the carbohydrate and fat reserves of pine needles

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Summary. The amounts of starch, soluble sugars, triacylglycerols, diacylglycerols and free fatty acids were studied in Scots pine (*Pinus sylvestris* L.) during an annual cycle in current-year needles and in 1-, 2- and 3-year-old needles collected shortly after bud break. Determination of the compounds was performed using specific enzymatic assays, capillary gas chromatography and thin layer chromatography. Newly emerging needles contained relatively large amounts of starch, but only trace amounts of fat. During autumn and winter, fat content rose, while starch content decreased; amounts of both these reserve materials were very high the next spring shortly before bud break and decreased again during shoot elongation. Concentration of intermediates in triacylglycerol biosynthesis (diacylglycerols and free fatty acids), were low in summer and high in winter. The same pattern was observed for fructose and glucose (the predominant soluble sugars), galactose/arabinose and raffinose/melibiose. In contrast, sucrose concentrations were highest in spring and in autumn. Mature needles of different ages collected in May showed significant differences only in their triacylglycerol and starch content. Concentration changes of reserve materials are discussed in relation to season, mobilization and translocation processes, dormancy, frost resistance and the possibility of carbohydrate-fat interconversions.

Key words: Food reserves – *Pinus sylvestris* – Starch – Sugars – Triacylglycerol

Introduction

Storage is a characteristic phenomenon of perennial plants. Storage material is synthesized in leaves and other green tissues like twigs, parts of flowers or developing fruits by photosynthesis. At times when production exceeds consumption, assimilates are converted into sucrose or other transport sugars, and translocated into the sink-regions of the tree. There they are utilized for vegetative and reproductive growth or stored in living cells (Kozlowski and Keller 1966). All of the living perennial organs of a woody plant may serve for storage. Recycling (or mobilization) is an important feature of storage and used to distinguish true storage substances from other compounds accumulated, like condensed tannins or terpene resins.

In trees, carbon is stored as nonstructural carbohydrates (starch, fructosans, soluble sugars) and fat (triacylglycerol). According to the predominant storage material in them, trees were classified as "starch trees" and "fat trees", respectively (Fischer 1891; Sinnott 1918). Based on this early classification, most ring-porous angiosperms, including some gymnosperms (like species of *Abies* and *Picea*), belong to the group of "starch trees", while most diffuse-porous angiosperms (including the genus *Pinus*) are regarded as "fat trees".

Seasonal changes in reserve material have been followed mainly in deciduous trees (Bonicel et al. 1987; Bonicel and de Medeiros 1990; Höll 1981; Höll and Priebe 1985; Jeremias 1968a, b; Sauter 1980; Yoshioka et al. 1988; older literature summarized by Kozlowski and Keller 1966). Usually, starch is high in summer and low in winter, whereas soluble sugars show the opposite behaviour. In general, storage by deciduous trees is more pronounced than by evergreen species. The lack of an intact photosynthesizing tissue in early spring increases the asynchrony of supply and demand in deciduous trees. Fluctuations of storage compounds in conifers have scarcely been investigated (Bernard-Dagan 1988; Höll 1985; Pomeroy et al. 1970; Saranpää and Nyberg 1987; Senser and Beck 1979) and reveal that starch is low in winter and high in spring, whereas fat and soluble sugars both increase during the cold period. Most investigations on conifers were restricted to either carbohydrate or fat reserves and to either needles or wood.

In the present paper we describe the seasonal fluctuation of carbohydrate and fat reserves (starch, glucose, fructose, sucrose, galactose/arabinose, raffinose/melibiose, triacyl-

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Table 1. Air temperature data during the period of the investigation

Month	Temperature (°C)			
	Mean	Minimum	Maximum	Mean temperature of collection day
January	0.1	− 8.2	10.2	− 3.2
February	1.0	− 7.3	11.3	− 0.2
March	2.6	−12.5	15.0	4.8
April	8.4	− 3.3	21.2	3.6
May[a]	14.2	2.0	24.8	14.8
June[a]	15.2	5.5	26.0	15.0
July	17.8	8.6	33.3	23.0
August	17.5	6.4	31.1	12.4
September	13.2	4.5	27.1	15.0
October	9.6	− 3.9	20.5	7.9
November	1.5	−12.9	11.4	− 0.8
December	1.9	−11.0	9.7	6.1
May[b]	13.5	1.5	24.8	16.8
June[b]	14.4	2.8	27.2	18.8

[a] Newly emerging needles collected
[b] One-year-old needles collected
Data (measured 2 m above ground) were provided by the German Weather Centre, Munich

glycerol, diacylglycerol and free fatty acids) in current-year needles of Scots pine trees during their 1st year and in 1-, 2- and 3-year-old needles collected in May. In a following paper, we will report on the seasonal changes in sapwood and the radial distribution of storage material the trunkwood of Scots pine.

Materials and methods

Needle material. Current-year needles of seven Scots pine trees (approximately 30 years old) were collected at monthly intervals in a forest stand near Munich. The collection dates were in 1988; Feb 22, Mar 23, Apr 24, May 24, Jun 24, July 22, Aug 23, Sept 23, Oct 25, Nov 24, Dec 23 and in 1989: Jan 26, May 19 and Jun 26. On 19 May 1989, newly emerging, 1-, 2- and 3-year-old needles were collected. For temperature data of the sampling period see Table 1. Immediately after collection, the needles were frozen in dry ice, lyophilized and homogenized with liquid nitrogen using a micro-dismembrator (Braun, Melsungen, FRG). Prior to extraction of carbohydrates and lipids, the needle powder was heated for 10 min at 100° C to inactivate hydrolytic enzymes.

