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Storage, mobilization and interrelations of starch, sugars, protein and fat in the ray storage tissue of poplar trees

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Abstract. The seasonal pattern in starch, various sugars, protein, and fat, and their interrelationship, has been followed in 3-year-old branch wood of poplar trees (*Populus* x *canadensis* Moench '*robusta*') under natural site conditions. The deposition of starch, protein and fat proceeds at different times. Starch accumulates from May until October, fat mainly during the summer months, and protein when the leaves are yellowing in September and October. The maximum concentrations in the branch wood were 15–18 µg starch, 6–9 µg protein, 4–8 µg fat, 10–15 µg sucrose, and up to 30 µg total sugars per milligram dry weight (DW). During starch deposition periods no increased sucrose level is found in the tissue. The maximum daily starch deposition rate was 0.2–0.4 µg starch/day/mg DW of wood. During starch hydrolysis in late autumn and winter, a dramatic increase in sucrose and its galactosides is measured (up to 15–27 µg/mg DW in total). In early spring, before budbreak, the concentrations of these sugars diminishes sharply. In contrast to this clear-cut starch-to-sugar conversion in autumn no significant starch-to-fat conversion is detected. An elevated content of free glycerol, however, is found in winter. In spring, starch and storage protein are mobilized completely, or almost completely, in poplar twig wood. A noteworthy pool of maltose is found transiently during autumn (up to 8 µg/mg DW) and again in spring. The results demonstrate that the individual storage materials, e.g. starch, protein, and fat, are accumulated fairly independently in the wood storage parenchyma. Tissue sugar levels, in contrast, appear to be closely related to the seasonal variations in starch content, on the one hand, and to the acclimation and deacclimation of the cells, on the other. The interrelations of the storage materials and sugars are discussed.

Key words: Cold acclimation – Fat – *Populus* – Protein – Sugars – Storage parenchyma

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Introduction

Perennial plants like trees depend on a well ordered transient accumulation of photosynthates and of various other storage compounds that are gained during favourable periods, stored throghout the dormant season, and mobilized for re-use in growth and reproduction when the appropriate time comes. The parenchymatous cells of the wood and the 'bark' in both the stem and the root serve as essential vegetative storage tissues in which starch, sugars, protein and fat accumulate seasonally. Although innumerable studies have been made on the accumulation and mobilization of stored material in trees (see Ziegler 1964; Kramer and Kozlowski 1979; Kozlowski 1992) little is known on the factors controlling initiation and regulation of deposition of the individual compounds (Jeremias 1964; Dickson 1989; Titus 1989; Sauter and Neumann 1994). For instance, storage protein deposition has recently been found by some authors to be under photoperiodic control (e.g. Coleman et al. 1991, 1992; Langheinrich and Tischner 1991) while others gave evidence that further factors are of great significance, for example the nitrogen level, and the temperature (Tromp and Ovaa 1984, 1985; van Cleve and Apel 1993; Sauter and Neumann 1994; Stepien et al. 1994). The insight into the interconversion of individual compounds, for example of starch and fat, of sugars and starch, and the prominent role of temperature on these events also is still limited (Jeremias 1968; Höll 1985; Sauter 1988; Fischer and Höll 1991, 1992; Sauter and van Cleve 1991). Finally, the factors involved in determining the height of the accumulation level of individual compounds are still poorly known (cf. Sauter and Neumann 1994). The intracellular space of a parenchyma cell that becomes dedicated to the storage of starch, fat droplets, or protein bodies can be very different as was demonstrated by micromorphometry (Sauter and van Cleve 1989a). Studies in which various storage compounds have been followed simultaneously in more detail (Nelson and Dickson 1981; Höll 1985; Dickson 1989; Pregitzer et al. 1990; Fischer and Höll 1991, 1992; Sauter and van Cleve 1990, 1991; Harms and Sauter 1992) also showed that there are considerable differences among

the deposition behaviour of individual compounds, among various tree species and even among various clones. Much more insight into the timing of these events under natural site conditions is needed as basic information for studies on the factors regulating the initiation and the height of deposition of individual storage compounds. In the present paper therefore the pattern of deposition and mobilization of starch, fat, and protein, as well as the changes in the level of individual sugars have been followed in parallel in poplar wood in order to gain insight into existing relationships and causal relations.

Materials and methods

Materials. The material for electron microscopy and for the biochemical analyses was taken from 3-year-old twig segments of 8- to 10-year-old poplar trees (*Populus* x *canadensis* Moench 'robusta') growing in the Botanical Garden of Kiel University. The samples were collected monthly, or, depending on the physiological stage in spring and in autumn, at shorter intervals. For comparison, a period of 3 years (1987–1989) has been investigated. In order to have a measure of the sugar content of the living cells of the wood, sugars present in the apoplast have been removed from twig segments by thoroughly flushing the xylem vessels with tap water before the biochemical analysis was done.

