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Seasonal fluctuations of starch in root and stem tissues of coppiced *Salix viminalis* plants grown under two nitrogen regimes

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Summary Seasonal changes in starch were studied at the tissue and cellular levels in roots and stems of *Salix viminalis* L. cuttings. Cuttings were planted in pots containing sand and grown in a controlled environment chamber in which seasons were artificially induced by changes in temperature and photoperiod. Nitrogen was supplied at optimum and low rates, and during dormancy, one-half of the plants were decapitated. Starch concentrations in root and stem tissues were determined regularly during shoot extension growth, dormancy and resprouting after dormancy. We used light microscopy (LM) combined with image analysis (IA) to determine the cellular localization and amount of starch in different cell types of stem and root tissues. Chemical analysis confirmed that starch concentrations were lower in plants receiving a high-N supply rate than in plants receiving a low-N supply rate. In all plants, the highest concentration of starch was in the roots. Light microscopy and IA showed that starch accumulated mainly in the phloem and cortical cells of both root and stem tissues. Starch grains were also regularly found in ray parenchyma cells. The amount of starch as well as the size of the grains showed strong seasonal fluctuations. In both roots and stems, starch concentrations were highest during predormancy and lowest during periods of shoot extension growth. At the time of resprouting, root cells of decapitated plants were more depleted of starch than root cells of intact plants, supporting the hypothesis that starch reserves in roots are important during the early phase of resprouting in coppice systems.

Keywords: accumulation, decapitation, energy forestry, mobilization, nutritional status, resprouting, storage compounds.

Introduction

Some *Salix* species have a high capacity for resprouting and are used for biomass production in short rotation coppice plantations (Sennerby-Forsse and Christersson 1994). After harvest of the coppice crops, the regenerating stems rely on internal nutrient reserves in roots and stumps during initiation and early growth (Dickmann and Pregitzer 1992, Sennerby-Forsse et al. 1992). These reserves are mainly composed of carbohydrates (starches and different types of sugars), lipids and proteins (Sauter et al. 1988, Wetzel et al. 1989).

Carbohydrates are the major storage compounds in woody plants (Kramer and Kozlowski 1979, Tromp 1983, Dickson 1991), and the carbohydrate reserves in roots and stumps are believed to play an important role in resprouting of coppiced trees (Dickmann and Pregitzer 1992, Kozlowski 1992). New stem growth in woody plants has been directly associated with rapid depletion of carbohydrates in roots. This does not necessarily mean that root reserves are translocated or used mainly for new growth. Sennerby-Forsse et al. (1992) observed a major allocation of mobilized reserve carbohydrates for use as respiratory substrate, indicating the importance of stored carbohydrates for maintenance processes in plants.

Starch is the most common storage form of carbohydrates in trees (Tromp 1983, Kozlowski 1992) and has often been used as an indicator of tree carbohydrate status (Ford and Deans 1977, Adams et al. 1986). Starch is also a more sensitive indicator of sprouting ability than carbohydrates. Seasonal variation in starch concentrations have been found in stems and branches of deciduous trees in the temperate zone, where starch concentration usually has two maxima, in the fall and in the spring (Nelson and Dickson 1981, Essiamah and Eschrich 1985, Sennerby-Forsse and von Fircks 1987, Dickson 1991, Sauter and van Cleve 1991, Kozlowski 1992, Johansson 1993). In roots, the seasonal pattern of starch accumulation seems to differ both among species and among different site conditions (Dickson 1991, Kozlowski 1992, Johansson 1993). Starch accumulation and mobilization are thought to be influenced by nutrient availability, particularly nitrogen (Adams et al. 1986) and some investigators have found a negative correlation between starch concentration and plant N-status (Waring et al. 1985, McDonald et al. 1986, Rytter and Ericsson 1993, Wetzel et al. 1995).

A better understanding of the role of nutrient reserves in coppice plants would help elucidate the mechanisms underlying coppice yields and provide a more reliable set of criteria for selecting and improving coppice plants. To test the hypothesis that carbohydrate reserves in roots are important sources of energy during the early stages of resprouting, we studied seasonal variation in starch accumulation and mobilization in root and stem tissues of coppiced willows in relation to nutrient availability. To localize and assess starch concentra-

tions at the cellular level we used light microscopy and image analysis to complement the chemical analysis.