Extraction and determination of soluble sugars. Five milligrams of each sample was extracted with 1 ml bidistilled water for 30 min. After mixing with 50 mg polyvinylpyrrolidone (Polyclar AT: Serva, Heidelberg, FRG) and centrifugation (4000 *g*, 1 min), sugars were assayed spectrophotometrically using coupled enzyme reaction methods. All enzymes and co-substrates were purchased from Boehringer (Mannheim, FRG). Optical density was measured at 334 nm.

Glucose and fructose. Fifty microlitres of the supernatant was treated with 1 ml of triethanolamine hydrochloride (0.3 *M*, pH 7.6) containing $MgSO_4$ (3 m*M*), ATP-$Na_2H_2 \cdot 3\ H_2O$ (300 m*M*), β-NADP (24 m*M*) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49, from yeast, 1.4 units). Glucose was calculated based on the change in absorbance after addition of 1.4 units hexokinase (EC 2.7.1.1. from yeast; Kunst et al. 1985). Addition of 3.5 units phosphoglucose isomerase (EC 5.3.1.9, from yeast) initiated a second increase in optical density, which was proportional to the amount of fructose present (Beutler 1985 c).

Sucrose. Fifty microlitres of the supernatant was hydrolysed at room temperature for 25 min with 100 µl acetate buffer (0.1 *M*, pH 4.6) containing 30 units β-fructosidase (EC 3.2.1.26, from yeast). The hydrolysate was measured for glucose as described above. For calculation, absorbance difference was corrected for the presence of free glucose (Outlaw and Tarczynski 1985).

Galactose/arabinose. Two hundred microlitres of the needle extract was treated with tris (hydroxymethyl)-aminomethane (1 *M*, pH 8.2) containing 15 m*M* NAD. The increase in optical density after addition of β-galactose dehydrogenase (EC 1.1.1.48, from *Pseudomonas fluorescens*, 0.5 units) was equivalent to the amount of galactose and arabinose present (Hjelm and de Verdier 1985).

Raffinose/melibiose. Two hundred microlitres of the supernatant was hydrolysed at room temperature for 45 min in citrate buffer (50 m*M*, pH 4.6) with 5 units α-galactosidase (EC 3.2.1.22, from green coffee beans). After incubation, galactose was assayed as described above. Absorbance differences were corrected for effects of free galactose and arabinose (Beutler 1985 b).

Extraction and determination of starch. Five milligrams of the needle powder was treated with 200 µl bidistilled water for 10 min at 100° C. For hydrolysation, 11 units amyloglucosidase (EC 3.2.1.3, from *Aspergillus niger*) was added. Mixtures were incubated at 37° C over night. Final volume was 1 ml. After centrifugation (4000 *g*, 1 min), 20 µl of the supernatant was assayed for glucose as described above. Values were corrected for the amount of free glucose (Beutler 1985 a).

Extraction of triacylglycerols (TAG), diacylglycerols (DAG) and free fatty acids (FFA). Twenty milligrams of the needle powder was extracted with 1 ml chloroform/methanol (2:1, v/v) containing 20 µ*M* pentadecanoic acid and 20 µ*M* γ-linolenic acid as internal standards for 30 min and washed twice with 0.5 ml chloroform/methanol (2:1, v/v). In a second step, the needle powder was washed three times with 1 ml diethyl ether. The combined extracts were dried under a stream of nitrogen. In addition, every third sample was extracted with chloroform/methanol (2:1; v/v) containing 50 µ*M* dioleate and 50 µ*M* trioleate as internal standards to check quantitative recovery.

Separation of TAG, DAG, and FFA by thin layer chromatography. Lipid extracts were redissolved in 50 µl chloroform/methanol (2:1, v/v) and applied to TLC plates (0.2 mm Silica gel 60 on aluminum). The remaining residues were dissolved twice in 50 µl diethyl ether and combined with the chloroform/methanol-soluble compounds of the samples on the plates. Authentic dioleate, trioleate, oleic acid and linolenic acid served as standards. Plates were developed twice in petroleum ether (boiling range 40–60° C)/diethyl ether/acetic acid − 90:20:1 v/v/v (Mangold and Malins 1960, modified). Standard lipids at the margins of the plates were visualized by spraying with sulphuric acid/acetic acid (1:1, v/v) and heating (3 min at 160° C) according to Kates (1972). Unsprayed plate areas corresponding to the standard lipids were scraped off. DAG was eluted three times with 1 ml chloroform/methanol (2:1, v/v), and TAG and FFA were eluted three times with 1 ml diethyl ether. Samples were taken to dryness under a stream of nitrogen.

Enzymatic hydrolysis and quantification of DAG and TAG. Samples were treated with 200 µl tris (hydroxymethyl)-aminomethane buffer (100 m*M*, pH 8.2) containing sodium cholate (8 m*M*), magnesium chloride (30 m*M*) and Genapol-X-80 (2 g/l). After vigorous mixing for 30 s (Vortex mixer) 10 µl lipoprotein lipase (EC 3.1.1.34, from *Pseudomonas sp.*: Merck, Darmstadt FRG) was added to the mixtures ("incubation mixture"). Final enzyme concentration was 6.8 units/ml. Specimens were incubated overnight at 37° C. Quantitative hydrolysis was checked by co-incubation of 50 nmol distearate and tristearate.

