

# Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees

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**Summary** We tested the hypothesis that broad-leaved forest species with contrasting wood anatomy and hydraulic system (ring-porous versus diffuse-porous) also differ in distribution and seasonal dynamics of carbohydrate reserves in stem wood. Total nonstructural carbohydrate (TNC) reserves (starch and sugars) were measured enzymatically in the 10 youngest stem xylem rings of adult oak (*Quercus petraea* (Matt.) Liebl.) and beech (*Fagus sylvatica* L.) trees during an annual cycle. Radial distribution of carbohydrates was investigated according to ring age. On all dates, oak trees had twofold higher TNC concentration than beech trees (41 versus 23 mg g<sub>DM</sub><sup>-1</sup>), with starch accounting for the high TNC concentration in oak. Seasonal dynamics of TNC concentration were significantly ( $P < 0.05$ ) more pronounced in oak (20–64 mg TNC g<sub>DM</sub><sup>-1</sup>) than in beech (17–34 mg TNC g<sub>DM</sub><sup>-1</sup>). A marked decrease in TNC concentration was observed in oak trees during bud burst and early wood growth, whereas seasonal fluctuations in TNC concentrations in beech trees were small. The radial distribution of TNC based on ring age differed between species: TNC was restricted to the sapwood rings in oak, whereas in beech, it was distributed throughout the wood from the outermost sapwood ring to the pith. Although the high TNC concentrations in the outermost rings accounted for most of the observed seasonal pattern, all of the 10 youngest xylem rings analyzed participated in the seasonal dynamics of TNC in beech trees. The innermost sapwood rings of oak trees had low TNC concentrations. Stem growth and accumulation of carbon reserves occurred concomitantly during the first part of the season, when there was no soil water deficit. When soil water content was depleted, stem growth ceased in both species, whereas TNC accumulation was negligibly affected and continued until leaf fall. The contrasting dynamics and distribution of carbohydrate reserves in oak and beech are discussed with reference to differences in phenology, early spring growth and hydraulic properties between ring-porous trees and diffuse-porous trees.

**Keywords:** annual growth, interspecific comparison, starch, stem wood, sugar.

## Introduction

Carbohydrate reserves are mobilized in trees to fuel maintenance respiration during winter (Ögren 2000), build leaves and support new growth in spring and to provide the energy expended in making adaptive responses to soil water deficits, pathogen attacks and herbivory (Wargo 1981, Oren et al. 1988, Dunn et al. 1990, Canham et al. 1999). The proportion of photosynthate allocated to different uses, including stored compounds, changes over the growing season and sometimes with the environment (Kramer and Kozlowski 1979, Terziev et al. 1997). Seasonal variations in carbohydrate reserves have been investigated in young trees (Wargo 1979, Bonicel et al. 1987, Sauter and van Cleeve 1994, Höll 1997, Sauter and Wellenkamp 1998) and a variety of fruit trees (Priestley 1960, 1977). However, few studies have focused on the seasonal dynamics of carbohydrate reserves in mature broad-leaved trees growing under field conditions.

Sessile oak (*Quercus petraea* (Matt.) Liebl.) and common beech (*Fagus sylvatica* L.) are sympatric deciduous European tree species that differ in wood anatomy (Gasson 1987). In oak, a ring-porous species, trees achieve a large part of annual radial stem growth (including the formation of early wood with large vessels) before leaf expansion in spring (Dougherty et al. 1979, Hinckley and Lassoie 1981, Bréda and Granier 1996). This type of phenology, involving stem growth before leaf unfolding, may be associated with winter embolism of large xylem vessels, necessitating that the water flow pathway be restored each spring before the onset of transpiration (Essiamah and Eschrich 1986, Sperry et al. 1994, Bréda and Granier 1996). In beech, a diffuse-porous species with small vessels, winter embolism has less impact on total tree hydraulic conductivity and leaf expansion starts before the beginning of stem growth (Essiamah and Eschrich 1986).

Another wood characteristic that differs between ring-porous and diffuse-porous species is the radial distribution of living tissues in which reserve compounds are located (Saranpää and Höll 1989, Höll 1997). The ring-age-related distribution of carbohydrates reserves may differ between diffuse-porous and ring-porous trees, because ring-porous species exhibit a

clear delineation between sapwood and heartwood whereas heartwood is negligible or absent in diffuse-porous species and sap flows through the entire xylem.

We determined if seasonal use of carbohydrate reserves in stem wood differed between mature diffuse-porous trees (common beech) and ring-porous trees (sessile oak) growing in pure stands. We quantified effects of species on both the seasonal dynamics of starch and sugar concentrations and the radial distribution of starch and sugar concentrations to evaluate if differences in seasonal dynamics of carbohydrate reserves could be unequivocally identified as species diagnostic characteristics. Specifically, we tested the hypothesis that oak trees rely entirely on the remobilization of reserves stored during the previous growing season to support early stem radial growth (Lacointe et al. 1993), whereas reserve remobilization is of minor importance for early stem radial growth in beech. We also postulated that the radial distribution of nonstructural carbohydrates differs between oak and beech as a consequence of the different distribution of living wood in the two species.