Electron microscopy. Radial longitudinal tissue sections were prepared of the wood and fixed in glutaraldehyde (5%)/paraformaldehyde (4%) in phosphate buffer (0.1 *M*, pH 7.2) at 0–2° C overnight and postfixed in OsO_4 (2%, overnight). Dehydration with isopropanol and propylene oxide, embedding in Spurr's low-viscosity resin, and staining with bismuth after sectioning was done as described elsewhere (Sauter and van Cleve 1990). The ultrathin sections were viewed in a Siemens 101 electron microscope.

Starch. Starch was extracted with 1 N HCl (1 h, 60° C) or 2 N HCl (1989) of tissue sections (thickness 40 µm, approx. 20 mg) prepared with a microtome. After hydrolysis with amyloglucosidase starch was determined in four parallel assays using the hexokinase method of Boehringer (1984).

Sugars. Sugars were extracted from oven-dried (80° C) tissue sections (100 µm thickness; H_2O, 20° C, 1 h) prepared from the flushed twig segments (see above) and determined qualitatively and quantitatively with the aid of a Biotronic ZA 5100 sugar autoanalyser. The total sugar content on the graphs is given as the sum of the contents measured for glucose, fructose, xylose, galactose, maltose, sucrose, raffinose, and stachyose. Although present in noteworthy amounts, maltotriose and verbascose could not been quantified and are not included.

Protein. Protein content was measured with the Lowry method after its extraction with a Laemmli buffer of wood powder prepared with a Retsch mixer mill of tangential wood sections (thickness 40 µm; for details see Sauter et al. 1989).

Fat. Free glycerol and glycerol of glycerolipids (after alkaline hydrolysis) were determined enzymatically using the glycerokinase method in the form described by Eggstein and Kuhlmann (1974). Their extraction was done from 20 mg wood powder with 2 ml bidistilled water or alcoholic 0.5 N KOH, respectively (30 min at 70° C in water bath; after addition of 0.4 ml perchloric acid and centrifugation for 5 min at 3000 rpm, 0.2 ml supernatant was used). Because the glycerol of phospholipids and of galactolipids were found to contribute little (less than 5%) to that bound in fat (Saranpää and Sauter, unpublished data), fat was computed as 'fat-bound glycerol' from total glycerol minus free glycerol multiplied by a factor of 8.