For enzymatic determination of glyceride glycerol, 950 µl "reaction mixture" was added to plastic cuvettes containing 100 µl "incubation mixture". The "reaction mixture" consisted of tris (hydroxymethyl)-aminomethane (100 m*M*, pH 8.2), sodium cholate (8 m*M*), $MgCl_2 \cdot 6\ H_2O$ (30 m*M*), NADH (sodium salt, 0.25 m*M*), ATP-$Na_2H_2 \cdot 3\ H_2O$ (0.1 m*M*), phosphoenolpyruvate (tricyclohexylammonium salt, 0.3 m*M*), $NaHCO_3$ (0.2%, w/v), pyruvate kinase (EC 2.7.1.40, from rabbit muscle, 150 units) and lactate dehydrogenase (EC 1.1.1.27, from rabbit muscle,

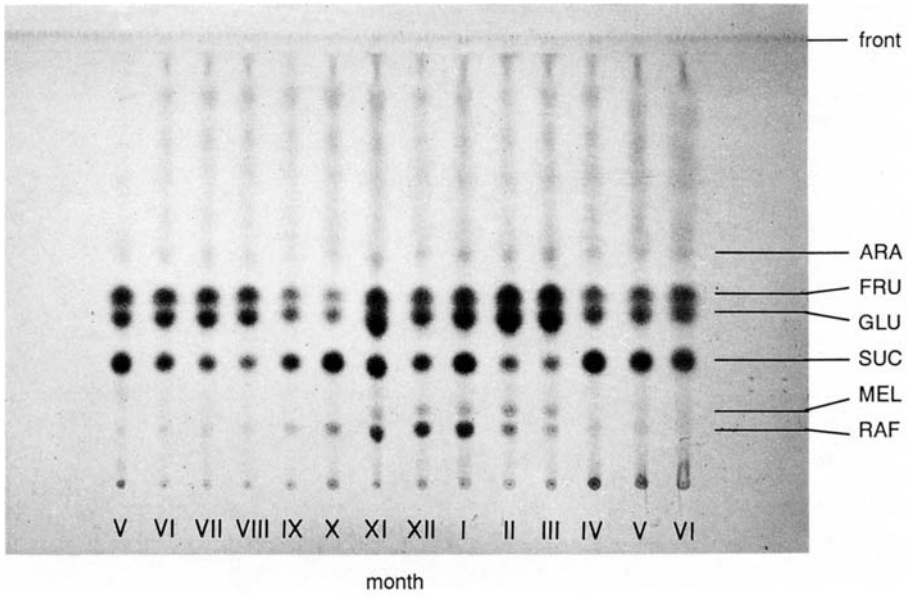

Fig. 1. Thin layer chromatogram of soluble sugars in needles of Scots pine during the first 14 months after their formation. *ARA*, arabinose; *FRU*, fructose; *GLU*, glucose; *SUC*, sucrose; *MEL*, melibiose; *RAF*, raffinose. *Roman numbers* indicate months of collection. Each *spot* represents sugars extracted from 0.6 mg needle material (dry weight)

120 units). The increase in optical density after addition of glycerokinase (EC 2.7.1.30, from *Candida mycoderma*, 100 kU/l) was measured at 334 nm. All enzymes and substrates were purchased from Boehringer Mannheim (Nägele et al. 1985, modified).

Derivatization and gas chromatography of fatty acids. For synthesis of fatty acid methyl ester (FAME) FFA as well as TAG and DAG samples after hydrolysis were treated with 0.1 ml of boron trifluoride (14% in methanol) for 30 min at 80° C. After adding 1 ml water, the mixtures were extracted three times with diethyl ether. The combined ether extracts were washed twice with 1 ml water, taken to dryness, and redissolved to a final concentration of approximately 50 ng/µl of FAME with chloroform.

A DANI 8500 Capillary Gas Chromatograph with a Programmed Temperature Vaporizer (PTV) injection system and flame ionization detector was used. The FAME was determined on a fused silica 30 m DB-225 (50% cyanopropylphenyl – 50% dimethylpolysiloxane) column (J&W Scientific) with an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The column temperature program was 2 min at 150° C, followed by a 10° C/min ramp to 210° C. This final temperature was held for 15 min. Helium served as carrier gas with an inlet pressure of 2.0 bar (flow rate: 3 ml/min). Detector gases were hydrogen at 56 ml/min and air at 180 ml/min; the make-up gas was nitrogen at 40 ml/min. We used the splitless injection mode with solvent split, the split valve was opened 30 s after injection. The injection volume was 1 µl, the split flow 40 ml/min (split ratio 1:13). The temperature of the PTV was 60° C for 6 s, followed by a very rapid heating approximately (900° C/min) to 230° C for 15 min. The detector temperature was 240° C. A Shimadzu C-R3A integrator was used to collect data. Fatty acids were identified by co-chromatography of authentic FAME and by gas chromatography-mass spectrometry.

TLC of annual fluctuations of sugars and lipids. For sugar TLC, the plate was developed twice in chloroform/acetic acid/water (5/7/1, v:v:v). Spots were visualized by spraying with diphenylamine/aniline/*ortho*-phosphoric acid/acetone (1/1/0.1/100, w/v/v/v) and heating to 130° C for 3 min (De Stefanis and Ponte 1968). The start-front distance was 9 cm. Micro-TLC of lipids was performed as described above, with the exception of the mixture ratio of the solvent (petroleum ether/diethyl ether/acetic acid: 35:20:1, v/v/v). The start-front distance was 4 cm. Spots were identified by co-chromatography of authentic sugars and lipids and by methods used for quantification (see coupled enzyme reaction methods for sugar and glyceride glycerol determination and gas chromatography of fatty acids).