## Materials and methods

### *Site and stand descriptions*

The study was carried out in an almost pure stand of sessile oak in the state forest of Champenoux, France (48°44' N, 6°14' E, elevation 237 m) and in an almost pure stand of common beech in the state forest of Hesse, France (48°40' N, 7°05' E, elevation 300 m). We chose to study two separate stands, even though they were 60 km apart, to avoid possible effects of interspecific competition. Mean annual temperature is 9.2 °C in both oak and beech stands, whereas mean annual precipitation is 744 and 820 mm, respectively. In 1998, the oak trees were 45 years old and the beech trees were 35–40 years old. The small age difference between stands was insignificant because both stands were grown from natural regeneration lasting several years and both were young high forests. The oak stand was growing on a luvisol, developed on a deep loam (for details see Bréda et al. 1995). Stand density was 2553 stems ha<sup>-1</sup>, basal area was 26.7 m<sup>2</sup> ha<sup>-1</sup> and dominant height was 17 m in 1998. The beech soil type was intermediate between a luvisol and a stagnic luvisol (for details see Granier et al. 2000b). Stand density was 3800 stems ha<sup>-1</sup>, basal area was 19.6 m<sup>2</sup> ha<sup>-1</sup>, and dominant height was 13 m in 1998. Maximal stand leaf area index (LAI), estimated by litter collection during autumn 1998, was 5.6 in the oak stand and 7.4 in the beech stand. Dates of maximum LAI and beginning of leaf fall were assessed from measurements of global radiation interception made at each plot with pyranometers (Delta-T devices, Cambridge, U.K.). Bud burst was recorded from the beginning of April to the end of May on 10 dominant trees from each stand every second day. Bud development was described according to a six-stage scale (dormant winter buds, swollen buds, broken buds, just-unfolded leaves, unfolded leaves, developed leaves with shoot elongation). Bud burst was achieved when the tree reached the unfolded leaf stage (Bréda and Granier

1996), which corresponded to the onset of both leaf expansion and increases in LAI.

### *Measurement of soil water content*

We measured soil water content with a neutron probe (Nordisk Elektrisk Apparatfabrik, Denmark). Six 1.60-m and two 2.60-m access tubes were installed in each stand. Counts were logged every 10 cm for the upper 100 cm of soil and every 20 cm below 100-cm depth. A polyethylene shield was used for the measurements made at a depth of 10 cm. Measurements were made weekly during the growing season. Soil water content for each soil layer was calculated as the product of volumetric soil water content and the thickness of the soil layer and then cumulated over the total profile for each measurement date. Extractable water in the root zone (EW; mm), i.e., the difference between soil water content at field capacity ( $W_F$ ) and minimum soil water content (i.e., lower limit of water availability,  $W_m$ ) was 180 mm in the uppermost 160 cm of soil in the beech stand (Granier et al. 2000a) and 165 mm in the uppermost 200 cm of soil in the oak stand (Bréda et al. 1995). With  $W$  denoting soil water content at a given date, we calculated the relative extractable water (REW) of the total soil depth as the ratio  $(W - W_m)/(W_F - W_m)$ , i.e.,  $(W - W_m)/EW$ .

### *Seasonal dynamics of carbohydrate reserves in tree rings (Experiment 1)*

In 1998, seasonal dynamics of carbohydrate reserves were assessed on three oaks and three beeches of dominant or codominant status. The seasonal circumference increment at breast height was measured with dendrometer bands (Table 1). One stem core (5-mm diameter and 10 cm long) was sampled at breast height every month from March to December from two dominant trees per species. The third oak and beech were codominant trees (see Table 1) and were sampled every second month. Samples were taken at midday to minimize variation in response to diurnal fluctuations in carbohydrate pools. The first sample was taken in the north direction and the following monthly cores were shifted a few centimeters to the left of the previous one. A total of 10 monthly samplings were thus distributed along the stem circumference at breast height. A preliminary experiment on variations in carbohydrate concentration within a tree was performed on other dominant trees to quantify azimuthal variations (for four cardinal orientations) and height variations (from base of the stem to 2.2 m). The concentrations of nonstructural carbohydrates in the stem varied slightly with stem height (0, 1.3 and 2.2 m) and for the four orientations (coefficient of variation 10%), whatever the date (August or December) and species (data not shown). Cores were stored at -20 °C until freeze-dried. After freeze-drying, ring widths were measured on each core with the aid of a binocular microscope. To quantify the dynamics of carbohydrate reserves from current to older rings, each core was cut, weighed and ground (Mixer Mill MM200, Retsch, Germany). We analyzed each ring separately along the visually identified sapwood for oaks and the last ten rings for beeches. The selected oaks exhibited different numbers of sapwood rings: Oak 302 had eight rings with a sapwood width of 31.1 mm;

Table 1. Characteristics of three 45-year-old sessile oak trees and three 35-year-old beech trees.

	Oak			Beech		
	Tree 302	Tree 139	Tree 118	Tree 1	Tree 2	Tree 3
Social position	Dominant	Dominant	Co-dominant	Dominant	Dominant	Co-dominant
Diameter at 1.30 m (mm)	216	206	190	149	156	150
Sapwood width at 1.30 m (mm)	31.1	24.2	26.4			
Number of sapwood rings	8	10	10			
Width of the 10 youngest rings (mm) at 1.30 m				45.4	38.9	41.3
Height (m)	19	18.25	18	16	16.5	15.25
Radial annual growth (mm)	2.7	1.6	1.8	3.82	3.09	3.25

Oaks 139 and 118 had 10 rings with a sapwood width of 24.2 and 26.4 mm, respectively. Because no visible sapwood–heartwood boundary was detected in the beech trees, we analyzed 10 rings, i.e., a width ranging from 38.9 to 45.4 mm, depending on the tree. Bark was removed from the samples and the analysis was restricted to the wood.

