

Genetic and environmental control of seasonal carbohydrate dynamics in trees of diverse *Pinus sylvestris* populations

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Summary We explored environmental and genetic factors affecting seasonal dynamics of starch and soluble non-structural carbohydrates in needle and twig cohorts and roots of Scots pine (*Pinus sylvestris* L.) trees of six populations originating between 49° and 60° N, and grown under common garden conditions in western Poland. Trees of each population were sampled once or twice per month over a 3-year period from age 15 to 17 years.

Based on similarity in starch concentration patterns in needles, two distinct groups of populations were identified; one comprised northern populations from Sweden and Russia (59–60° N), and another comprised central European populations from Latvia, Poland, Germany and France (49–56° N). Needle starch concentrations of northern populations started to decline in late spring and reached minimum values earlier than those of central populations. For all populations, starch accumulation in spring started when minimum air temperature permanently exceeded 0 °C. Starch accumulation peaked before bud break and was highest in 1-year-old needles, averaging 9–13% of dry mass. Soluble carbohydrate concentrations were lowest in spring and summer and highest in autumn and winter. There were no differences among populations in seasonal pattern of soluble carbohydrate concentrations. Averaged across all populations, needle soluble carbohydrate concentrations increased from about 4% of needle dry mass in developing current-year needles, to about 9% in 1- and 2-year-old needles. Root carbohydrate concentration exhibited a bimodal pattern with peaks in spring and autumn. Northern populations had higher concentrations of fine-root starch in spring and autumn than central populations. Late-summer carbohydrate accumulation in roots started only after depletion of starch in needles and woody shoots. We conclude that Scots pine carbohydrate dynamics depend partially on inherited properties that are probably related to phenology of root and shoot growth.

Keywords: defoliation, needle, phenology, provenance, root, soluble carbohydrates, starch, shoot.

Introduction

The temporal pattern of long-term changes in nonstructural

carbohydrates in tree tissues is an important characteristic that reflects the relative balance between source (net canopy assimilation) and sinks (the use of assimilates for growth and respiration). Carbohydrate dynamics are also related to seasonal fluctuations in cold hardiness (Parker 1959). Because of the relationship between carbohydrate concentration and CO₂ exchange, carbohydrate concentration is frequently used as a measure of environmental and physiological constraints on plant yield (Linder and Flower-Ellis 1992, Stockfors and Linder 1998, Tjoelker et al. 1999).

Scots pine (*Pinus sylvestris* L.) and other coniferous species are characterized by pronounced seasonal changes in non-structural carbohydrate concentrations in needles, stems and roots. Generally, a substantial accumulation of starch is found in needles before bud break, followed by starch depletion after the onset of shoot growth (Ericsson 1979, Chung and Barnes 1980). Numerous external and internal factors such as fertilization and irrigation (Adams et al. 1986, Linder 1995, Stockfors and Linder 1998), drought (Parker and Patton 1975, Sudachkova et al. 1994), low temperatures (Ericsson 1984), defoliation or shoot pruning (Ericsson et al. 1980, 1985, Långström et al. 1990), atmospheric CO₂ and pollutant concentrations (Kainulainen et al. 1998, Tjoelker et al. 1999), and reproduction (Sweet 1979) affect sink–source relationships, thereby altering the seasonal dynamics of nonstructural carbohydrates in trees.

Shoot phenology can also play an important role in the dynamics of carbohydrate reserves in trees. For example, *Pseudotsuga menziesii* (Mirb.) Franco trees displayed different seasonal starch patterns at two different altitudes, and these differences were correlated with timing of bud burst (Webb and Kilpatrick 1993). Compared with trees at the lower altitude, trees exhibiting later bud burst at the higher altitude were characterized by later increases in starch accumulation. However, it is not clear whether the observed seasonal patterns resulted from differences in climatic conditions at the two altitudes or from genetic factors.

In many tree species with a large distribution area or altitudinal range, or both, selection has resulted in substantial genetic diversity. Thus, if comparisons are made across both environment and genotype, broad-scale environmental heterogeneity across the species' range usually complicates interpre-

tation of observed phenotypic patterns and their genetic basis. Common garden experiments provide an effective way to examine genotype differences. This approach has been used to show that different populations of Scots pine, a common and important timber species in Eurasia, differ widely in such traits as needle and shoot phenology, root to shoot ratio, biomass production and ecophysiology (Giertych 1991, Reich et al. 1996a, Oleksyn et al. 1998). Based on these differences, we postulated that populations originating from diverse climatic conditions differ in seasonal patterns of nonstructural carbohydrate concentration and distribution.

An early attempt to address these questions in populations of Scots pine seedlings failed because of methodological constraints (Langlet 1936). Besides Langlet's study, we have identified only one other published study where the interaction between genotype and seasonal changes in carbohydrates was tested in a coniferous species (Cranswick et al. 1987). In that study, it was found that genotypic differences in carbohydrates among clones and crosses of *Pinus radiata* D. Don were minor; however, the study is of a species with an extremely narrow area of natural distribution. There have been no studies of seasonal patterns of nonstructural carbohydrate concentrations in different tissue types of diverse tree populations. Furthermore, seasonal patterns of carbohydrates in woody tissues and fine roots have not been studied extensively and have usually been considered separately from those of needles (Gholz and Cropper 1991).