Experimental design and statistics. Experiments were designed as single factor experiments with repeated measures. Populations were checked for Gauss distribution using the Kolmogoroff-Smirnoff test. Homogeneity of variance was checked using the F_{max} test. Means were tested for significance using a single factor analysis of variance for repeated measures. For multiple comparisons, the LSR test was performed. Pearson's correlation coefficient was tested for significance using the *t*-test according to Fisher.

Experimental errors of three different measurements using the same sample (expressed as coefficients of variation) were 2–4% for glucose and fructose, 2–8% for galactose/arabinose and melibiose/raffinose, 10–20% for sucrose and 5–10% for starch. In the case of lipid analysis experimental errors were 5–10% for DAG and TAG and 10–20% for FFA. Tree-to-tree variations were quite large and are specified in the figure legends.

Results

Soluble sugars

The composition of soluble sugars in current-year needles of Scots pine during the first 14 months after their formation is shown in Fig. 1. Raffinose, melibiose, sucrose, glucose/galactose, fructose, arabinose and other pentoses were detectable. Arabinose, fructose, glucose/ galactose and sucrose were present all year. In contrast, raffinose and melibiose disappeared in summer. Due to their identical chromatographic behaviour, galactose could not be separated from glucose.

Quantitative measurement and changes of the two predominant hexoses, fructose and glucose, are depicted in Fig. 2. During the cold period, from November to March, needles contained up to twice the amounts of both monosaccharides than from April to October. Maximum values occurred in January and February (differences are significant at the 99.9%-level). No significant differences (95% level) could be detected between specimens collected during the warm period (April to October) of the year.

Quantification procedures used did not distinguish between galactose and arabinose and between galactosides of sucrose (raffinose) and galactosides of glucose (melibiose). Concentrations of galactose/arabinose were very

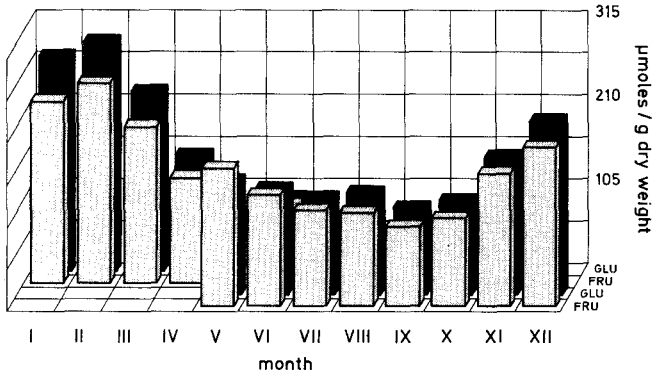

Fig. 2. Seasonal changes of fructose (*FRU*) and glucose (*GLU*) in current-year needles of Scots pine. Tree-to-tree variation, expressed as 95% confidence interval, was 16–32% of the mean for glucose and 10–56% for fructose. *Columns* represent average values of seven trees. Bud break occurred at the end of April, and needle maturation was in August. *Roman numbers* indicate months of collection; May and June needles initiated in different years are shown in different rows. For significance of differences, see Results

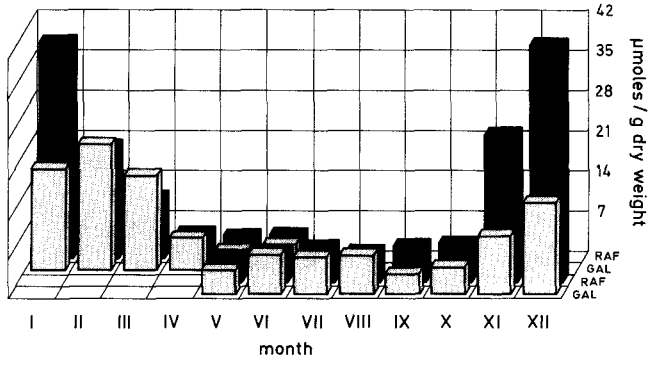

Fig. 3. Seasonal changes of galactose/arabinose (*GAL*) and raffinose/melibiose (*RAF*) in current-year needles of Scots pine. Tree-to-tree variations, given as 95% intervals, were 22–78% of the mean for galactose/arabinose and 20–65% for raffinose/melibiose. For further explanations see Fig. 2

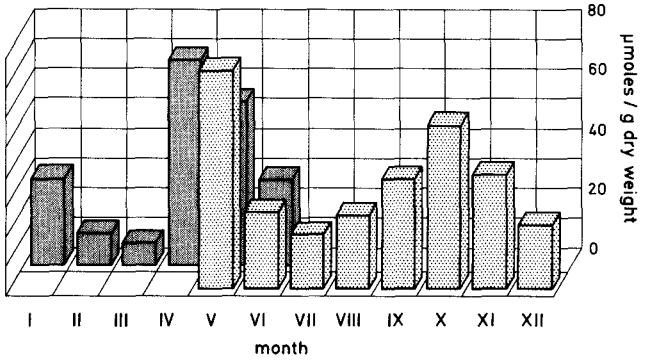

Fig. 4. Seasonal changes of sucrose in current-year needles of Scots pine. Tree-to-tree variation, given as 95% confidence interval, was 22–200% of the mean. See Fig. 2 for further explanations