#### Variation in carbohydrate concentrations among trees (Experiment 2)

Variation in carbohydrate reserves in stem wood within each stand was assessed among ten dominant or codominant oaks and ten beeches. Each tree was cored (5-mm diameter cores sampled at breast height) on three sampling dates in 1999 (April 27, June 1 and October 14, Day of Year 117, 152 and 287, respectively) to quantify tree, date and species contributions to the variations in carbohydrate concentrations. The three sampling dates were selected on the basis of phenological development: April was before bud break, June was when LAI was maximum and October was when leaf fall occurred. Carbohydrate concentrations were determined based on the whole visible sapwood for oaks and the last 10 rings for beeches.

#### Carbohydrate analysis

Soluble sugars were extracted twice from 5–10 mg of powdered core with 80% boiling ethanol (1 ml) for 30 min. The extracts were centrifuged at 13,000 g for 10 min; combined and dried overnight with a vacuum-evaporator to eliminate ethanol. The dried extracts were rehydrated in 0.5 ml of 0.32 M citrate buffer, pH 4.2, and soluble sugars (glucose, fructose and saccharose) were determined colorimetrically at 340 nm (Boehringer 1984). Starch was extracted from the dried extracts by boiling in 0.02 N NaOH solution for 1 h. Starch was hydrolyzed to glucose with  $\alpha$ -amylglucosidase (EC 3.2.1.3, Boehringer Mannheim Biochemicals, Mannheim, Germany) in 0.32 M citrate buffer, pH 4.2 at 48 °C for 30 min. Glucose was assessed as described above (Boehringer 1984). Starch was quantified as glucose equivalents. Total nonstructural carbohydrates (TNC) were calculated as the sum of total soluble sugars and starch.

#### Statistics

Data were analyzed by ANOVA (SAS software package, Ver-

sion 6.12). Statistically significant differences were tested with the Student-Newman-Keuls multiple range test at  $P = 0.05$ . For statistical analysis of the seasonal dynamics of starch and sugars, the following hierarchical model was used:

$$[C]_{ij(i)k} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + \delta_{ik} + E_{ijk}, \quad (1)$$

where  $[C]_{ij(i)k}$  is the measured concentration of TNC, starch or sugars for tree  $j$  within species  $i$ , at date  $k$ ,  $\mu$  is a constant,  $\alpha_i$  is the effect of species  $i$  in its site,  $\beta_{j(i)}$  is the effect of tree  $j$  within species  $i$ ,  $\gamma_k$  is the effect of date  $k$ ,  $\delta_{ik}$  is the effect of interaction between species  $i$  and date  $k$ , and  $E_{ijk}$  is an error term.

The species effect was tested separately on residuals from the general model, leading to a one-factor analysis of variance (i.e., species in its site). For the inter-tree variability of starch and sugars, the model used depended on species, date and tree effects. For the TNC distribution between rings at two dates, the following hierarchical model, including several double or triple interactions, was used:

$$[C]_{ij(i)kl} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + \delta_l + \lambda_{ik} + \rho_{il} + \sigma_{kl} + \omega_{ikl} + E_{ijkl}, \quad (2)$$

where  $[C]_{ij(i)kl}$  is the measured concentration of TNC for tree  $j$  within species  $i$  in its site, at date  $k$  for ring formation year  $l$ ,  $\mu = \text{constant}$ ,  $\alpha_i$  is the effect of species  $i$ ,  $\beta_{j(i)}$  is the effect of tree  $j$  within species  $i$ ,  $\gamma_k$  is the effect of date  $k$ ,  $\delta_l$  is the effect of the ring formation year  $l$ ,  $\lambda_{ik}$  is the effect of interaction between species  $i$  and date  $k$ ,  $\rho_{il}$  is the effect of interaction between species  $i$  and ring formation year  $l$ ,  $\sigma_{kl}$  is the effect of interaction between date  $k$  and ring formation year  $l$ ,  $\omega_{ikl}$  is the effect of interaction between species  $i$ , date  $k$  and ring formation year  $l$ , and  $E_{ijkl}$  is an error term.

## Results

#### Sources of variation in concentrations of TNC, starch and sugar (Experiment 1)

Species and trees within species had significant effects on TNC concentration (Table 2, Model 1). Interspecific variation represented 54% of the total variance, whereas 10% was attributed to intraspecific variability. Because the species were

Table 2. Analysis of variance of the effects of species in its site, tree within species, date and the interaction between date and species on seasonal dynamics of TNC, starch and sugar concentrations (Model 1). The number of observations in the data set is 50. Abbreviation: SS = sum of squares.

Source of variation	Degrees of freedom	TNC		Starch		Sugars	
		SS	<i>P</i>	SS	<i>P</i>	SS	<i>P</i>
Species (+ site)	1	38.4	0.0001	32.6	0.0001	0.2	0.0046
Tree (species)	4	7.0	0.0260	7.1	0.0048	0.2	0.1358
Date	9	9.3	0.0893	8.1	0.0348	9.5	0.0001
Species × date	9	2.5	0.8483	5.3	0.1644	1.5	0.0001
Error	26	13.8		9.5		0.6	
Model	23	57.1	0.0001	53.1	0.0001	11.4	0.0001