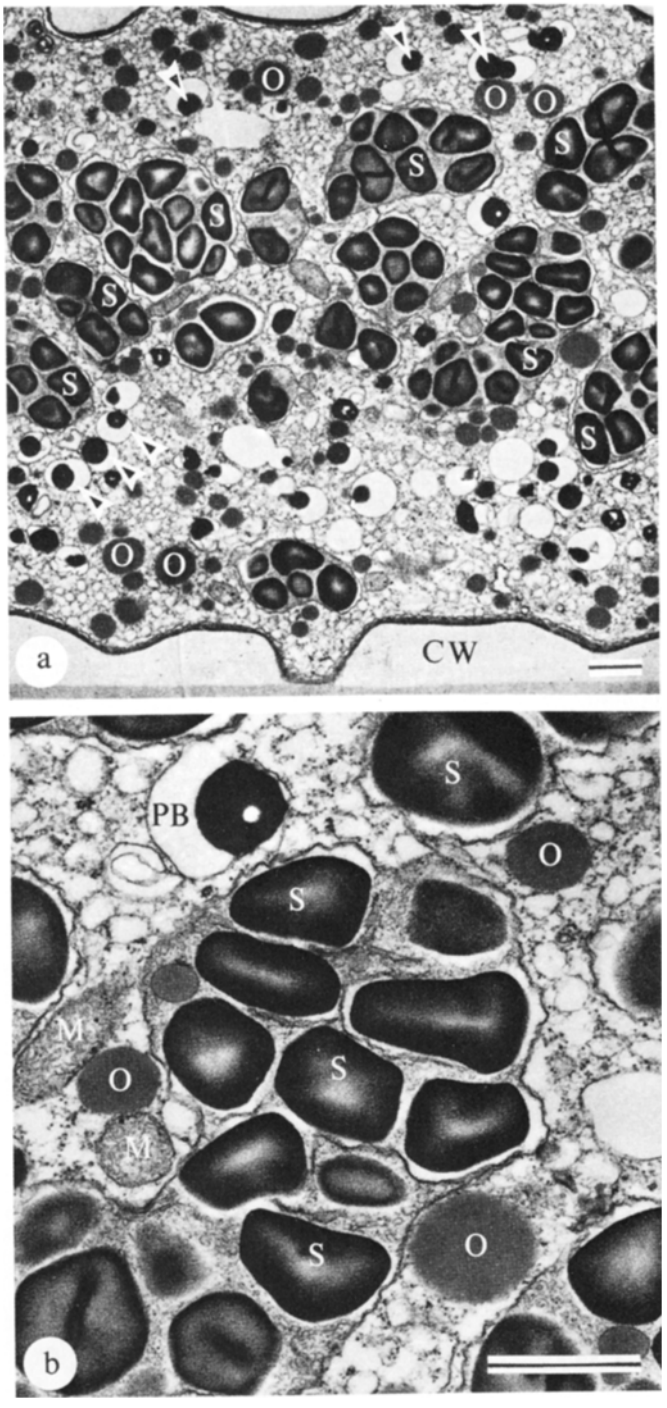

Fig. 1 a–b. Ray cells of poplar wood in late autumn (November 1988) showing storage of starch (*S*) in amyloplasts, fat in oleosomes (*O*), and of protein in protein bodies (*arrow heads* in **a**; *PB* in **b**). *M* = mitochondrion, *CW* = cell wall. Note accumulation of small vesicles in cytoplasm. **a**, × 8000; **b** × 24000. *Bar* = 1 µm

Results

Electron microscopy

The storage parenchyma of poplar wood is almost exclusively formed by the rays. The biochemical data therefore reflect the changes in this tissue. In comparison, axial

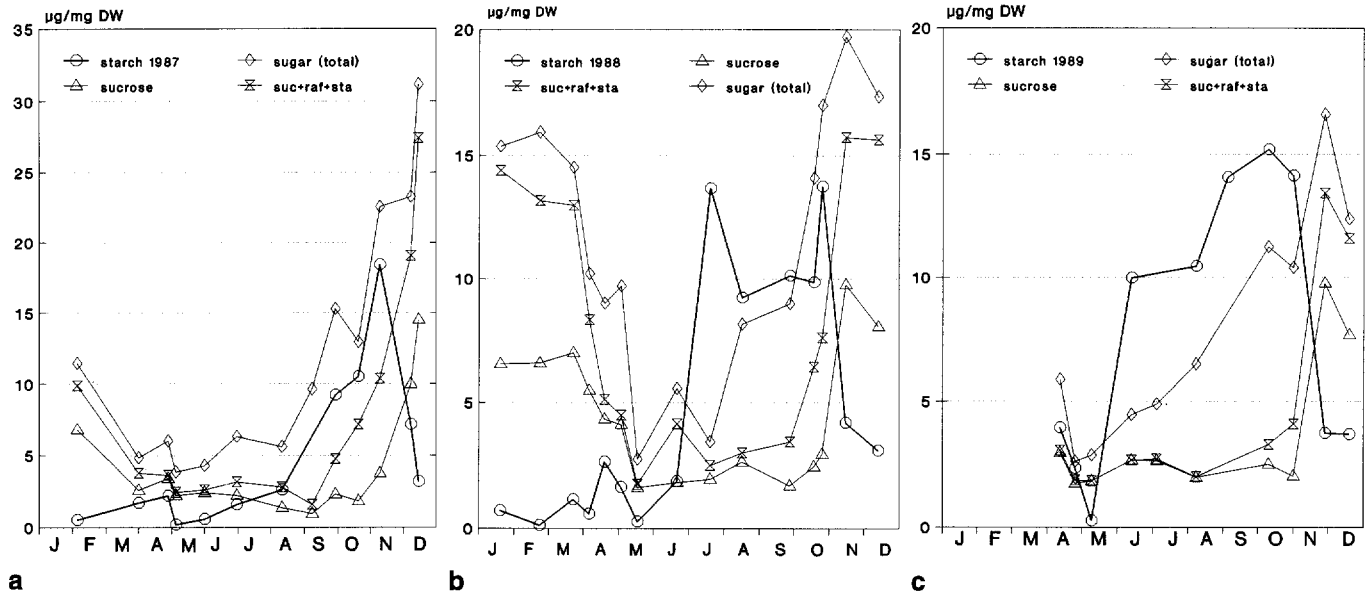

Fig. 2a–c. Seasonal variation of starch in the wood of poplar twigs in relation to the content of various sugars (total of eight different sugars; sucrose; sucrose+raffinose+stachyose). **a** = 1987, **b** = 1988, **c** = 1989

parenchyma is neglegible and is only very scarcely present in the form of single paratracheal and terminal cell strands.