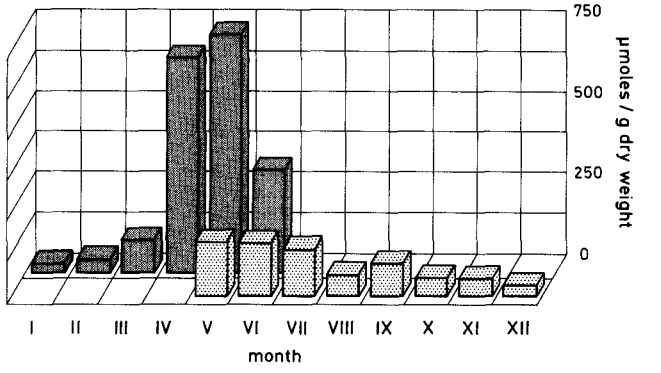

Fig. 5. Seasonal changes of starch, expressed as glucose equivalents, in current-year needles of Scots pine. Tree-to-tree variation, given as 95% confidence interval, was 16–122% of the mean. See Fig. 2 for further explanations

small at all times and followed the same seasonal pattern (Fig. 3). In the case of raffinose and melibiose, similar fluctuations to monosaccharides could be detected, but maximum values were observed in December and January. These maximum values compared to all other months investigated, and values measured during the warmer (March to October) and the colder period (November to February) were significantly different at the 99.9% level.

Newly emerging needles contained very high levels of sucrose (Fig. 4). During needle differentiation amounts decreased, then reached a second maximum in autumn. In March, needles contained the lowest levels of sucrose, but 4 weeks later, a third maximum value was measured. In June, amounts of sucrose had decreased again. Due to the very high variability of the investigated trees, only maximum and minimum values are significantly different compared to values measured in all other months at the 95%-level.

Needles 1, 2 and 3-years old, collected shortly after bud break, were very similar in their amounts of soluble sugars (Table 2). The glucose, fructose and galactose/arabinose content of newly emerging needles was higher than that of the older needles (95% level). No significant differences between all needle age classes could be detected in case of sucrose and raffinose/melibiose.

Starch

Compared to winter needles, elongating and differentiating needles (collected between May and July; Fig. 5) contained relatively large amounts of starch (expressed as glucose equivalents), differences were significant at the 95% level. During autumn and winter, starch content decreased to a very small level. In spring next year, needles accumulated extremely high amounts of this reserve carbohydrate. When new twigs emerged, the starch content of the now 1-year-old needles decreased again; these differences were significant at the 99.9% level.

In Table 2 the amounts of starch of newly emerging needles are compared with those of older (1-, 2- and 3-year-old) needles. It is clear that older needles contained more starch on a dry weight basis than differentiating ones. Within the 1-, 2- and 3-year-old needles, however, no

Table 2. Carbohydrate and lipid content (μmol/g dry weight) of newly emerging (0), 1-(1), 2-(2), and 3-(3)-year-old needles from Scot's pine collected shortly after bud break ($n = 7$)

	0			1			2			3		
	Mean	CV		Mean	CV		Mean	CV		Mean	CV	
Glucose	121.8	21.2	a	89.0	17.2	b	82.9	23.3	b	87.2	45.2	b
Fructose	151.3	24.2	a	90.8	16.9	b	85.5	19.8	b	89.1	43.5	b
Galactose/arabinose	5.2	19.2	a	3.7	32.4	b	3.0	30.0	b	2.9	48.3	b
Sucrose	59.6	37.7	a	55.3	28.4	a	37.4	28.9	a	38.5	49.3	a
Raffinose/melibiose	4.1	26.8	a	4.1	19.5	a	2.7	25.9	a	3.5	45.7	a
Starch	124.8	15.4	a	738.7	12.7	b	473.3	20.9	c	624.8	45.3	d
Free fatty acids	10.3	24.3	a	9.0	41.1	a	16.5	64.8	a	22.1	51.1	a
Diacylglycerols	2.3	21.7	a	1.7	29.4	a	1.4	21.4	a	1.7	47.0	a
Triacylglycerols	1.2	16.7	a	6.3	23.8	b	4.6	19.6	bc	3.1	45.2	c

Means within a row not followed by the same letter are significantly different from each other ($P < 0.05$).
Values were calculated on a dry weight basis, water content of newly emerging needles was 71%, of mature needles 55%

clearcut tendency could be observed. Differences between all age classes were significant at the 99.9% level.

Lipids

The neutral lipid composition of Scots pine needles during the first 14 months after their formation is shown in Fig. 6. Steryl esters, TAG, FFA, DAG and free sterols could be detected and were present in all months investigated.

Quantitative changes of DAG are depicted in Fig. 7. From November to March, needles contained significantly higher amounts of DAG, compared to all other months investigated (mostly 99.9% level).

Figure 8 shows the seasonal fluctuations of FFA. Levels were low in summer and autumn, and higher in winter and spring. Variability between the seven trees investigated was very high: significant differences (95% level) could be detected only between needles collected in spring (February/March) and those collected in summer and autumn (May to October).

Only traces of TAG were present in elongating and differentiating needles (May to August; Fig. 9). In winter and spring, fat content rose and was at a maximum in April. When new twigs emerged, amounts decreased again to a very small level. Differences between summer and autumn months (including December) were not significant at the 95%-level; differences between all other months investigated were significant at the 99.9% level.

FFA and DAG contents of newly emerging and 1-, 2- and 3-year-old needles collected in May were not significantly different at the 95% level (Table 2). In contrast, the fat (TAG) content of newly emerging needles was very small, compared to older needles. One-year-old needles contained the highest TAG amounts. With increasing needle age, the fat content decreased; differences were significant (99.9%-level mostly) with the exception of the 1/2-year-old and the 2/3-year-old comparisons.