To obtain conclusive information about these relationships, we studied needle and twig (wood + bark) cohorts, and root nonstructural carbohydrates in geographically diverse populations for three consecutive years in 15-year-old Scots pine grown in a common garden experiment in western Poland. The experimental design provided a means to explore genotypic differences in nonstructural carbohydrate dynamics in different organs of Scots pine under uniform climatic conditions. We hypothesized that both climatic and genetic factors affect carbohydrate partitioning and phenology. Based on the lower growth rates and earlier growth cessation of northern populations, we hypothesized that these populations have lower seasonal concentrations of nonstructural carbohydrates and shorter periods of starch accumulation in needles than populations from the central part of the species' range.

Materials and methods

Plant material and study site

Seeds of Scots pine were collected between 1978 and 1980 from 20 locations in Europe as a part of an international collaborative experiment established under the auspices of the International Union of Forestry Research Organizations. Detailed information about this experiment is presented elsewhere (Oleksyn 1988, Giertych and Oleksyn 1992, Oleksyn et al. 1999a).

In April 1984, 2-year-old seedlings of 19 populations of Scots pine were planted in a seven-block, permanent site in the experimental forest, Zwierzyńiec, near Kórnik in central Po-

land (52°15' N and 17°04' E, altitude 70 m). Every provenance was planted in three to seven replicated plots (one per block), 7.2 × 5.2 m, each with 48 plants (4 rows × 12 plants). The original spacing was 0.6 m within and 1.3 m between rows, and original stocking was 12,834 trees ha⁻¹. In 1994, thinning was conducted and more than 60% of the trees were removed. Soils at this site are light sands.

A total of six geographically diverse populations were selected for this study (Table 1). The selected populations represented sites with mean annual temperatures ranging from 4.5 °C (15-Sweden) to 10 °C (14-France), and latitudes from 60.2 to 48.8° N. Previous studies have revealed that the selected populations differ significantly in number of trees per ha, basal area, aboveground standing biomass, net primary production, CO₂ exchange, growth phenology and foliage nutrient concentration (Table 1, Oleksyn and Bialobok 1986, Reich et al. 1994, 1996a, Oleksyn et al. 1998, 1999a, 1999b, 2000).

Environmental conditions

The climate of the region is transitional between maritime and continental. Mean annual precipitation is 526 mm and mean temperature is 7.7 °C, with a mean growing season length of 220 days, calculated as the number of days with mean temperature ≥ 5 °C. Meteorological data were obtained from a local meteorological station approximately 2 km from the experimental forest.

The study was conducted over a 3-year period from 1996 to 1998 (Figure 1). Climatic patterns differed over the study period. In 1996, mean annual air temperature was 7.1 °C (0.6 °C below normal), whereas in 1997 and 1998 mean annual temperatures were 9.1 and 9.7 °C, respectively (1.4 and 2.0 °C higher, respectively, than the long-term mean). Mean annual precipitation was 526 mm in 1996, 516 mm in 1997 and 634 mm in 1998, and occurred predominantly during the summer months. On July 8 and 9, 1996, rainfall totaled 78 mm (17% of annual sum). The period from June 20 to July 20, 1997 was also unusually wet, with 156 mm of precipitation (about 30% of mean annual rainfall for that year) (Figure 1).

The winter of 1996 was relatively long and cold for the region and minimum air temperature was below 0 °C until early April, whereas in both 1997 and 1998 the minimum air temperature remained below 0 °C only until late February. For 39 consecutive days from January 15 to February 24, 1997, maximum daily temperatures varied from 4 to 11 °C, but the ground remained frozen. During this period, significant damage to crop plants, trees and shrubs was observed in the region (Oleksyn, unpublished observations). Similar climatic periods in 1996 and 1998 were much shorter and did not exceed 10 days.

In mid-May 1997, almost all 2-year-old needles were shed, which is typical for all populations at this site (Reich et al. 1996b). At the beginning of July 1997, from 20 to 30% of 1-year-old needles were abscised in all populations at the site. The culmination of needle abscission occurred in mid-August. A similar phenomenon was not noted before or after 1997.

Table 1. Origin of seeds of *Pinus sylvestris*, number of trees and basal area at age 16. Provenances are ordered by latitude of origin.

Population	Stand origin information						Common garden data	
	Country	Latitude (° N)	Longitude (° E)	Altitude (m)	Precipitation (mm)	Temperature (°C)	Trees, 1997 (no. ha ⁻¹)	Basal area (m ² ha ⁻¹)
15	Sweden	60.18	15.87	185	608	4.5	3701	10.9
3	Russia	58.83	29.12	80	616	5.0	3930	19.4
4	Latvia	55.75	26.67	165	619	5.4	5227	27.9
7	Poland	51.60	20.20	160	575	7.4	5266	34.7
12	Germany	49.50	8.50	97	645	9.6	4541	38.4
14	France	48.80	7.78	150	850	10.0	4184	27.5
Mean							4475	26.5

Phytopathological observations conducted in July and August 1997 by K. Przybyl (Department of Forest Pathology of the Institute of Dendrology, Kórnik, Poland) excluded biotic factors as a cause of the observed phenomenon. A low-level presence of fungi (*Lophodermium pinastri* (Schrad. ex Hook.) Chev., *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter, *Phoma* sp. or *Sclerophoma pithyophila* (Corda) Hoehn.) and insects (*Nuculaspis abietis* Schrank, *Brachonyx pineti* Paykull, *Cinara pini* L., etc.) was noted but was not responsible for the needle loss. We note this unusual July–August needle loss because carbohydrate concentrations at this time will be contrasted with other more typical periods.