Material and methods

Plant material and growth conditions

Woody cuttings of *Salix viminalis* L., Clone 78183, were obtained from one-year-old dormant stems. The cuttings (5 cm long and 0.5–1.0 cm in diameter) were planted in 0.6 dm^3 pots containing quartz sand (mean particle diameter 0.6 mm). The pots were randomly arranged in a controlled environment chamber. Light intensity was maintained at 300 μmol m^{-2} s^{-1} and relative humidity was 60–70%. Artificial seasons were created by prolonging the night from 6 to 16 h per day and decreasing the day/night temperatures from 25/15 to 4/4 °C (Table 1). Starch concentrations in stems and roots were studied during five growth phases (shoot extension growth, predormancy, dormancy, postdormancy and shoot extension growth phases) of the artificial seasonal cycle.

Treatments included two nitrogen (N) supply regimes and decapitation. The nitrogen treatments were: (a) high-N = daily saturation of the rooting medium with a complete nutrient solution of 100 mg N l^{-1} and other essential mineral nutrients in proportions to N found optimal for *Salix* (Ericsson 1981); and (b) low-N = 1/3 of the potential requirement under the given environmental conditions. Nitrogen was added daily during Phases 1 and 2 (shoot extension growth and predormancy, Table 1) by pipetting small volumes of the complete nutrient solution after saturating the rooting medium with deionized water. Nitrogen was added in amounts according to previously calculated dose curves (Ingestad and Lund 1986). Before the experiment and after every harvest, the N concentration of the plants was analyzed. In all cases, plants receiving the high-N treatment had high concentrations of N and plants receiving the low-N treatment had low concentrations of N. Half of the plants in each N treatment were decapitated at the end of Phase 3 (dormancy), leaving about 2 cm of the cut stem.

Table 1. Temperatures and photoperiod regimes of the artificial growth cycle.

	Day/night temperature (°C)	Photoperiod (h)	Duration (weeks)
Establishment	25/15	18	3
Shoot Extension Growth (H1)	20/15	18	4
Predormancy (H2)			
Step 1	20/10	14	1
Step 2	20/10	12	1
Step 3	20/10	10	1
Step 4	20/10	8	3
Dormancy (H3)			
Step 1	15/4	8	5
Step 2	4/4	8	2
Postdormancy (H4)	18/15	18	7
Shoot Extension Growth (H5)	20/15	18	7.5

Plants were harvested at the end of each of the five growth phases. At each harvest, 10 plants from each treatment were randomly selected and separated into roots, cuttings, stems and leaves. Samples for anatomical examination with light microscopy and image analysis were then removed and the remaining plant parts were dried for chemical analysis.

Starch analysis

All sampled roots (including fine and medium roots, and excluding coarse roots) and stems were oven-dried at 70 °C for 48 h. The samples were ground, and 50-mg samples were then extracted with 5 ml of 0.1 M acetate buffer (pH 5.0) at 60 °C for 40 min and centrifuged. After withdrawal of a 1-ml aliquot, 0.1 ml of thermostable α-amylase (Termamyl 3001, NoVo A/S, Bagsvaerd, Denmark) was added and the extraction was continued at 90 °C for 60 min. Glucose, fructose, sucrose, fructan and soluble starch were determined in the first extract, and the second extract was used for determination of residual starch. Sucrose and fructans were hydrolyzed in 0.037 M sulfuric acid at 80 °C for 70 min. Sucrose was defined as the increase in glucose after hydrolysis, whereas fructans were defined as the excess of fructose liberated. The partially hydrolyzed or soluble starch was hydrolyzed to glucose by amyloglucosidase at 60 °C for 60 min. Glucose and fructose were determined by the glucose phosphate dehydrogenase method. Details of the methods are described by Steen and Larsson (1986).

Light-microscopy studies and image analysis

On each sampling occasion, sections of stems and medium-sized roots (0.5 to 1.5 mm in diameter) were cut with a razor blade. Samples were taken from the middle part of stems and roots and were immediately fixed in 4% glutaraldehyde in 0.1 M phosphate buffer. The samples were then dehydrated through an alcohol series and embedded in Historesin (Leica Instruments GmbH, NuBloch, Germany). The sections (2–3 μm) were cut with an LKB ultratome. Sections used to study cell structure were stained with toluidine blue and the sections used for starch determinations were treated with iodine solution (I_2KI) (Wargo 1975). Samples were placed on glass slides and photographed with a Zeiss light microscope.