The three main storage compounds found in ray cells during late autumn are shown in Fig. 1. While starch accumulates in the form of several starch grains within large amyloplasts of increasing size (Fig. 1a), a peculiar storage protein is deposited within small vacuoles called 'protein bodies' (Fig. 1b). It is seen as electron-dense masses (arrowheads in Fig. 1a). Fat is stored in small oleosomes of 0.2–1 µm in diameter. Furthermore, enrichment of cytoplasm with tubular and vesicular ER cisternae is most characteristic for the late autumn stage illustrated. Because these cisternae originate concomitantly with the prominent rise in sucrose and its galactosides shown in Fig. 2, they have been suggested to be the peculiar sites of sugar accumulation (cf. Sauter and Kloth 1987; Sauter and van Cleve 1991).

Starch

The seasonal accumulation pattern in the 3-year-old branch wood is shown for three individual years in relation to the content of individual sugars (Fig. 2a–c). At the time of budburst and growth of leaves in spring, starch is completely mobilized (end of April, begin of May). Immediately thereafter, starch again accumulates continuously from May until October. The deposition in these years proceeds at different speeds, suggesting its dependency on the climatic conditions of the individual year. The delayed deposition seen in 1987, for instance, coincided with a very cool and rainy early summer and with a noteworthy infection of leaves by *Melampsora*. No relationship to the tissue sugar level is observed (Fig. 2a). In all 3 years, however, a similar maximum of about 15–18 µg/DW is reached at the time of leaf fall. Thereafter, starch decreases rapidly to a winter minimum which usually persists from December to February (Fig. 2b). The starch level maintained is dependant therefore on the winter temperatures (cf. Sauter and Kloth 1987; Sauter 1988; Sauter and van Cleve 1991). The speed of starch hydrolysis in November was remarkably identical in all 3 years (cf. the slopes in Fig. 2a–c). It is at least as fast as the maximum synthesis rates observed in summer. Finally in late March and in April, starch content rises transiently again before spring mobilization begins (Fig. 2a, b). When the daily starch deposition rates in the wood are computed from the slopes of starch content in Fig. 2, deposition rates up to 0.24 to 0.42 µg/day/mg DW are obtained.

Sugars

Total sugar content. The total sugar content is at its minimum in late April/early May during leafing out (Fig. 2a–c). It decreases to only 2–4 µg/mg DW. During the following summer months no clear-cut causal relationship is observed between the intensity of starch deposition and the height of tissue sugar level as could be suspected. Only in late summer, are increasing sugar levels observed parallel to higher levels of starch. However, when starch disappears in autumn, the tissue sugar level begins to increase dramatically, reaching 17–32 µg/mg DW (Fig. 2a–c). It would be interesting to have information on the sugar level of the living cells during this period: because rays make up only 8–10% of poplar wood, their sugar level might reach 10-fold this value.

Sucrose, raffinose, stachyose. Figure 2a–c illustrates that the total sugar content of the tissue is governed by the combined content of sucrose and its galactosides during most times of the year, in autumn, winter and spring. Only in April and particularly in autumn, does maltose make a substantial contribution to the tissue sugar content (Fig. 4). The content of sucrose and its galactosides remains low during summer, e.g. at 3 µg/mg DW. Sucrose thereby

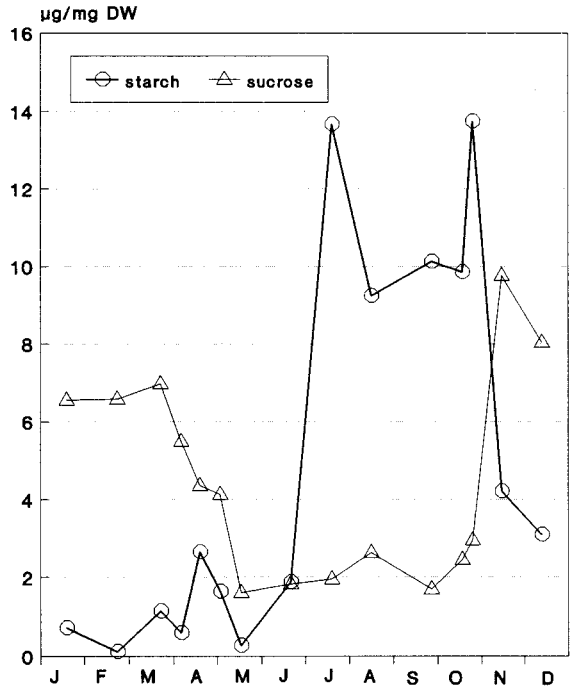

Fig. 3. Seasonal pattern of sucrose content in poplar twig wood in relation to changes in starch content during deposition (May–October), starch-to-sugar-conversion (October–December), starch resynthesis (April), and mobilization (April–May)