Sampling scheme

Needles and twigs were sampled 49 times between January 8, 1996 and September 7, 1998 (Figure 1). Samples were taken twice per month, except during the winter months, when one sample was taken each month. To avoid excessive defoliation, samples were taken from the sunlit portion of the crowns of different trees on each date. For each sampling date, provenance was represented by samples taken from two trees in two different blocks.

Roots were extracted from core samples collected between rows and otherwise randomly located within the plot. The soil core sampler was 15 cm long and 4.7 cm in diameter (Arts Mfg. & Supply, American Falls, ID). Root samples were taken 18 times (eight in 1996, nine in 1997, and one in January 1998) at approximately monthly intervals from mineral soil, after removing the A₀ horizon. Root sampling was limited to times when the ground was not frozen or covered by snow. On average, between 81 and 85% of fine root biomass is distributed in the 0–15 cm soil depth at this site (Oleksyn et al. 1999a). On any given date, each population was represented by six cores taken in two blocks (three per block). To limit soil and root disturbance during the study, samples were taken from four blocks.

Soil samples with roots were washed over 1-mm sieves, and roots were manually separated from soil and, if necessary, from roots of annual plants. There were no other tree species and very few other plants present in the experimental plots. All visibly dead roots were removed. Roots were then separated into two categories: ≤ 3 mm and > 3 mm in diameter, and oven-dried at 65 °C. For further analyses, only the fine root (≤ 3 mm) fraction was used.

During each shoot sampling, all buds were removed to calculate bud water content. Bud water content was measured to assess dormancy release and establish the time of bud burst (Rinne et al. 1994, Myking 1998).

All needle, twig and root samples were taken at the same time of day, approximately 4–5 h after sunrise. After collection, samples were placed on ice in a cooler for transportation to the laboratory (distance of 2 km) for further processing.

Twig carbohydrates were evaluated on two populations, 3-Russia and 7-Poland. For each shoot-age class, twig samples (2 to 3 cm long) were taken from the middle of the shoot. Anal-

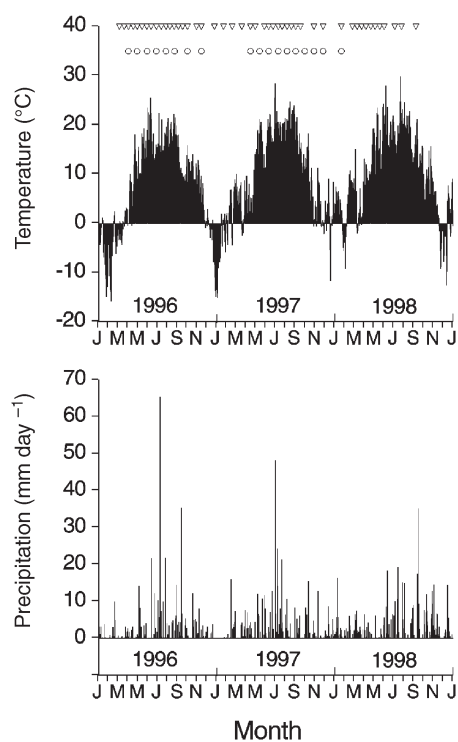


Figure 1. Seasonal variability in mean daily temperature (top) and daily precipitation (bottom) in the Scots pine (*Pinus sylvestris* L.) provenance plantation in Kórnik, Poland (52° N). Symbols represent needle (∇) and root (○) sampling dates.

yses were conducted on wood + bark tissue that was dried and ground.

Measurements of nonstructural carbohydrates

Total nonstructural carbohydrates (TNC) were determined by a modification of the method described by Haissig and Dickson (1979) and Hansen and Møller (1975). Sugars were extracted from oven-dried (65 °C, 48 h) tissue powder in methanol:chloroform:water (12:5:3 by volume), and the residue was used for starch determination. Extracted soluble sugars were determined colorimetrically with anthrone reagent at 625 nm within 30 min. Starch in the insoluble material was gelled and converted to glucose with amyloglucosidase. Glucose was measured with glucose oxidase by mixing the sample with peroxidase-glucose oxidase-*o*-dianisidine dihydrochloride reagent. Absorbance was measured at 450 nm after a 30-min incubation at 25 °C. Concentrations of soluble sugars and starch are expressed as percent of tissue dry mass. Soluble carbohydrate concentrations were calculated from standard regression equations based on glucose standard solutions. Data are means of two replicates consisting of one composite sample from each of two blocks sampled.

For all variables, statistical differences among provenances and sampling dates were calculated by analysis of variance. Because different trees and blocks were sampled during the study, and samples were pooled by block, the experimental design was considered completely random. Relationships between sampling day and studied traits were examined by correlation and regression analyses. For presentation, both correlation and regression are used, but we do not assume that direct causal relationships are involved. A Ward's hierarchical clustering method was used to compute cluster groups of Scots pine populations based on needle starch concentrations on different sampling dates. All statistical analyses were conducted with JMP software (version 3.1.5, SAS Institute, Cary, NC).