Palmitic acid was the dominant fatty acid in the DAG fraction (Fig. 10). Stearic acid, oleic acid, and linoleic acid represented about 10% of total DAG acyl moieties. In the FFA fraction, however, linolenic and linoleic acid were the major components (approximately 25%) throughout the year, followed by palmitic acid (12%) and oleic acid (10%). Oleic (27%), linoleic (23%) and palmitic (17%)

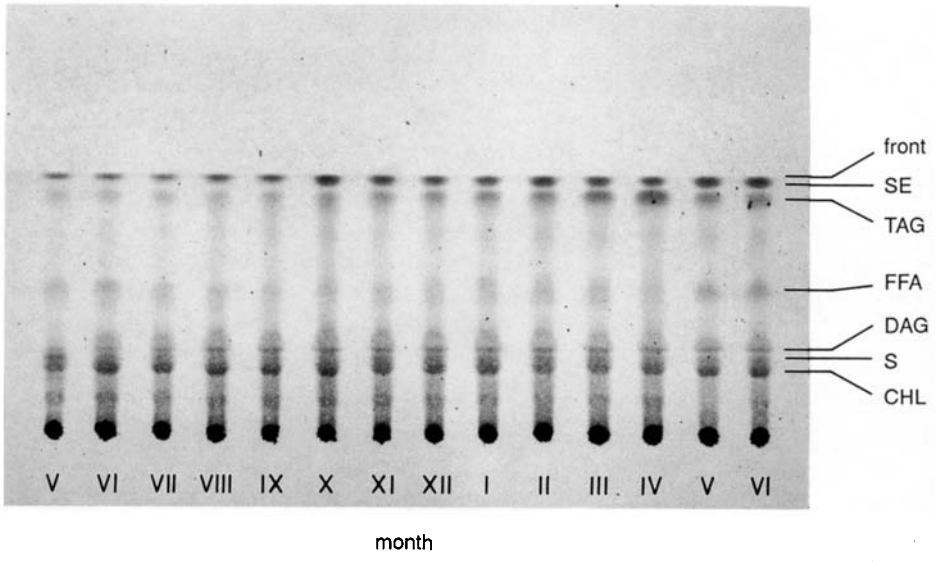

Fig. 6. Thin layer chromatogram of lipids in needles of Scots pine during the first 14 months after their formation. *SE*, Steryl esters; *TAG*, triacylglycerols; *FFA*, free fatty acids; *DAG*, diacylglycerols; *S*, free sterols; *CHL*, chlorophyll. *Roman numbers* indicate months of collection. Each *spot* represents lipids extracted from 1.25 mg needle material (dry weight)

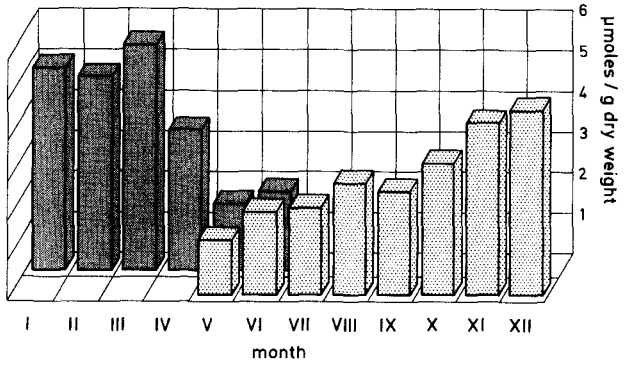

Fig. 7. Seasonal changes of diacylglycerols in needles of Scots pine. Tree-to-tree variation, expressed as 95% confidence interval, was 25–92% of the mean. See Fig. 2 for further explanations

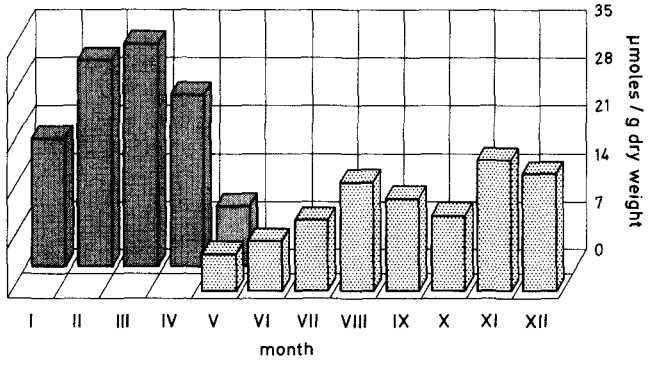

Fig. 8. Seasonal changes of free fatty acids in needles of Scots pine. Tree-to-tree variation, given as 95% confidence interval, was 40–176% of the mean. See Fig. 2 for further explanations

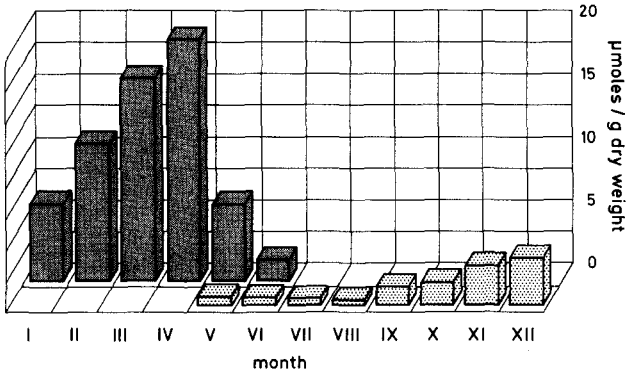

Fig. 9. Seasonal changes of triacylglycerols in needles of Scots pine. Tree-to-tree variation, given as 95% confidence interval, was 25–86% of the mean. See Fig. 2 for further explanations

acid showed highest relative amounts in the TAG throughout the year.