Image analysis was used for quantitative determination of starch at the cellular level. The image analysis system consisted of a high-resolution scanner (Sharp JX-325) and a computer equipped with an SIS software package (Soft-Imaging Software, GmbH, Münster, Germany). The light microscope photographs were scanned into the computer and starch grain area in relation to total cell area was measured and the percentage starch calculated. Ten slides were selected randomly for each harvest and treatment. Five slides were taken of roots and another five were taken of stems. Four photographs (two of the xylem and two of the phloem) were taken from each slide. Altogether, 400 photographs from intact plants and 160 photographs from decapitated plants were scanned for image analysis.

Statistical analyses

Statistical analysis was performed by Systat for Windows Version 5 (Systat, Inc., Evanston, IL). Pair-wise comparisons were made by the Least Significant Difference (LSD)-method at a 95% confidence level.

Results

Effect of nitrogen availability on starch concentrations

In both intact and decapitated plants, roots in the high-N treatment contained less starch than roots in the low-N treatment (Table 2). The biggest difference between the two treatments was found during predormancy when the amount of starch reached a maximum, and the smallest difference was found during dormancy. During the shoot extension growth period, high-N plants had less starch in the stem than low-N plants. There were no differences between treatments in stem starch concentrations from predormancy until the end of the experiment (Table 2).

Fluctuations in starch concentrations in roots and stems of intact plants

In intact plants, the N treatments had no effect on seasonal variation in starch concentration. Except during Phase 1 of the growth cycle, roots contained more starch than stems (Table 2). In roots, starch was mainly deposited in phloem and cortical parenchyma cells and also in xylem cells (Figure 1A). In stems, most of the starch accumulated in phloem and cortical parenchyma cells and in xylem ray cells (Figure 2A).

Table 2. Seasonal variation of starch concentrations (% of dry weight ± SD) in stems and roots of intact and decapitated plants grown at two different nitrogen supply rates. For roots and stems, means followed by the same letter within each column are not significantly different (LSD at 0.05 level, $n = 3$) (data for intact plants, L. Bollmark, Dept. Short Rot. For., Swedish Univ. of Agric. Sci., Uppsala Sweden, personal communication). Successive growth phases are given as: H1 = Shoot Extension Growth; H2 = Predormancy; H3 = Dormancy; H4 = Postdormancy; H5 =Shoot Extension Growth.

	Starch concentration (mg g^{-1})			
	Low-N		High-N	
	Intact	Decapitated	Intact	Decapitated
Stem				
H1	4.17 ± 0.6 b		2.83 ± 0.7 bc	
H2	6.67 ± 1.9 c		6.87 ± 0.1 d	
H3	3.87 ± 0.2 b		3.50 ± 0.7 c	
H4	2.30 ± 0.0 ab	2.83 ± 0.3 b	1.80 ± 0.4 ab	2.23 ± 0.1 b
H5	1.40 ± 0.2 a	1.20 ± 0.3 a	1.50 ± 0.4 a	1.07 ± 0.2 a
Roots				
H1	3.00 ± 0.2 a	1.67 ± 0.1 a		
H2	18.67 ± 3.2 c	14.8 ± 1.3 d		
H3	9.90 ± 1.0 b	9.70 ± 0.7 c		
H4	8.00 ± 0.6 b	8.47 ± 1.2 b	7.00 ± 0.3 b	7.90 ± 0.7 b
H5	7.20 ± 0.4 b	6.33 ± 0.1 a	6.23 ± 0.2 b	5.43 ± 0.6 a

During the period from shoot extension growth to predormancy, total starch concentrations increased significantly in both roots and stems (Table 2), reaching a maximum in both organs at the time of predormancy. The amount of starch almost doubled in root phloem and cortical cells during the period from shoot extension growth to predormancy (Figure 3A). Starch concentrations also increased in root and stem xylem cells, but decreased slightly in the stem phloem and cortex (Figures 3A and 3B).