selected in different forests, the variance attributed to the species may also include a site influence. Date had a significant effect ( $P < 0.1$ , 13% of total variation), but the interaction between date and species was not significant. Model 1 explained 81% of total TNC variance. For starch, Model 1 explained 85% of total variance; 52% was due to species effect, whereas date and intraspecific variability accounted for 13 and 11%, respectively. For sugars, Model 1 explained 95% of total sugar variance where the main effect was attributed to date (78% of total variation), but species and the interaction between date and species were also significant. A one-factor analysis of variance of TNC, starch and sugar concentrations confirmed that the species effect was significant at  $P < 0.05$ . Oak and beech trees exhibited similar seasonal dynamics in starch and TNC, but the concentrations were higher in oak than in beech (41 versus 23 mg g<sub>DM</sub><sup>-1</sup> for TNC and 29 versus 13 mg g<sub>DM</sub><sup>-1</sup> for starch). In contrast, oaks and beeches differed in both seasonal dynamics and sugar concentration (12 versus 10 mg g<sub>DM</sub><sup>-1</sup> in oak and beech, respectively). Starch and TNC concentrations also differed among trees within a species.

#### *Leaf area index, stem growth and soil water content during 1998 (Experiment 1)*

In both species, bud swelling was observed from Day 105 (April 15), bud burst occurred on Day 121 (May 1) and the leafy period occurred from Days 140 (mid May) to 300 (end of October, leaf fall). The growing season (based on stem girth increment) started on Day 115 (end of April) for oak, i.e., 10 days before bud burst (Figure 1A), but not until Day 130 (mid-May) for beech, i.e., a few days after bud burst (Figure 1B).

Soil water content exhibited a similar seasonal pattern in both stands: REW was at field capacity on Day 100 (April 10) and decreased progressively as LAI increased. In both stands, REW dropped below 0.4 (i.e., the soil water deficit threshold leading to stomatal closure, Granier et al. 1999) on Day 200 (mid-July) and the onset of the soil water deficit was paralleled by a reduction or cessation in stem increment growth. The period of soil water deficit lasted until leaf fall (Day 300 and Day 280 in the oak and beech stands, respectively).

#### *Seasonal dynamics of TNC, starch and sugar concentrations*

Starch and sugar concentrations averaged over the analyzed rings as well as the extent of seasonal variation were larger in

oaks than in beeches (Figure 2). On all dates, the high TNC concentrations in oaks were a result of high starch concentrations. Both species exhibited minimum starch and sugar concentrations on Day 159, corresponding to the time when maximum LAI was reached. For oaks, starch concentration decreased from Day 126 to Day 159 concomitantly with early wood growth and leaf expansion. In contrast, only limited starch depletion was observed in beeches in spring; however, a progressive decrease in the concentration of sugars coupled with an equivalent increase in starch concentration occurred from Day 70 to Day 159. After maximum LAI was reached, starch and sugars began to accumulate concomitantly with the continuation of stem growth. In both species, the accumulation of carbohydrate reserves continued during the period of soil water deficit, whereas stem increment growth slowed or stopped. For both species, maximum concentrations of TNC and starch concentration were found on Day 302 (end of October), i.e., after the start of leaf fall. In both species, a large increase in the concentration of sugars was observed during the winter (Days 355 and 399), whereas the opposite pattern occurred for starch. Conversion of starch to sugars was observed during the last two sampling dates after stem temperature beneath the bark dropped progressively from 0 to -8.9 °C from Day 320 to Day 330, indicating that this 10-day period below 0 °C was sufficient to induce starch hydrolysis.

All sapwood rings in oak underwent seasonal changes in TNC concentration, ranging from 10 to 85 mg g<sub>DM</sub><sup>-1</sup>, whereas the range of variation in TNC concentration was lower in beech (from 15 to 35 mg g<sub>DM</sub><sup>-1</sup>) (Figure 3). For the three beech trees, seasonal changes in TNC concentration were detected in the 10 rings analyzed, but the magnitude of the changes was higher for the three youngest rings than for the other rings. Oaks 139 and 118 exhibited a greater decrease in TNC concentration during spring than Oak 302. The latter tree also had a lower early wood/ring width ratio and later bud burst than Oaks 139 and 118. In the three oak trees, the youngest rings had a higher TNC concentration than the older rings and the range of seasonal changes in TNC concentration was smaller for older rings than for young rings, with a lower TNC increase during refilling. In contrast to Oaks 118 and 302, the sapwood TNC continued to increase in Oak 139 for a number of weeks after leaf fall, before beginning to decline (Figure 3).