Discussion

In contrast to deciduous trees, conifers possess intact photosynthesizing tissue in leaves of the previous year. With increasing day temperatures in early spring, photosynthesis

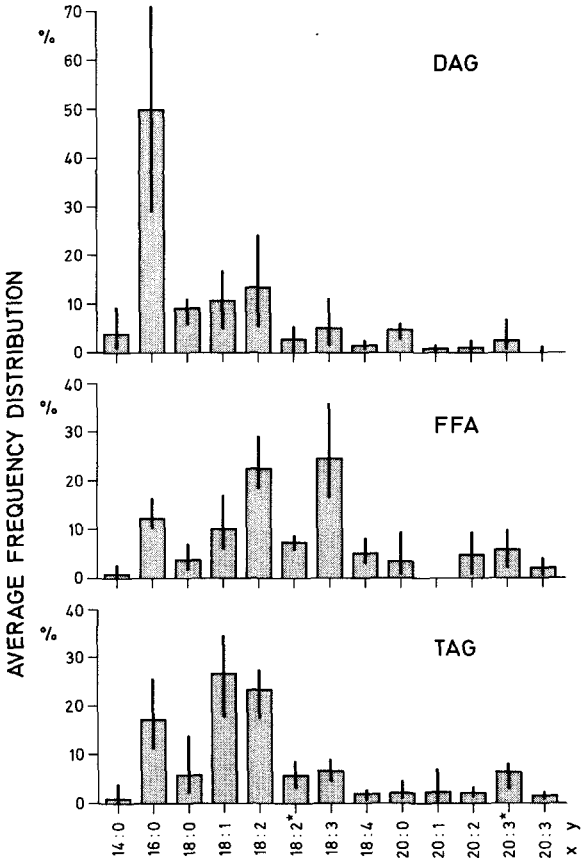

Fig. 10. Average frequency distribution of free fatty acids (*FFA*), diacylglycerol fatty acids (*DAG*) and triacylglycerol fatty acids (*TAG*) extracted from Scots pine needles (relative amounts). Current-year needles were harvested at monthly intervals from May (shortly after bud break) to June the next year. *Columns* represent average values of 14 months ($n = 7$). *Vertical bars* indicate maximum and minimum values for the 14 months; *X : Y*, number of carbon atoms: number of double bounds; *asterisks* indicate isomers

in evergreen trees becomes apparent, long before shoot and root growth starts. As a result of low night temperatures, dark respiration in needles is low (Kozlowski and Keller 1966; Little 1970a; Ursino et al. 1968). As a consequence, a pre-budbreak accumulation of starch has been reported in needles of *Picea abies* (Senser and Beck 1979). *Pinus resinosa* (Pomeroy et al. 1970) and *Abies balsamea* (Little 1970a, b). Our findings indicate that the high levels of starch and fat in Scots pine needles reflect current photosynthetic activity. Due to the lack of active sinks (e.g. new needles or the onset of radial growth), reserve substances are accumulated in the form of sucrose (Fig. 4), starch (Fig. 5), and fat (Fig. 9) in early spring.

With the onset of shoot expansion at the end of April, tissues of intense sink strength are formed. Allocation and redistribution of carbon after pulse-labelling of the photosynthates have been studied in *Pinus sylvestris* (Hansen and Beck 1990), *Pinus strobus* (Ursino et al. 1968) and *Pinus resinosa* (Gordon and Larson 1968) elucidating source-sink relationships during bud break. In general, previous-year needles played a significant role in exporting assimilates to the developing new shoots, stored material in

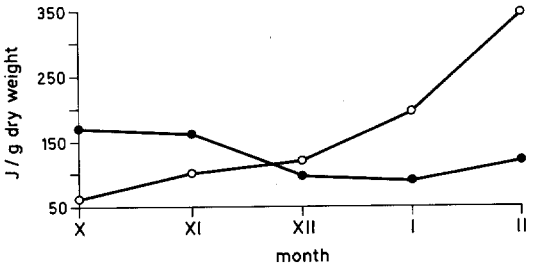

Fig. 11. Heat of combustion (given in J/g dry weight) of starch (●) and fat (○) stored in Scots pine needles during the period October – February

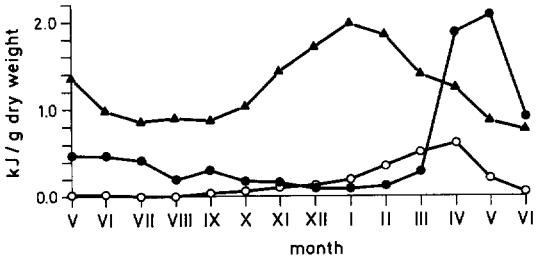

Fig. 12. Heat of combustion (given in kJ/g dry weight) of soluble sugars (▲), starch (●) and fat (○) stored in young Scots pine needles during the first 14 months after their formation. Roman numbers indicate month of collection

the ray parenchyma cells of the wood is of minor importance, if at all. In agreement with this data, starch and fat decreased dramatically in the 1-year-old needles of Scots pine during expansion of the new twigs (Figs. 5, 9), and sucrose, the major transport sugar in trees (Zimmermann and Brown 1971) peaked in both mature and newly emerging needles (Fig. 4). The sucrose content of the 2- and 3-year-old needles was similar to that measured in 1-year-old needles, and their starch and fat content was very high, compared to elongating needles (Table 2). It seems likely, that 2- and 3-year-old needles are important sources of energy-rich compounds as well.