From predormancy to postdormancy, total starch concentrations decreased in all cell types containing starch in both the roots and stems (Table 2), with the exception of xylem cells in the roots (Figures 3A and 3B). The size of the starch grains decreased in both roots and stems (on average from 5 to 1 μm and 3 to 0.6 μm, respectively) during the period from predormancy to postdormancy (Figures 1B–D, Figures 2B–D).

From postdormancy to shoot extension growth, the total amount of starch continued to decrease in phloem and cortical cells of both roots and stems, whereas it increased slightly in root and stem xylem cells (Figures 3A and 3B).

Effect of decapitation on root starch concentrations

During the resprouting period, from postdormancy to shoot extension growth, starch concentration in roots of decapitated plants decreased more rapidly than in roots of intact plants (Table 2). During this period, the amount of starch in root xylem cells decreased in decapitated plants, whereas it increased in root xylem cells of intact plants (Figure 3C). In root phloem and cortical cells, the amount of starch decreased during the same period in both intact and decapitated plants, with a higher rate of decrease in decapitated plants (Figure 3C). The decrease in root starch followed the same trend in all plants, but was slightly more pronounced in high-N plants than in low-N plants (Table 2).

Discussion

During shoot extension growth, high-N plants had lower stem starch concentrations than low-N plants. Similar results were obtained by Wetzel et al. (1995). We conclude that greater N availability increased shoot growth, which led to increased utilization of photosynthate and lower carbohydrate availability for storage in high-N plants compared with low-N plants. During predormancy, roots of low-N plants contained significantly more starch than roots of high-N plants, whereas starch concentrations in stems were similar in low-N and high-N plants. This finding indicates that roots play an important role in starch storage under low-N conditions. During dormancy, the N treatments had little effect on starch concentrations in roots; however, between predormancy and dormancy, starch concentrations decreased more rapidly in low-N plants than in high-N plants, perhaps indicating that roots of plants grown under N-poor conditions are more sensitive to temperature changes than roots of plants grown under N-rich conditions and that starch degradation accompanies the development of frost hardiness. From dormancy to resprouting, the decrease in root starch was more pronounced in the high-N treatment