Figure 4 shows the mean radial distribution of TNC among



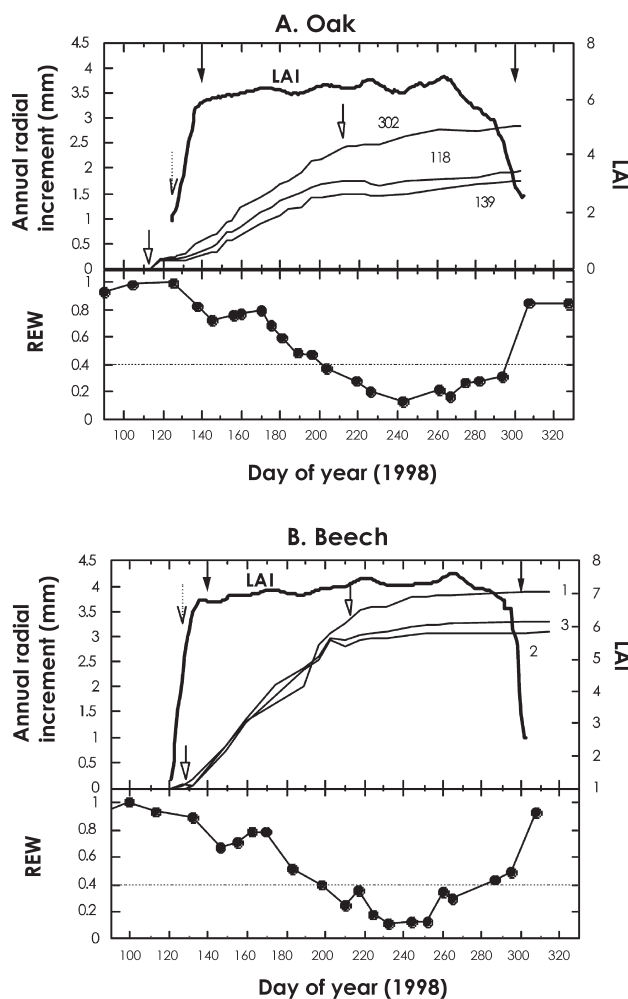


Figure 1. Seasonal dynamics of relative soil extractable water (REW) and phenological stand development including leaf area index (LAI) and stem circumference growth of three oaks (A) and three beeches (B) studied in 1998. The dotted arrows correspond to time of bud break. Black arrows delimit the leafy period from maximum LAI to leaf fall. White arrows delimit the stem growth period. The dotted line for REW = 0.40 corresponds to the threshold for soil water deficit leading to reductions on leaf and canopy gas exchange as a result of stomatal closure (Granier et al. 1999).

rings of different ages between Day 159 (maximum TNC) and Day 302 (minimum TNC) and in April (Day 113, before bud break) for each species. Differences in TNC concentrations between June and October were significant in all oak rings from 1993 to 1998 ( $P > 0.0001$ ) and for the 10 beech rings except the 1997 ring. Storage of TNC was highest in the youngest oak rings and low or no TNC was detected in the 1990–1991 oak rings, first heartwood rings or in the heartwood–sapwood transition zone. In contrast, in beech, the distribution of total TNC was less dependent on ring age. All 10 beech rings analyzed contributed to TNC storage and the minimum TNC concentration in June was similar among the oldest rings. Mobilization of TNC for bud break and early wood formation (from April to June) differed between species: oak mo-

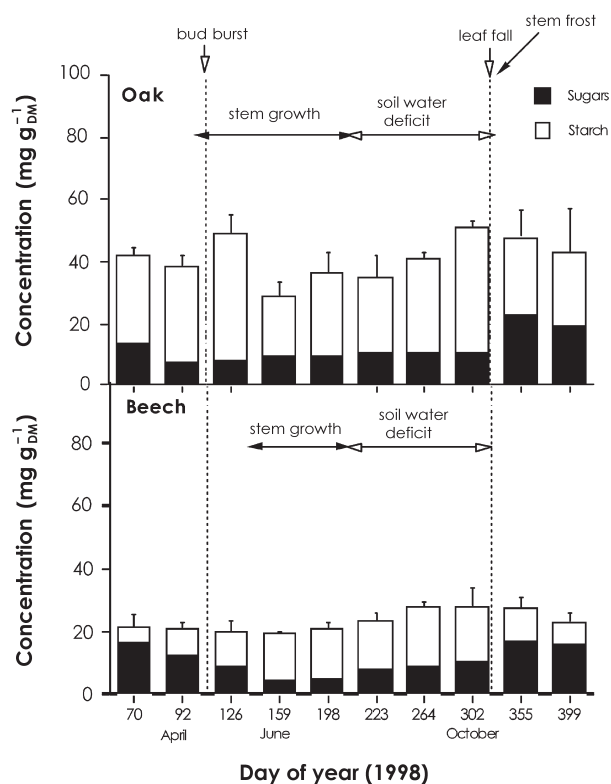


Figure 2. Seasonal variations in weighted mean of starch (in white) and sugar (in black) concentrations ( $\text{mg g}_{\text{DM}}^{-1}$ ) for all sapwood rings averaged for three oak trees and for the 10 last rings for three beech trees. Vertical bars indicate the standard errors for TNC concentration. The vertical dotted lines indicate times of bud burst and leaf fall. Soil water deficits lasted from the end of growth to leaf fall. Stem frost indicates a 10-day period when temperature beneath the bark was less than  $0^\circ\text{C}$ .

bilized more TNC than beech in all of its sapwood rings. In beech, mobilization was restricted to the oldest rings. Analysis of variance showed that species in its site, tree in species, date, ring and interactions had significant effects on TNC, starch and sugar concentrations (Table 3). Model 2 explained 91% of TNC variance, 89% of starch variance and 95% of sugar variance. The distribution of carbohydrate reserves among rings of different ages explained from 33 to 47% of total variation, depending on the reserve component. Model 2 also confirmed that the difference between minimum and maximum concentrations of carbohydrate reserves was significant for both species, and that concentrations of carbohydrate reserves differed significantly between species (Figure 4).