Results

Pattern of needle nonstructural carbohydrates

In all populations, soluble carbohydrate concentrations increased during the life span of the needles from about 3–6% in developing current-year needles to about 6–14% in 1- and 2-year-old needles (Figure 2, for 1996 cohort, $r = 0.46$, $P = 0.003$). Maximum soluble carbohydrate values were observed in 1-year-old needles in early spring, just before needle starch concentrations reached maximum values (Figure 2). On average, soluble carbohydrate concentrations in 1-year-old needles were lowest in spring and summer and highest in autumn and winter (Figure 2). For 1-year-old needles, all seasonal differences and differences among populations were statistically significant (Table 2). There were no sampling date \times population interactions, indicating that seasonal patterns of soluble carbohydrates were similar among all populations.

Mean soluble carbohydrate concentrations of 1-year-old needles were higher for low-latitude populations than for high-latitude populations, and increased linearly with in-

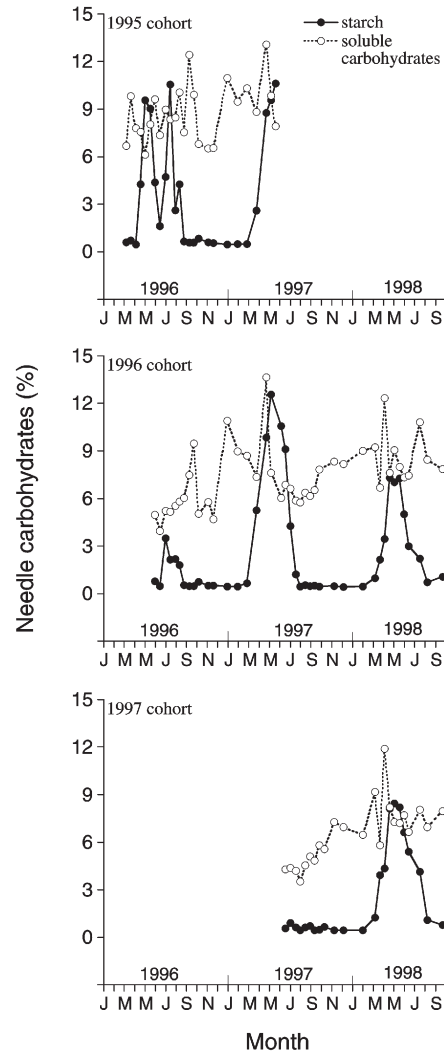


Figure 2. Seasonal changes in concentrations of starch and soluble sugars for 1995, 1996 and 1997 needle cohorts in Scots pine populations growing in a common garden in Kórnik, Poland (52° N). Each value is the mean of six populations. Data for the 1996 cohort cover the entire life span of the needles.

creases in mean annual temperature at the seed origin (Figure 3). A similar pattern of changes was noted for nonstructural carbohydrates for the entire life span of the needles (1996 cohort, data not shown).

Needle starch concentration had a pronounced seasonal pattern (Table 2, Figure 2). Starch was detected in needles only during the February–August period. In 1997 and 1998, the presence of starch in needles was noted at the end of February and beginning of March, whereas in 1996, starch was not detected in needles until the beginning of April (Figure 2). Year-to-year variation in the onset of starch accumulation in needles in spring was closely related to seasonal air temperature (Figure 4). Accumulation of starch in needles was noted only after minimum air temperature permanently exceeded 0 °C, and no starch was found in needles when minimum air

Table 2. Analysis of variance probabilities ($P > F$) of main effects of population origin (P), time of sampling (T) and $P \times T$ interactions on carbohydrate concentration in different age-classes of needles and woody shoots, and in fine roots of Scots pine. Observations on needle and roots were conducted on all populations, and those on stems on two populations (3-Russia, 58.8° N, and 7-Poland 51.6° N). Abbreviation: SE = standard error of population effect in ANOVA.

Tissue	Age	Carbohydrate	± SE	Population (P)	Sampling time (T)	$P \times T$
Needle	Current	Soluble	0.18	0.47	< 0.0001	0.53
		Starch	0.11	0.004	< 0.0001	0.75
		TNC	0.21	0.10	< 0.0001	0.29
	1-year-old	Soluble	0.18	0.02	< 0.0001	0.92
		Starch	0.15	< 0.0001	< 0.0001	0.16
		TNC	0.23	< 0.0001	< 0.0001	0.72
	2-year-old	Soluble	0.25	0.21	< 0.0001	0.12
		Starch	0.30	0.0003	< 0.0001	0.20
		TNC	0.40	< 0.0001	< 0.0001	0.21
Entire life span (1996 cohort)	Soluble	0.16	0.32	< 0.0001	0.61	
	Starch	0.14	0.0002	< 0.0001	0.14	
	TNC	0.21	0.002	< 0.0001	0.16	
Twig (wood + bark)	Current	Soluble	0.26	0.57	< 0.0001	0.03
		Starch	0.10	0.84	0.0005	0.30
		TNC	0.31	0.59	< 0.0001	0.03
	1-year-old	Soluble	0.15	0.73	< 0.0001	0.60
		Starch	0.14	0.61	< 0.0001	0.10
		TNC	0.20	0.55	< 0.0001	0.67
	2-year-old	Soluble	0.19	0.89	0.0006	0.26
		Starch	0.19	0.76	< 0.0001	0.19
		TNC	0.27	0.73	< 0.0001	0.21
Root	Soluble	0.12	0.75	< 0.0001	0.67	
	Starch	0.25	0.31	< 0.0001	0.17	
	TNC	0.29	0.75	< 0.0001	0.22	