Newly emerging needles contained considerable amounts of sucrose and starch, but only traces of fat (Figs. 4, 5, 9). The fat reserves of the older needles might have been converted to soluble sugars for translocation. With maturation of the new needles, the starch and fat content of the young needles remained very low during summer and autumn, while at the same time there was a rise in sucrose. This phenomenon probably reflects a large increase in export activity of photosynthate out of the current-year needles into other parts of the tree. In agreement with our data, transport almost exclusively downwards into stem and roots in summer and autumn has been shown for *Pinus resinosa* (Gordon and Larson 1968) and *Pinus strobus* (Ursino and Paul 1973).

With onset of the cold period, starch decreased, while fat, DAG, FFA and soluble sugars (with the exception of sucrose) rose (Figs. 3–9). This breakdown of starch, accompanied by an accumulation of soluble sugars is in good agreement with reports in the field (Pomeroy et al. 1970; Senser and Beck 1979; Senser et al. 1971). Fat accumulation in winter needles has been scarcely investigated, but was observed for *Pinus pinaster* (Bernard-Dagan 1988).

DAG and FFA (as CoA esters) are known to be intermediates in TAG biosynthesis, but no significant correlation could be found between the amounts of fat and fat precursors (FFA and DAG) in needles of Scots pine. Additionally, the fatty acid composition of the TAG does not reflect the composition of the FFA and DAG (Fig. 10). Other acyl lipids like steryl esters or polar lipids, which are also built from DAG or fatty acids, should also be considered in this context. A detailed description of acyl changes in different lipids during the season will be given in a following paper.

It is an open question whether starch is converted into fat during dormancy. Ziegler (1964) suggested that this process takes place in "fat trees". He supported the view that reconversion of fat into carbohydrates in spring may be of selective advantage in avoiding embolism. Transformation of fat into sugars consumes oxygen, which (due to its low solubility in water) might be a contributing factor in causing embolism in xylem vessels.

If starch is converted into fat during winter, starch hydrolysing enzymes must be active. In fact, in needles of Scots pine, amylolytic activity is at its maximum during the cold period (W. Höll and A. Grätzlmaier, unpublished data). Further, the energy contents of starch stored in early winter, and of fat stored in late winter needles must be more or less equal. Neglecting entropy, we used the heat of combustion to compare energy contents of Scots pine reserves (Fig. 11). Interestingly, energy of fat stored in February needles is twice as high as energy of starch stored in October needles. If soluble sugars (they also could originate from starch) are taken into account, the increase in energy stored in Scots pine needles during winter becomes dramatic (Fig. 12).

Translocation of energy-rich compounds from wood into needles could explain this phenomenon. Our results of food reserve changes in Scots pine sapwood, however, exclude the wood as a source of energy. Also studies of carbon allocation do not indicate a transport out of the wood (Hansen and Beck 1990; Ursino and Paul 1973; Ursino et al. 1968). Another explanation for this energy increase in winter needles is the production of assimilates despite low temperatures. Winter photosynthesis in evergreens has been documented (reviewed by Kozlowski and Keller 1966) but due to the high respiration activity, no increase in food reserves was observed in *Pinus sylvestris* (Troeng and Linder 1982; Ungerson and Scherdin 1965). Numerous data contradict the assumption of functional photosynthesizing tissue in needles of Scots pine in winter; the chloroplasts assume their characteristic winter form, they become swollen and their membrane system is disorganized and reduced (Martin and Öquist 1979). Using electron paramagnetic resonance, no reaction centres for photosystem II could be detected in the cold period, indicating absence of full photosynthesis at that time. In contrast, the reaction centres of photosystem I are operating, and thus cyclic phosphorylation and ATP formation may take place (Tsel'niker and Chetverikov 1988). It is possible, that the striking increase of soluble sugars and fat observed in Scots pine needles in winter is a consequence of energy

supply based on cyclic phosphorylation. Other carbon sources like hemicelluloses and fructans (not analysed in our investigation) as well as CO_2 dark fixation (PEP carboxylase) might contribute to the overall increase in the total quantity of carbon in reserves in winter.

The seasonal variations in the carbohydrates of trees have often been investigated in relation to cold acclimation. In general, starch disappears during artificially induced or naturally occurring low temperatures, while soluble sugars, especially the lower molecular weight members of the raffinose family, rise (Alberdi et al. 1989; Kandler et al. 1979; Sauter 1988; Senser et al. 1971; Siminowitch 1953).

With the exception of sucrose, all soluble sugars investigated were significantly correlated with temperature ($r = -0.857$ for glucose, $r = -0.808$ for fructose, $r = -0.835$ for galactose/arabinose and $r = -0.788$ for raffinose/melibiose; in all cases $P < 0.001$ – Figs. 2–4). The maximum concentrations of raffinose and melibiose (needles did not contain stachyose, Fig. 1) occurred before the yearly temperature minimum was reached (Table 1). In contrast, maximum concentrations of glucose, fructose and galactose/arabinose were measured more or less simultaneously with the onset of the lowest temperatures, probably reflecting the particular role of raffinose in frost hardening (Kandler and Hopf 1980). Raffinose, a 1.6-α galactoside of sucrose is accompanied by melibiose, a 1.6-α galactoside of glucose. To our knowledge, melibiose had never been reported to play a role in frost resistance, but the strong correlation between raffinose and melibiose may indicate such a role.

In seeds, sugars (sucrose, trehalose) have been shown to be protective components during desiccation. They act as substitute for water in biomembranes, preventing contact between the collapsing membranes and membrane lipid fusions (Chen and Burris 1990). The tendency of sucrose to crystallize lessens its effectiveness, but can be overcome by addition of small amounts of raffinose (Caffrey et al. 1988). Since frost injuries are based mainly on desiccation processes, this may help to explain the physiological role of raffinose sugars in frost resistance of conifers.

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