#### Carbohydrate variability between trees during the season (Experiment 2)

In 1999, the seasonal dynamics observed for the three trees of each species initially studied was confirmed in a larger sample of trees (Figure 5). The soil water shortage lasted from Day 200 to Day 270 in 1999, but soil water content was less depleted than in the previous year (data not shown). Variability in carbohydrate concentration among the 10 trees of each spe-

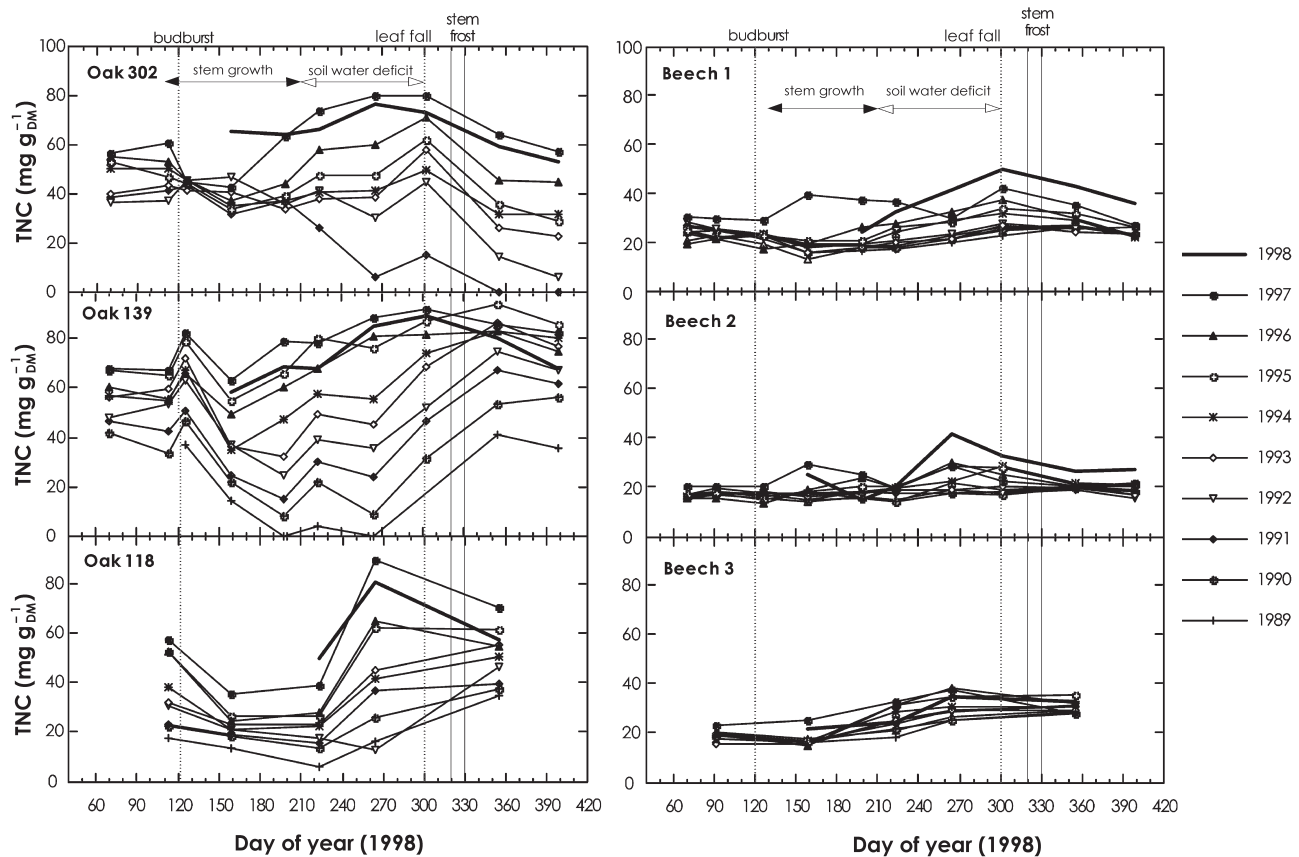


Figure 3. Seasonal variations in total nonstructural carbohydrate concentrations (TNC;  $\text{mg g}_{\text{DM}}^{-1}$ ) in each sapwood ring of three oak trees (Oak 302, 139, 118) and in each of the 10 last rings of three beech trees (Beech 1, 2, 3). The vertical dotted lines indicate times of bud burst and leaf fall; the vertical solid lines delimit the period of stem frost; the horizontal arrows indicate the period of stem growth. Soil water deficits lasted from the end of growth to leaf fall.

cies was low, irrespective of date and species (coefficient of variation of 15%, Figure 5). Carbohydrate concentrations were higher in oak than in beech on all sampling dates in 1999. For oak, carbohydrate concentrations differed significantly between the three measurement dates, with a sharp decrease in starch concentration in spring. In both species, there was a significant increase in TNC concentration in October as a result of an increase in sugars in beech and increases in both starch and sugars in oak. The general linear model indicated that 46% of the variability in carbohydrate concentration was due to species, 31.2% to date and 1% to the tree effect. The tree effect was not significant when each species was considered separately: date accounted for 75.6 and 76.2% of the variability in carbohydrate concentration for *Quercus* and *Fagus*, respectively, whereas the tree component was only 6.4 and 6.9%, respectively.