temperatures fell below 10 °C in the late summer. The total period when starch was present in the needles varied from 137 days in 1996 to 164 days in 1998 (Figure 5). The onset of starch accumulation in needles coincided with increasing bud water content (Figure 5). Peak starch concentration occurred in May and was highest for 1-year-old needles, averaging 9–13% of dry mass (Figure 2). Peak starch concentrations in 2-year-old and current-year needles were about 7–8% and < 4%, respectively. In 1997 when 20 to 30% of 1-year-old needles were shed, only traces of starch were observed in current-year needles. Needle starch concentration started to decrease after bud-break and the beginning of shoot elongation.

In 1997 and 1998, the pattern of starch accumulation in needles was unimodal. In contrast, in 1996, a depletion of starch reserves associated with intensive growth occurred in June, followed by an unexpected peak in mid-July (Figure 2). The increase in starch concentration in needles in July coincided with a rainy and cold period. Mean daily temperature during that time was about 15 °C and minimal temperature varied between 5 and 10 °C.

To determine whether populations differed in the timing of the onset of starch accumulation in needles, data for the early spring period from February to April were analyzed separately for each needle cohort and sampling year. In all combinations,

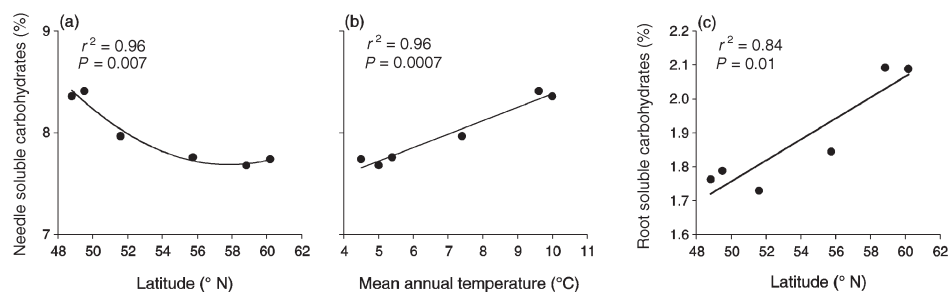


Figure 3. Mean soluble carbohydrate concentrations in 1-year-old needles of Scots pine populations growing in a common garden in Kórnik, Poland (52° N), in relation to latitude (a) and mean annual temperature of origin (b). Mean soluble carbohydrate concentration of fine roots (< 0.3 mm) for the November–January period in relation to latitude of origin is shown in (c).

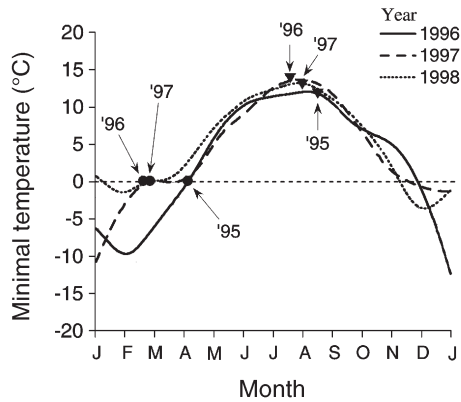


Figure 4. Seasonal trends in minimum air temperature at the Kórnik site. Symbols: ● = the time of year when spring accumulation of starch in needles was noted; ▲ = the time of year when starch disappeared. Abbreviations: '95, '96 and '97 indicate needle cohorts originating in 1995, 1996 and 1997, respectively.

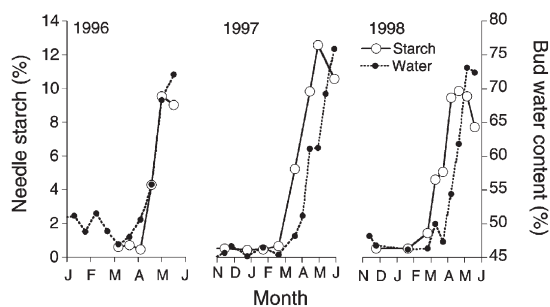


Figure 5. Mean starch concentration of 1-year-old needles and mean bud water content in 1996, 1997 and 1998 in Scots pine populations growing in a common garden in Kórnik, Poland (52° N).

the sampling date \times population interaction was not statistically significant ($P \geq 0.14$), indicating that the populations did not differ with respect to the onset of starch accumulation.

Figure 6 shows a dendrogram summarizing cluster analyses of populations based on the time course of starch concentration in needles. Two distinct groups were identified; one group comprised northern populations from Sweden and Russia (59–60° N), and the other group comprised central European populations from Latvia, Poland, Germany and France (49–56° N). There was a significant sampling date \times group interaction ($P = 0.001$), indicating differences between the two groups in seasonal patterns of starch accumulation in needles. The seasonal pattern of starch concentration for different years and needle cohorts is shown in Figure 6. To illustrate the temperature component, seasonal changes in needle starch concentrations were plotted against degree-days. In general, starch in needles of northern populations started to decline and reached low values earlier than in needles of central populations.