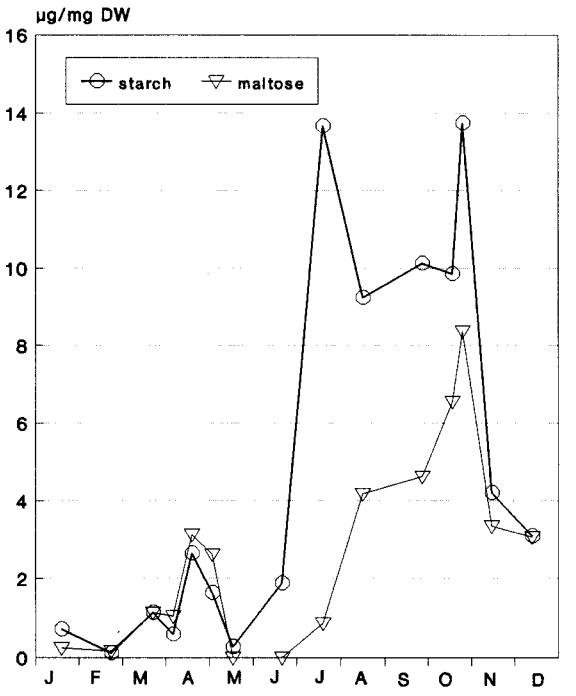

Fig. 4. Seasonal pattern of maltose content in relation to changes in starch content

dominates by far while its glactosides are still insignificant (see difference of suc and suc+raf+sta in Fig. 2a, c). In autumn, a sharp increase in these sugars begins. It parallels starch hydrolysis, both in time and in quantity. At the end of autum between 14 and 27 µg/mg DW were measured. The high sugar level of the living cells in poplar wood during winter is thus always brought about by these sugars.

Sucrose. Because sucrose is considered to be the main transport sugar in the ray parenchyma (cf. Sauter and Kloth 1986; van Bel 1990) it is of interest to compare its level with the observed intensities of starch deposition (Fig. 3). No link between starch accumulation and the sucrose level of the tissue, however, is detectable. Sucrose clearly remains at a low level during summer even at periods of accelerated starch deposition (see for instance October 1987, July 1988, and June and September 1989 in Fig. 2a–c). In contrast, there is a sharp increase in late autumn, to 7–10 µg/mg DW, concomitantly with a prominent rise in its galactosides. During a frost period of –5° C 15 µg/mg DW of sucrose were reached (December 1987, Fig. 2a). The increased sucrose levels commonly last until the next spring.

Maltose. Maltose content is usually low in the tissue, from May until July and in winter. During two periods, however, it may reach a higher level (Fig. 4). In early spring, before starch mobilization begins, a smaller pool is observed. From late summer onwards, again a most conspicuous pool is built up which reaches about 8 µg/mg DW in autumn. It vanishes rapidly in late autumn and early winter parallel to the rise of sucrose and its galactosides (cf. Figs. 2, 4).

Free and fat-bound glycerol

Fat content is followed by analysing the glycerol of total glycerolipids which is mainly fat-bound glycerol (cf. Materials and methods). Glycerol content of 0.5–1 µg/mg DW is multiplied by a factor of 8, in order to have a measure for the content of neutral fat in the tissue (4–8 µg/mg DW). Fat content increases in summer in the twigs (Fig. 5b), mostly in July and August (1987 and 1988), seldom later (1989). There is no increase in fat parallel to the prominent starch hydrolysis in autumn (Fig. 5a) demonstrating the absence of a noteworthy starch-to-fat conversion during this period. At a later winter stage, a minor increase might occur (see December in Fig. 5b). Furthermore, there is no consistent relationship to periods of extensive starch accumulation observed (Fig. 5a). On the other hand, a decrease in fat-bound glycerol is always found during budbreak period (Fig. 5a, b). Free glycerol, in constrast, always stays at a very low level, e.g. from April until October mostly below 0.1 µg/mg DW. Only in autumn and winter does glycerol content increase sharply in the tissue, by a factor of 10–15 (Fig. 5c).

Protein

The seasonal variation in twig wood protein content shows remarkable differences to both starch and fat. There is a rapid and almost complete mobilization of protein during budbreak in spring, followed by a continuous low level throughout the vegetation period, in contrast to the starch and fat, and a renewed sharp increase in autumn (Fig. 6).