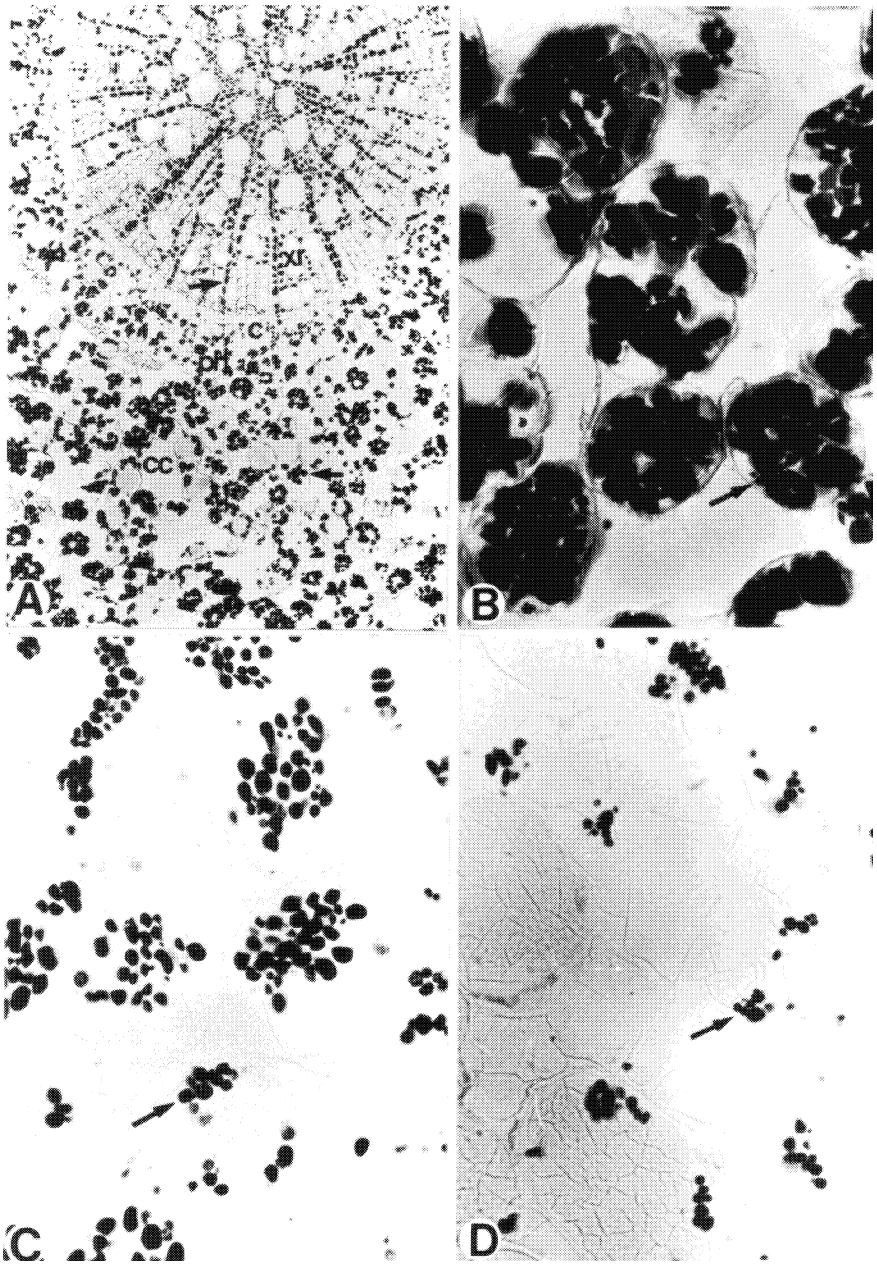

Figure 1. Cross sections of root of *Salix viminalis*, stained with iodine solution (I_2KI). (A) Root tissues 70×. Xylem ray cells (xr), cambium (c), phloem (ph) and cortex (cc). Starch is (arrows) visible in xylem ray cells and in phloem and cortex cells. (B) Starch deposition in cells in the phloem and cortex area during predormancy 441×. (C) Starch deposition in cells in the phloem and cortex area during dormancy 441×. (D) Starch deposition in cells in phloem and cortex area during postdormancy 441×.

than in the low-N treatment in both intact and decapitated plants, suggesting that a high plant nitrogen status enhances starch dissolution so that high-N plants resprout earlier in spring than low-N plants.

The seasonal variations in starch concentrations of *S. viminalis* plants were similar in roots and stems although roots generally contained more starch than stems. Similar results have been reported for *Populus* spp. (Nguyen et al. 1990, Wetzel et al. 1995). We found that higher concentrations of starch were stored in phloem and cortical cells of the root tissue than in root xylem cells. This observation differs from the results reported by Keller and Loescher (1989) for sweet cherry and by Kile (1981) for *Eucalyptus.* They reported that root wood had a higher starch concentration than root bark. The reason for this difference might be associated with plant age, we used young plants with small root diameters and low wood/bark ratio.

We combined chemical analysis with light microscopy (LM) and image analysis (IA) to determine which cell types accumulated starch, and to monitor the seasonal variation in starch accumulation in roots and stems. There was a rapid accumulation of starch from the period of shoot extension growth to predormancy. At the end of the predormancy period, starch concentration reached its maximum and parenchyma cells in the phloem and cortex were heavily loaded with starch, suggesting translocation of carbohydrates from leaves to stems and from stems to roots. From predormancy to dormancy, both root and shoot starch decreased. A similar