Carbohydrates in twig cohorts

Changes in twig (wood + bark) starch paralleled those observed for needles, with the beginning of starch accumulation, peak concentration and disappearance of starch occurring at the same times (Figure 7). The concentrations of starch in needles and twigs were remarkably similar at all times (Figure 8).

Soluble carbohydrates in twigs did not increase with time as in needles and the overall concentration of starch in wood + bark was typically 50% lower than in needles. There was no significant population effect on carbohydrate concentration in twigs (Table 2). The only interaction between population and sampling time for soluble carbohydrates (or TNC) concentration was in current-year twigs. This was mainly a result of differences in the time of spring peaks in soluble carbohydrates (data not shown).

Similar to the soluble carbohydrate pattern in needles, the soluble carbohydrate concentration in twigs was higher during winter compared with the other seasons (Figure 7). Because the seasonal variation in soluble carbohydrates was small, temporal patterns in TNC concentration followed those of starch concentration (data not shown).

Root carbohydrates

In roots, soluble carbohydrate concentrations changed little with season, from 1 to 3% of dry mass (data not shown); however, the changes were statistically significant (Table 2). In both years, the lowest concentration of soluble carbohydrates was observed in August and the highest in early spring and late autumn. In late autumn and early winter, soluble carbohydrate concentration increased linearly with increases in latitude of seed origin (Figure 3).

Starch concentration varied over time from 0.5 to 4% of fine root dry mass. In general, the highest values were observed in spring and autumn–winter (Figure 9). The lowest starch concentrations in roots were in August–September. In contrast to aboveground organs, starch was present in roots during the autumn and winter periods. In 1996, an increase in root starch concentration immediately followed the disappearance of starch in needles and woody stems (Figure 9). In the succeeding year, the late-season increase in root starch was not observed until December 1997–January 1998.

There were no population \times sampling time interactions on root carbohydrate concentration (Table 2). However, there was a significant interaction between population clusters (similar to Figure 6) and sampling time on root starch concentration that was mainly a result of higher concentrations of starch in spring and autumn in northern (15-Sweden, 3-Russia) populations than in central European populations (data not shown).

Discussion

Patterns of needle and stem nonstructural carbohydrates

Little is known about genetic variation in seasonal carbohydrate concentration patterns. Scots pine populations grown in

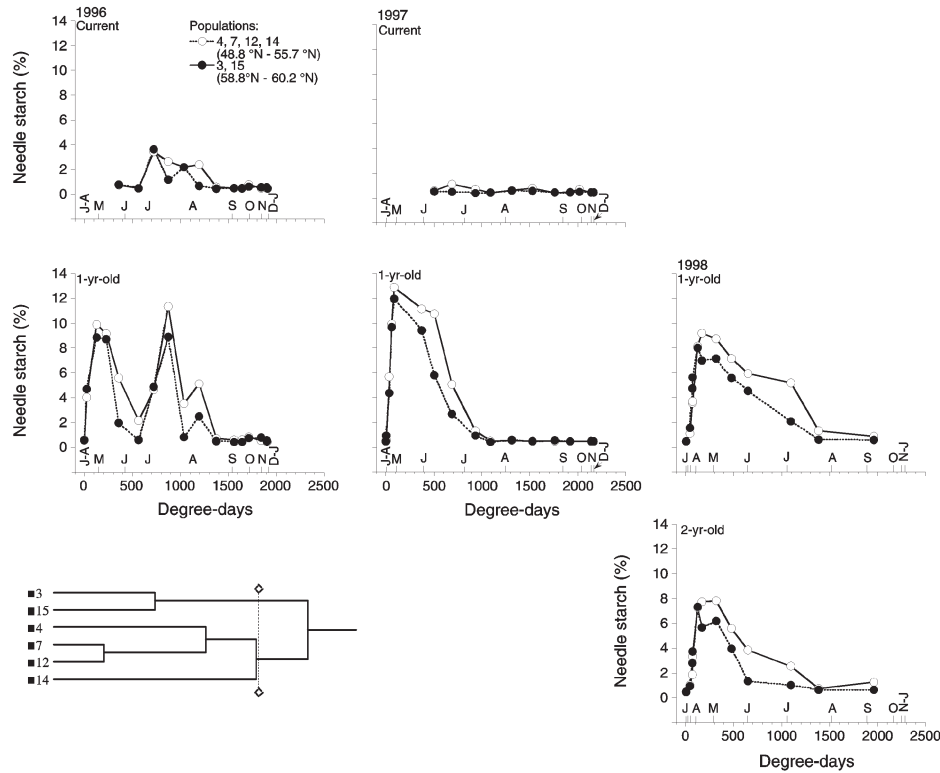


Figure 6. Seasonal pattern of needle starch for two cluster groups of Scots pine populations growing in a common garden in Kórnik, Poland (52°N) plotted against degree-days (sum of temperatures above 5 °C). A dendrogram of cluster groupings of provenances of Scots pine based on similarity of starch concentration is shown (left bottom corner). The concentrations of needle starch for different sampling dates and needle cohorts were used as clustering variables.