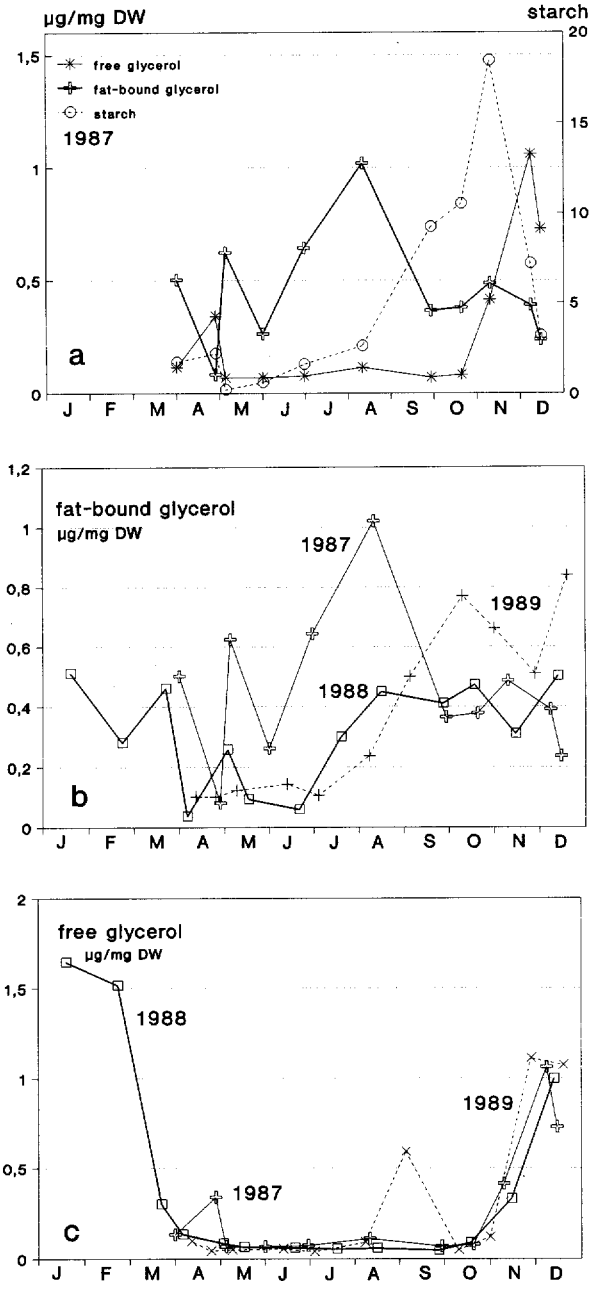

Fig. 5a–c. Seasonal cycles of fat-bound glycerol (**a–b**) and of free glycerol (**c**) in poplar twig wood. The relation to changes in starch content is given in **a**

The deposition of storage protein in autumn always coincides fairly exactly with the beginning of leaf yellowing. From the fluctuation of protein content by about 5 µg/mg DW in spring and autumn, the amount of storage protein deposited and mobilized in the 3-year-old twigs can be deduced. Only in 1989, when a hurricane damaged the foliage in late August, did much less storage protein accumulate during autumn (Fig. 6).

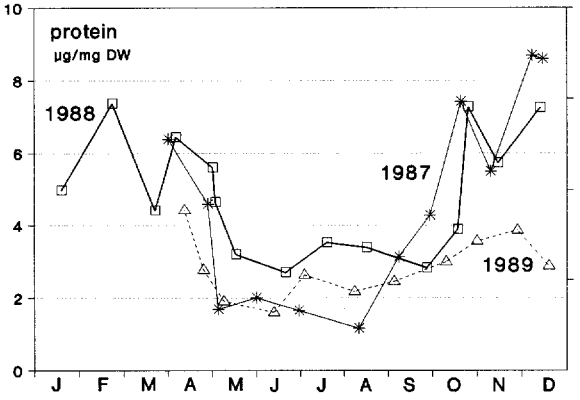

Fig. 6. Seasonal cycles of protein content in 3-year-old poplar twig wood

Discussion

The behaviour of various storage materials in poplar twig wood, e.g. starch, sugars, fat, and protein has been followed in parallel during the deposition, mobilization and winter acclimation period. In addition, the relationship of individual sugar levels in the tissue to these physiological events has been investigated. The vegetative storage parenchyma of poplar wood is composed almost exclusively of ray cells. Poplar, therefore, has the advantage that the biochemical data are characteristic for the changes proceeding at the cellular level in this fairly homogenous tissue (Sauter and Kloth 1987).