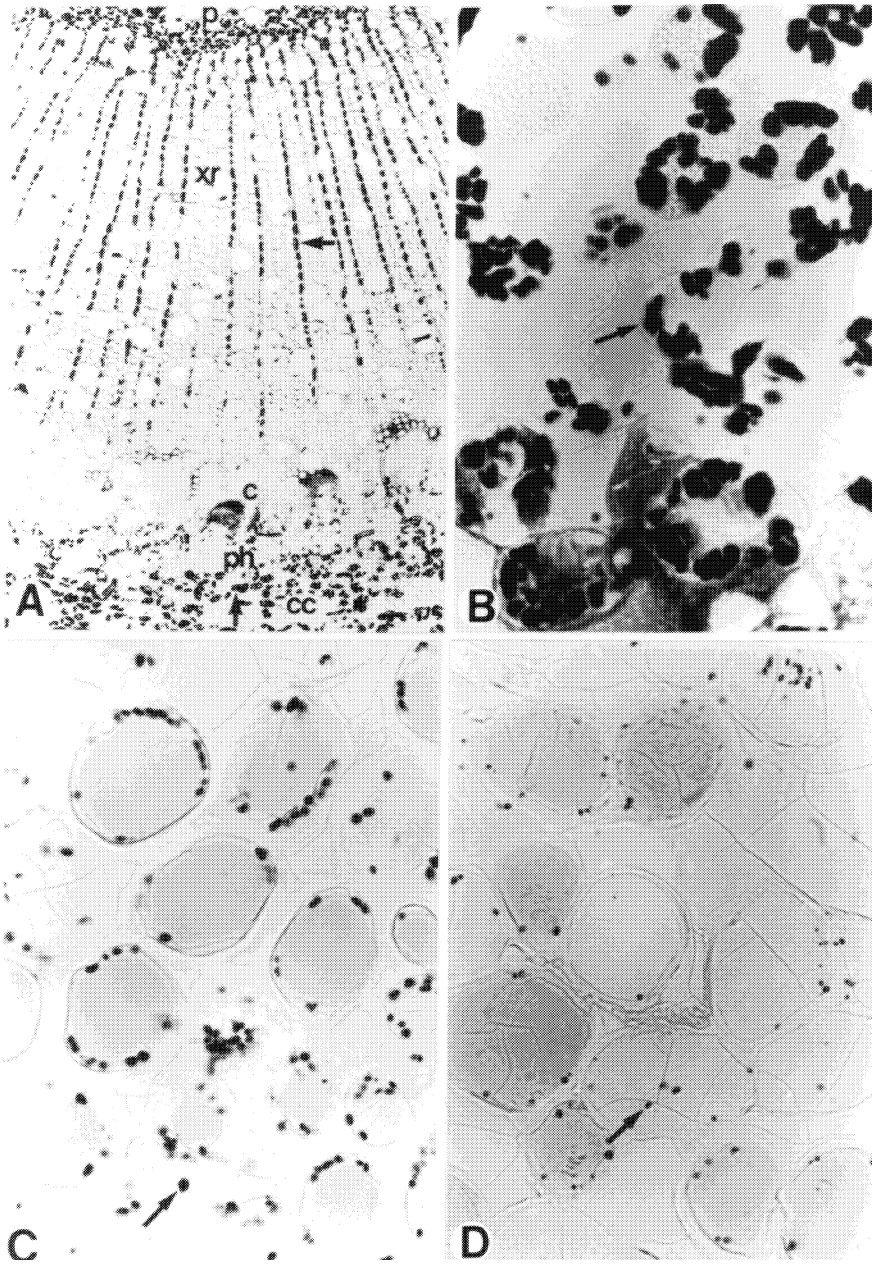

Figure 2. Cross sections of stem of *Salix viminalis*, stained with iodine solution (I_2KI). (A) Stem tissues 70×. Pith cells (p), xylem ray cells (xr), cambium (c), phloem (ph) and cortex (cc). Starch (arrows) visible in xylem ray cells and in phloem and cortex cells. (B) Starch deposition in cells in the phloem and cortex area during predormancy 441×. (C) Starch deposition in cells in the phloem and cortex area during dormancy 441×. (D) Starch deposition in cells in phloem and cortex area during post-dormancy 441×.

pattern has also been observed under field conditions in other tree species of the northern temperate zone (Kramer and Kozlowski 1979, Harms and Sauter 1992). The decrease in starch concentration in late autumn reflects the temperature dependency of starch to sugar conversion, which has been studied in *Populus* (Sauter 1988, Sauter and van Cleve 1991) and *Salix* (Sennerby-Forsse and von Fircks 1987). The dissolution of starch to sugar is believed to be important for binding water, maintaining membrane properties, and increasing freezing tolerance (Levitt 1980). From dormancy to regrowth, starch concentration continued to decrease, particularly in root phloem and cortical cells. This decrease could be explained by conversion of starch to sugars and transportation of the sugars to expanding buds for use in regrowth (Sennerby-Forsse 1986).