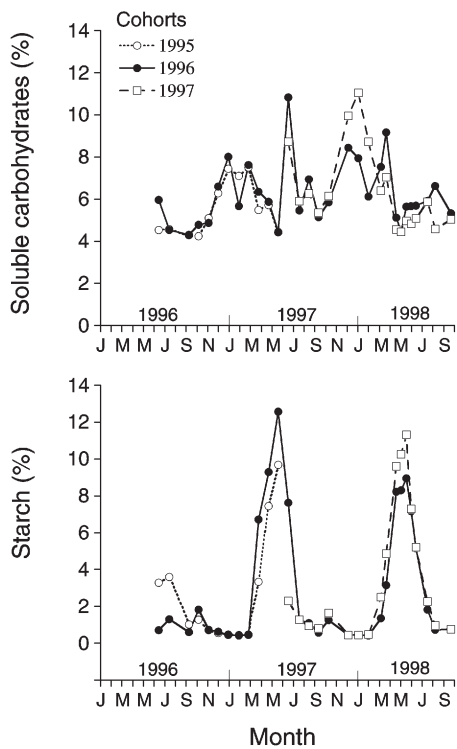


Figure 7. Seasonal patterns of twig (wood + bark) soluble carbohydrates and starch in Scots pine populations growing in a common garden in Kórnik, Poland (52° N). Each value is the mean for two populations (Nos. 3 and 7, see Table 1).

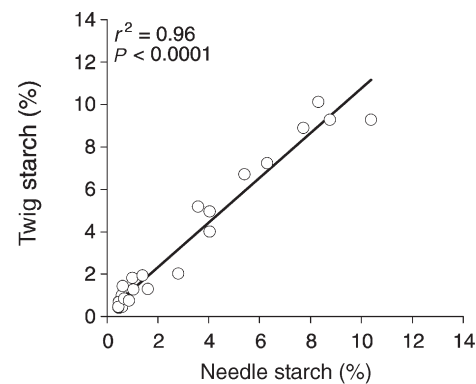


Figure 8. Mean starch concentration in 1-year-old needles of Scots pine populations growing in a common garden in Kórnik, Poland (52° N), in relation to 1-year-old twig (wood + bark) starch concentration. Each value is the mean of two populations on a given date.

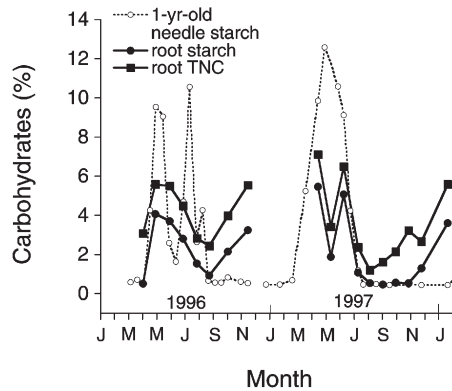


Figure 9. Mean seasonal pattern of concentrations of starch and total nonstructural (TNC) carbohydrates in fine roots (< 0.3 mm diameter) of Scots pine populations growing in a common garden in Kórnik, Poland (52° N). Each value is the mean of all populations. The difference between TNC and starch represents the soluble carbohydrate concentration. The dashed line represents the seasonal patterns of mean starch concentration in needles pooled for all populations. The needle starch values are taken from Figure 3.

shoot growth duration of the same populations (Oleksyn et al. 1992, 1998), indicating that differences in growth phenology are probably responsible for the observed starch patterns. Seasonal changes in starch accumulation in needles, with peaks observed before the onset of shoot growth, are commonly observed for Scots pine (Ericsson 1979, Ericsson and Persson 1980), and other coniferous tree species, such as *Pinus resinosa* Ait. (Pomeroy and Siminovitch 1970), *P. taeda* L. (Adams et al. 1986), *P. elliotii* Engelm. (Gholz and Cropper 1991), *Picea abies* (L.) Karst. (Linder 1995, Stockfors and Linder 1998), *Pseudotsuga menziesii* (Webb and Kilpatrick 1993) or *Abies balsamea* (L.) Mill. (Little 1970a, 1970b).

The carbohydrate pool that accumulated in needles in early spring started to decline shortly after the onset of shoot growth. In coniferous trees, the carbohydrates and recently formed photosynthates in previous-year needles support spring shoot growth until the current-year needles develop (Ursino et al. 1968, Hansen and Beck 1994, Lippu 1998). Because several strong sinks (roots, stem, developing shoots, flowers) require photosynthates in spring, Hansen and Beck (1994) hypothesized that partial immobilization of photosynthates as starch in needles and stems of Scots pine prevents assimilates from being translocated to other sinks.

Maximum needle carbohydrate concentrations were observed in 1-year-old needles in April or May and comprised up to 13% of needle dry mass for starch and 25% for TNC. At the end of summer, needle starch reserves were entirely depleted and the TNC concentration was only about 6% of needle dry mass (Figure 2). Studies of other coniferous species indicate that needle starch concentration can reach up to 30% of needle dry mass (Linder 1995). This temporal variation in carbohydrate concentration significantly changes foliage dry mass which, in turn, can be a source of variation in mass-based calculations of physiological processes such as CO₂ exchange,

specific leaf area and nutrient concentrations (Stockfors and Linder 1998, Oleksyn et al. 2000). However, the predictable nature of the seasonal variation in starch makes it possible to select the sampling period during which mass-based measurements will be less affected.