The electron micrographs show that a considerable area of the cells is devoted to the storage of starch, fat and protein. Earlier micromorphometric determinations of these compounds on electron micrographs showed that about 20–30% of the cell area was occupied in winter by amyloplasts, 12% by protein storing vacuoles, and 1.1–1.4% by oleosomes (Sauter and van Cleve 1989a). However, creating a surplus of photosynthates in summer, e.g. by ringing the branches, gave rise to a considerable increase in the deposition of these storage compounds (Sauter and Neumann 1994). This latter result indicates that the maximum accumulation levels observed now during 3 consecutive years for the starch (15–18 µg/mg DW), sucrose (10–15 µg/mg DW), maltose (8 µg/mg DW), sucrose+raffinose+stachyose (14–27 µg/mg DW), sugars in total (17–31 µg/mg DW), protein (6–9 µg/mg DW), fat (4–8 µg/mg DW; determined as fat-bound glycerol × 8), and free glycerol (1.6 µg/mg DW) might be fairly representative for the twigwood but probably could be increased under certain conditions. On the other hand, adverse conditions like a fungal attack, loss of leaves, or adverse climatic conditions can alter the deposition pattern considerably and lower the accumulation level (e.g. Sauter and van Cleve 1990 for storage protein). In *Fagus sylvatica*, however, Wiebe and Blaschke (1988) surprisingly found no correlation between starch content of the roots and the degree of stress seen by the crowns. Measurements of protein content in twigs and stems at sites of different age furthermore showed that there are noteworthy gradients in protein content in twigs, stemwood and roots (Cooper et al.

1972; Sauter et al. 1989). A decrease from younger to older parts of poplar shoots was also found for sugars and starch concentrations (Fege and Brown 1984; cf. Kozlowski 1992). Whether such differences in distribution must be attributed mainly to differences in sink strength of the individual site, to the relative proximity to source leaves, or even to differences in the amount of parenchyma cells, cannot be decided yet. Detailed studies on carbon and nitrogen allocation in red oak seedlings (Dickson et al. 1990), 1-year-old poplar 'trees' (Pregitzer et al. 1990; Nguyen et al. 1990), and 8-year-old pine trees (Hansen and Beck 1990) cannot yet answer this important question.

Information obtained from fruit trees about the influence of fertilizers on the accumulation level of storage materials shows that the nutritional status is also of great importance (Tromp and Ovaa 1985; Titus 1989). The values given above for the individual storage materials in poplar twigs thus must be taken with these implications.

When the seasonal pattern of starch is considered, consistency with present knowledge is found (cf. Kramer and Kozlowski 1979; Kozlowski 1992). Only the considerable variation in deposition during the individual years, which we attribute to peculiarities in climatic conditions, is unexpected. In winter, the degree of starch dissolution is particularly dependent upon winter temperatures (Sauter 1988). In 1987, when temperatures of $-5°$ C and below lasted for a longer period, almost all starch disappeared from poplar wood. Complete disappearance of starch from amyloplasts was also seen at the electron microscopical level (Sauter and Kloth 1987, Fig. 10). At the same time, the sugar content increased tremendously proving the starch-to-sugar conversion (Sauter and Cleve 1991). Our results give good evidence that up to 100–150 µg/mg of sucrose in addition to raffinose+stachyose are reached in the ray parenchyma cells. Together with parallel structural changes that are observed in the protoplast of poplar ray cells (Sauter and Kloth 1987; Sauter and van Cleve 1991), i.e., the origin of a vast amount of vesicular and/or tubular ER cisternae, this enormous increase in sugars undoubtedly takes part in the cold adaptation process of these cells. Structural changes and an increase in sugar content, particularly of sucrose and its galactosides, have mostly been found to parallel closely the cold acclimation of living cells in the wood (Jeremias 1964, 1968; Pomeroy and Siminovitch 1971; Senser et al. 1971; Nelson and Dickson 1981; Fege and Brown 1984; cf. Sakai and Larcher 1987; Sauter and van Cleve 1991; Fischer and Höll 1992).

There is little information on the rate of starch deposition in the wood as an indication for the carbohydrate synthesis capacity of the tissue. From the present results a maximum daily deposition rate of 0.24–0.42 µg/mg DW is obtained. For the parenchymatous cells themselves this value must be multiplied by a factor of 10–12 (see above). Earlier values obtained on poplar wood, e.g. 0.17 (Sauter 1982) and 0.18 µg/mg DW (Sauter and Kloth 1986), are in accordance with the present finding.