Essiamah and Eschrich (1985), in a study of seasonal variation of starch in wood and bark of six north European deciduous tree species, identified four phases in the starch cycle: (1) accumulation of starch in autumn; (2) dissolution of starch during dormancy; (3) resynthesis of starch at the end of dormancy; and (4) dissolution of starch during bud swelling and bud break. We identified all phases except Phase 3 in our study. The failure to identify Phase 3 may result from the sampling periods we employed. For example, we would have missed Phase 3 if plants that we sampled during resprouting had begun to break down starch at the end of dormancy. Ryugo (1988) reported that resynthesis of starch from midwinter to bud break lasts about 90 days in pecan, 50 days in kiwifruit, and only 7 to 10 days in peach. Our results may indicate that the period for starch resynthesis is short in *Salix*.

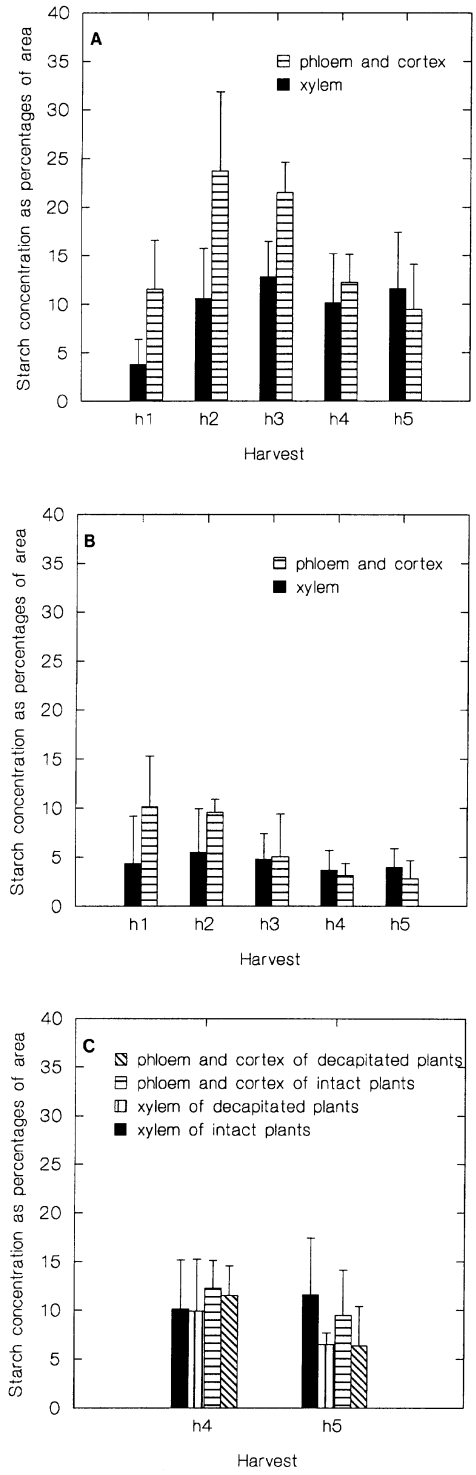

Figure 3. Seasonal variation of starch concentrations in root tissues and stem tissues of intact and decapitated *Salix* plants grown at high-N availability. Mean value and SE are shown, $n = 10$. (A) Root tissues of intact *Salix* plants. (B) Stem tissues of intact *Salix* plants. (C) Root tissues of intact and decapitated *Salix* plants.

There is little information about the effect of coppicing on starch formation and mobilization in trees. Tschaplinski and Blake (1994) investigated the influence of decapitation of actively growing hybrid poplars and observed that, 10 days after stem decapitation, stem starch concentration had declined to 50% of that of intact plants as a result of resprouting. Miyanishi and Kellman (1986) studied the role of root nutrient reserves in regrowth of two savanna shrubs and found that starch was significantly depleted in roots of burnt shrubs after resprouting started. We observed an intensified starch dissolution in roots after removal of the shoot system, supporting the hypothesis that starch reserves in roots are important for the early phases of resprouting in coppice systems. Large carbohydrate reserves in combination with a strong sink function of shoots during the early stages of resprouting may partly explain the capacity for rapid regrowth of coppiced *Salix* plants.

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