Our values of starch concentration are generally lower than those reported for Scots pine from more northern sites in Sweden (Ericsson 1979, Ericsson and Persson 1980). In contrast to these earlier studies, we did not find starch in needles during the period from late summer to early spring (Figure 2). Similarly, Martin and Öquist (1979) reported that starch was absent from chloroplasts of Scots pine during winter. A possible explanation for this discrepancy is that differences in starch accumulation could result from the lower number of needle cohorts observed in trees grown in southern locations compared with northern locations (Reich et al. 1996b). Also, differences in starch extraction protocol can result in the presence of structural carbohydrates in the extract (see Rose et al. 1991).

Mean carbohydrate concentrations over the life span of the needles showed a strong geographic pattern, with trees originating from warmer locations (low latitude) having up to 25% higher starch and up to 10% higher soluble carbohydrate concentrations than trees from colder locations (Figure 3). We found that the seasonal or lifetime mean needle carbohydrate concentrations were significantly correlated with previously reported growth parameters of the populations (data not shown). Storage of nonstructural carbohydrates in tree tissues over the year, or the tissue life span, reflects the relative balance between inputs through net assimilation and allocation to growth and respiration (Gholz and Cropper 1991). We conclude, therefore, that the lower seasonal nonstructural carbohydrate concentrations in needles of northern populations, coupled with a lower sink strength, reflects their overall lower productivity at this site compared with central populations. Both the shorter aboveground growing season (Oleksyn et al. 1998) and lower nonstructural carbohydrate accumulation rate contribute to the lower accumulation of carbohydrate reserves in needles of northern populations than in needles of central populations (Figures 3 and 6). Because northern populations of Scots pine are adapted to a short growing season, in common garden conditions they are usually outperformed in growth and productivity by populations from more southern locations (Giertych and Oleksyn 1981, 1992, Shutyaev and Giertych 1997). We suggest that the lower carbohydrate concentrations (Figure 3) of northern populations, along with a shorter growing season (Oleksyn et al. 1998, 1999a), higher respiration rates (Reich et al. 1996a), and higher biomass allocation to roots (Oleksyn et al. 1992, 1999a) are partly responsible for the lower aboveground productivity of populations originating from cold locations compared with that of populations originating from warmer locations.

General divisions of Scots pine populations based on needle starch patterns (Figure 6) are in good agreement with the cluster groupings of the same populations based on similarities of first-year growth and phenological responses (Oleksyn et al.

1992). Growth and physiological differences between the studied northern and central European populations are persistent and have been observed in all countries where this experiment was established (Gracan and Peric 1996, Stephan and Liesenbach 1996, Oleksyn et al. 1999a).

Root nonstructural carbohydrates and the needle defoliation effect

Needles and shoots had almost identical patterns of seasonal starch and similar patterns of soluble carbohydrates. In contrast, changes in starch concentrations in roots paralleled those of aboveground organs only until complete depletion of starch in needles and woody shoots. Subsequently, a substantial increase in root nonstructural carbohydrates was observed (Figure 9). The bimodal pattern of root carbohydrate concentration, with spring and autumn peaks, corresponded to periods of active root growth in Scots pine (Lyr and Hoffmann 1967) and paralleled a similar pattern of assimilate import (Smith and Paul 1988, Hansen and Beck 1994). Because root growth continues as long as soil temperature is above freezing, roots are the only major sink during the cold season. For example, Hansen et al. (1996) found that 7 weeks after a December pulse labeling of Scots pine trees with $^{14}\text{CO}_2$, 25% of the radiocarbon was recovered from the roots. Before bud break, the sink activity of roots was even greater, with more than 30% of assimilates being translocated to roots.

The loss of 20 to 30% of 1-year-old needles in mid-1997 had a profound effect on the carbohydrate patterns in needles and roots. The most striking effect was a total lack of starch in current-year needles in that year and in fine roots from June to December (Figure 9). A similar response to defoliation has been reported by Ericsson et al. (1980) who found that the buildup of starch reserves in needles of artificially defoliated 18-year-old Scots pine trees was less than normal and the reserves were more rapidly depleted, especially in trees defoliated in late season. However, our data do not support the idea (Ericsson et al. 1980) that current-year needles possess a mechanism that limits the export of a carbohydrate surplus (Figure 2).

The significant reduction in starch reserves in roots following partial defoliation may reflect a shortage of carbohydrates on a whole-tree basis. A decrease in starch concentration in roots as a result of defoliation was also found in deciduous and coniferous trees (Wargo et al. 1972, Parker and Patton 1975, Webb and Karchesy 1977). It is possible that low carbohydrate reserves in roots in autumn have an adverse effect on root growth the following spring, when the carbon demand by the growing shoots prevents its allocation to roots. A reduction in root carbohydrate concentration also has a dampening effect on soil microbial activity (B. Kieliszewska-Rokicka, Institute of Dendrology, Poland; unpublished observations).

The observed increase in foliar and root concentration of soluble carbohydrates in autumn and winter (Figures 2 and 9) was associated with increasing frost hardening (Aronsson et al. 1976, Amundson et al. 1992). A significant increase in root soluble carbohydrates with increasing latitude of seed origin

may indicate that northern populations possess greater frost resistance than populations from more southern locations.

In summary, we observed distinct differences among diverse Scots pine populations in nonstructural carbohydrate concentration and its seasonal changes. These patterns indicate that carbohydrate dynamics in Scots pine depend on inherited properties as well as growth phenology. The expression of this pattern depends on source–sink relationships that are altered by internal and environmental conditions.

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