The seasonal pattern of protein is quite simple (Fig. 6). It clearly reveals that there is a sharp and fairly rapid drop in spring which emphasizes the significance of storage protein for new shoot growth. This agrees with findings made at fruit trees and other deciduous trees (O'Kennedy and Titus 1979; Wetzel et al. 1989, 1991). Mobilization at an even earlier stage is known from tree species blossoming in late winter, e.g. the willows (cf. Sauter 1981; Sauter and van Cleve 1992). In contrast to starch, protein deposition does not occur before autumn. The close temporal relationship to leaf senescence (Côté and Dawson 1986; Sauter and van Cleve 1990) suggests that this is evoked mainly by the breakdown of leaf proteins and the rescue of their nitrogen in the wood and bark storage parenchyma. Although other inducing factors like nitrogen and low temperature (van Cleve and Apel 1993), short day treatment (Langheinrich and Tischner 1991; Coleman et al. 1991), and ringing of twigs (Sauter and Neumann 1994) have been reported, leaf senescence appears to be most closely involved in the induction of storage protein deposition. Such vegetative storage proteins of trees have already been characterized for poplar (van Cleve et al. 1988; Clausen and Apel 1991; Stepien and Martin 1992) and several other angiosperm and gymnosperm tree species (e.g. Wetzel and Greenwood 1989, 1991a, b; Wetzel et al. 1989; Harms and Sauter 1992). Interestingly, there are closely related storage proteins found in the wood and the bark of one species (Stepien et al. 1992), in related species, e.g. in Taxodiaceae (Harms and Sauter 1992), and in poplar and willow (Sauter and van Cleve 1989b).

The fat content of poplar twigs always reached its maximum in summer but was still prominent in winter (Fig. 5b). With an amount of 4–8 µg/mg DW it makes a significant contribution to the storage material in poplar wood. The clear-cut decrease during budbreak gives further evidence that fat is mobilized for new growth during spring. The lack of a prominent increase during autumn clearly excludes a noteworthy starch-to-fat conversion in poplar wood. By contrast, Jeremias (1968) found an increase of fat during the cold period in the bark of some poplar clones. However, in spruce trunkwood the content of triacylglycerol was also low in winter and high in spring and summer (Höll 1985). No great variation, with only a slight increase in November and again in February, was found in the wood of Scots pine (Fischer and Höll 1992). All these results support the conclusion that there is no large-scale conversion of starch into fat during autumn in poplar, and also that there is no fat maximum in winter.

On the other hand, a remarkable increase in free glycerol content during winter was found in our study for the first time (Fig. 5c), in contrast to earlier studies on trees (cf. Sakai and Larcher 1987). Because the apoplast has been washed out before the biochemical analyses were performed (cf. Materials and methods) this glycerol must be located in the parenchymatous cells themselves, but whether and to what extent it might take part in protecting the cells from freezing injury is still completely open.

Regarding the content of various sugars during individual physiological stages of the year, there are some interesting relationships. Firstly, there is apparently no relation between the intensity of starch deposition and the content of either sucrose or of total sugars in the tissue (Figs. 2a–c, 3). Secondly, there is a clear-cut relation between the content of the maltose and the period of starch resynthesis and starch mobilization in spring and again during the starch-to-sugar conversion in autumn. During

the latter period, a most prominent maltose pool is built up in the cells which could be shown earlier to persist at temperatures of about 10° C and to disappear at 0–5° C and below during the formation of sucrose and its galactosides (Sauter 1988; Sauter and van Cleve 1993). Neither the site of accumulation of this huge amount of maltose, nor details of its physiological regulation are known yet. Thirdly, there is a very close relationship between the degree of starch disappearance in poplar wood during late autumn and winter, the outdoor temperatures, and the level of sucrose+raffinose+stachyose reached in the tissue (cf. Sauter and Kloth 1987; Sauter and van Cleve 1991). Fourthly, there is no obvious relationship between fat synthesis in summer and the sugar level. Interestingly, however, fat content clearly increased above controls – as did starch – when a surplus of photosynthates was created by ringing (Sauter and Neumann 1994). Its causal involvement thus became obvious. The observation made at the cellular level that the fat content of contact cells in rays increases markedly in summer, parallel to their starch disappearance which proceeds in advance of that of the neighbouring ray cells (cf. Sauter and van Cleve 1989a), furthermore illustrates that there are noteworthy differences, even within various cell types of one tissue, and that we are far from understanding the regulation of these events at the cellular level. With the seasonal patterns of the individual storage materials now fairly well disclosed for poplar, we hope that further analyses at the enzymatic level will help to give deeper insight into the factors governing storage, mobilization and adaptation processes in trees.

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