## Discussion

### *Mobilization of carbohydrate reserves for spring reactivation*

Seasonal variation in TNC of stem wood is characteristic of

deciduous trees from temperate latitudes, for which bud burst and increases in LAI are strong carbon sinks (Dubroca 1983, Ashworth et al. 1993, Sauter and van Cleve 1994, Gansert and Sprick 1998). We found significant differences between the minimum TNC concentration after leaf expansion in early June and the highest TNC concentration just before leaf fall in both species (Figures 4 and 5). Both starch concentration and the magnitude of seasonal TNC dynamics were higher in oak than in beech, confirming our hypothesis that contrasting phenology and anatomy of the species are accompanied by differences in the dynamics of stored carbohydrates. Spring mobilization of TNC in oaks was especially high. High spring mobilization of TNC has been reported in young walnut trees, a ring-porous species (Lacointe et al. 1993). Oak stem growth started 10 days before bud burst and 25 days before maximum LAI. This result confirms our previous observations (Bréda and Granier 1996) and is in agreement with the typical phenology of ring-porous species where about 30% of the total annual stem increment is added before bud burst (Hinckley and Lassoie 1981). Spring mobilization of TNC in oak may be related its hydraulic properties. In ring-porous species, all of the previous year's early wood vessels are embolized by frost events each winter; therefore, the production of new early

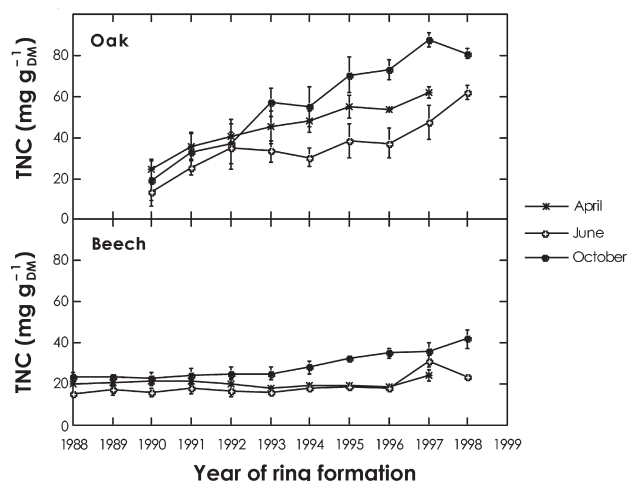


Figure 4. Mean total nonstructural carbohydrate (TNC) concentrations in the different sapwood rings of three oaks and the 10 last rings of the three beeches for three dates: before bud reactivation, (April 2, Day 92), at minimum (June 8, Day 159) and maximum (October 29, Day 302) observed TNC concentration, as indicated in Figure 2. Vertical bars indicate the standard errors.

wood large vessels before leaf expansion is necessary for the spring recovery of hydraulic conductivity (Bréda and Granier 1996, Hacke and Sauter 1996). In contrast, the small vessels in beeches are less sensitive to winter embolism, and so spring reactivation of growth is less dependent on stored carbon.

Beech showed limited changes in stem TNC concentration, supporting the results of Höll (1997) on several diffuse-porous tree species (*Betula verrucosa* J.F. Ehrh., *Tilia cordata* Mill. and *Acer pseudoplatanus* L.). We suggest that, because TNC mobilization from the stem was so limited, other tree compartments such as branches were probably also carbon sources. This suggestion is consistent with the report that cambium reactivation in diffuse-porous species proceeds from the upper part of the branches and occurs 3 weeks before cambium reac-

tivation in the stem (Lachaud and Bonnemain 1981). Another consequence of diffuse-porous anatomy is that only a few conduits are fully embolized during winter, even in the outermost growth rings in beech (Hacke and Sauter 1996). Thus, water transport from soil to crown is possible, even if not maximal, as soon as leaf area expands. Cochard et al. (2001) demonstrated that the formation of new functional vessels is largely responsible for the complete recovery of xylem conductivity in terminal branches. When we detected stem growth, all parts of the tree were already reactivated and xylem recovery was complete. We did not investigate the possibility that other reserve materials (e.g., storage proteins, glycerol forms, free fatty or amino acids) might be involved in supplying spring carbon sinks (Stepien et al. 1994). This possibility should be tested because diffuse-porous trees are regarded as “fat trees” (Höll 1997) and a decrease in stem wood TNC in spring is not clearly observed for diffuse-porous species.

#### Concentration of TNC and ring number

The contrasting anatomies of ring-porous and diffuse-porous species appeared to be associated with the specific radial distribution of TNC in stem wood. Higher TNC concentrations were found in oak than in beech on all measurement dates, but the carbohydrate reserves in oak were restricted to the sapwood, i.e., in the 8–10 most recent rings. Ring by ring analysis in oak showed that all sapwood rings contributed to the seasonal dynamics of xylem TNC, with a strong decrease in TNC concentration with ring age. The decrease in TNC with ring age has previously been observed in coniferous species (Fischer and Höll 1992, Terziev et al. 1997). Thus, in oak, the greater the sapwood area, the greater the sap flux and reserve carbohydrate storage. In contrast, TNC in beech was distributed in more than the 10 rings examined. Although most of the carbohydrate reserves were located in the outermost rings, TNC was also present in the innermost rings analyzed. This radial profile of TNC closely parallels the profiles of both wood water content and sap flux transport efficiency of beech trees from the same stand (Granier et al. 2000a). Wood water con-

Table 3. Analysis of variance of the effects of species in its site, tree within species, date, ring formation year and the several interactions on TNC, starch and sugar concentrations distribution among rings at maximum and minimum concentrations (Model 2). The number of observations in the data set is 128. Abbreviation: SS = sum of squares.

Source of variation	Degrees of freedom	TNC		Starch		Sugars	
		SS	P	SS	P	SS	P
Species (+ site)	1	93.7	0.0001	32.0	0.0001	5.0	0.0001
Tree (species)	4	23.9	0.0001	9.4	0.0008	0.3	0.0837
Date	1	64.3	0.0001	27.8	0.0001	1.0	0.0001
Ring <sup>1</sup>	10	176.2	0.0001	101.3	0.0001	28.7	0.0001
Species × date	1	4.0	0.0114	2.7	0.0149	2.1	0.0001
Species × ring	10	85.2	0.0001	42.2	0.0001	9.1	0.0001
Date × ring	10	23.4	0.0002	43.5	0.0001	11.1	0.0001
Species × date × ring	10	16.7	0.0047	17.6	0.0002	0.9	0.0188
Error	78	46.0		34.6		3.0	
Model	47	487.3	0.0001	276.5	0.0001	58.3	0.0001

<sup>1</sup> Ring = year of formation of the ring.

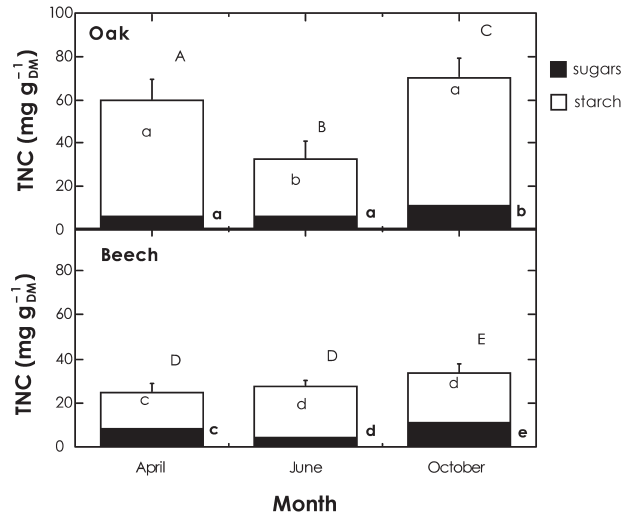


Figure 5. Variations in TNC (including starch and sugars) concentrations in the stem among 10 dominant trees from the oak and beech stands for three dates in 1999. Ten different trees were sampled at each date and the mean is presented. Vertical bars indicate the standard errors for TNC concentration. Capital letters indicate significant differences in TNC between sampling dates and between species for each sampling date ( $P < 0.05$ ,  $n = 10$ , Student  $t$ -test), lower-case light letters are for starch and lower-case bold letters are for sugars.

tent and sap flow densities are higher in the outermost 20 mm (which is equivalent to the width of 21.5 mm of the five outermost rings we studied), with a significant decrease in sap flux density in the innermost 20 mm (which is equivalent to the width of 20.3 mm of the five innermost rings studied) (Granier et al. 2000a). These data are consistent with the observation that beech sapwood could comprise 10 to 20 annual rings (Ladefoged 1960). We investigated 10 rings (equivalent to a width of 4.2 cm) and found that they were all involved in TNC dynamics. Nevertheless, the peripheral wood layers contributed more than the older wood to both TNC storage and translocation from ring to ring. Similarly, Schäfer et al. (2000) reported that 50% of the water used by adult beech trees is transported in the outermost 20 mm of the xylem.

#### Carbon distribution among stem growth and carbohydrate storage

Although spring reactivation differed between oak and beech, the seasonal courses of stem increment and carbohydrate refilling were similar. From June to July, stem growth and TNC storage occurred concomitantly in both species. Radial growth provides “room for reserve storage” (Lacointe 2000) and both dynamics are correlated (Wargo 1979, Lacointe et al. 1993). Despite small differences in extractable soil water, annual rainfall, and LAI between the two sites, a similar progression of soil drying was observed (Figure 1) and the period of soil water deficits was similar. In both species, increases in stem increment ceased or were severely reduced during the period when soil water content fell below a threshold value of 0.4 (cf. Bréda et al. 1995). We note that we measured stem radial growth as changes in dimension resulting from cell division

and enlargement, which are determined by tree water status. However, the subsequent maturation phase of wood elements, involving secondary cell wall synthesis and lignification, is initiated as soon as growth in width is completed. The maturation phase is probably less sensitive to tree water status than the growth phase. The current ring remains a carbon sink during the maturation phase, but the duration and extent of carbon investment for the maturation phase have not been quantified. Anatomical observations of beech branches during ring formation indicate that lignification continues at least 3 weeks after cell enlargement has occurred (Lachaud and Bonnemain 1981). Unlike stem growth, TNC accumulation in both species continued during the period of soil water deficit and until October; i.e., 2 months after stem growth cessation. This finding suggests that radial growth was more sensitive to soil water deficits than photosynthesis in these moderately drought-tolerant species (cf. Epron et al. 1992, Bréda et al. 1993, Granier and Bréda 1996, Granier et al. 2000b, 2000c, Leuschner et al. 2001). A significant part of the carbon fixed during the period of soil water deficits was allocated to TNC replenishment. Similar findings have been reported for other tree species (Dickson 1991). Oak mainly stored starch compounds in the stem wood, whereas beech accumulated sugars. Similar increases in starch and sugars over the season were observed by Sauter and van Cleve (1994) and Witt and Sauter (1994) who reported that starch accumulated continuously from May to October in 8-year-old *Populus*.

We quantified the seasonal dynamics of carbohydrate reserves and their spatial distribution on the basis of mass-based concentration. We did not scale up the tree carbon balance calculation from TNC concentration to TNC content at the stem and tree levels because scaling from concentration to content is difficult for mature trees. The living biomass of each organ has to be evaluated either by cutting trees or by using allometric relationships. For diffuse-porous trees, which lack a clear sapwood, additional information about the radial and longitudinal distribution of living cells are needed to quantify the living part of the wood. In addition, interannual dynamics of TNC in adult trees has to be compared with interannual variation in stem growth in order to determine both possible changes in carbon allocation and possible growth limitations imposed by available carbon reserves.

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