Evidence for a 26kDa vegetative storage protein in the stem sapwood of mature pedunculate oak

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EVIDENCE FOR A 26kDA VEGETATIVE STORAGE PROTEIN IN THE STEM SAPWOOD OF MATURE PEDUNCULATE OAK

Luis M. Valenzuela Núñez, Dominique Gérant, Pascale Maillard, Nathalie Bréda, Guillermo González Cervantes and Ignacio Sánchez Cohen

SUMMARY

The distribution of total soluble proteins was investigated in the sapwood of 20-year-old Quercus robur *L. at leaf shedding (October) and after leaf expansion (June) in order to assess seasonal changes in soluble protein content at whole tree level. The content of total soluble proteins was 10 times lower than that of starch. In October, the top sapwood segments stored significantly more soluble proteins than other organs. In the below canopy stem, up to 80% of the proteins present during October were mobilized for spring growth, as compared to only 50-60% of the starch. As oak trees produce a large fraction of annual radial growth before spring-leaf expansion, vegetative storage proteins (VSP) in the stem are hydrolyzed and a part of the resulting free amino acids pool should be used to sustain wood formation. A 26kDa VSP was isolated by SDS-PAGE. At leaf shedding, this VSP represented up to 50% of the soluble proteins, while it was absent in June. This suggests this protein was remobilized to sustain early growth. The use of this 26kDa VSP as a marker for spring growth reactivation capacity is proposed.*

Introduction

Since the 80s, trees are known to store large amounts of proteins before leaf shedding (Kang and Titus, 1980). Remobilization of these compounds during the following spring is necessary for the new growth while nutrient acquisition remains still limited (Staswick, 1994; Stepien *et al*., 1994). Vegetative storage proteins (VSP) correspond to a heterogeneous group and there is no specific biochemical assay. VSPs can only be identified by their relative transient pattern of temporary accumulation in dormant organs and loss during the growing season (Staswick, 1994). VSP are highly N-enriched proteins that are intensively mobilized for spring reactivation, including but burst, leaf expansion and cambial growth. Such storage proteins were first identified in fruit trees (O'Kennedy and Titus, 1979) and in poplar (Sauter and van Cleve, 1990; Stepien *et al*., 1994). In poplar, VSPs have been extensively characterized, and a 32kDa VSP was isolated and identified. The protein is encoded by a small multigene family from which one member has been cloned. Expression of these genes is influenced by shoot growth, photoperiod, nitrogen availability and wounding (reviewed by Cooke and Weih, 2005). Histochemical studies have previously shown the presence of VSP in vacuoles of parenchymateous bark cells, cambium and xylem of poplar (Wetzel *et al*., 1989; Clausen and Apel 1991; Stepien and Martin 1992) and of tropical tree species (Tian *et al*., 2003). VSPs have been identified due to changes in the overall protein pattern of bark tissues of two- to three-years old branches of several tree species between winter and spring (Sauter and van Cleve, 1990; Wetzel and Greenwood, 1991) and protein patterns are in agreement with histochemical studies (Wetzel and Greenwood, 1991). The seasonal patterns of VSPs at whole tree level are still poorly known (Tian *et al*., 2003) particularly in mature temperate forest trees in which carbon storage was well characterized (Hoch *et al*., 2003). A large amount of total non-structural carbohydrates (TNC) was shown to occur in twigs and coarse roots of adult *Quercus petraea* (Barbaroux *et al.,* 2003; Marçais and Bréda, 2006), but to our knowledge, no VSP has been identified until now in *Quercu*s. The aims of this study were to identify VSPs among soluble extracted proteins in perennial organs of mature *Quercus robur* L., and to elucidate 1) their distribution within the whole tree, 2) their quantitative importance in respect to other soluble N-compounds and 3) to C-storage compounds.

Materials and Methods

Study plot

The study plot was set up in an even-aged, 20-year-old stand of *Quercus robur* in a natural regeneration in the Champenoux communal forest, located 15km east of Nancy, NE France (48°44'N, 6°14'E, altitude 237m). Oaks were the dominant trees with an understory of *Carpinus betulus* L. The soil was homogeneous across the stand and was a hydromorphic clayey loam with a calcareous clay layer appearing 30-45cm below the soil surface.

KEYWORDS / C Storage / Mature Tree / N Storage / *Quercus robur* L / Sapwood / Vegetative Storage Protein /

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EVIDENCIA DE UNA PROTEÍNA DE DEPÓSITO VEGETATIVO DE 26kDA EN LA SAVIA DEL TALLO DE ROBLE PEDUNCULADO MADURO

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RESUMEN

La distribución de proteínas solubles totales fue investigada en la albura de Quercus robur *L. de 20 años, al momento de la caída de las hojas (octubre) y después de la expansión de las hojas (junio), a fin de evaluar los cambios estacionales en contenido de proteína soluble a nivel del árbol intacto. El contenido de proteínas solubles totales fue 10 veces menor que el de almidón. En octubre, los segmentos superiores del la albura almacenaron significativamente más proteínas solubles que otros órganos. En el tallo bajo el dosel, hasta el 80% de las proteínas presentes en octubre fue mobilizado para el crecimiento de primavera, comparado a solamente 50-60% del almidón. Como los robles producen una fracción grande del crecimiento anual antes de la expansión foliar de primavera, las proteínas de depósito vegetativo (PDV) en el tallo son hidrolizadas y parte del pool de aminoácidos libres resultante ha de ser utilizado para sustentar la formación de madera. Una PDV de 26kD fue aislada por SDS-PAGE. Al momento de la caída de las hojas, esa PDV representó hasta 50% de las proteínas solubles, mientras que en junio estuvo ausente. Esto sugiere que esta proteína fue remobilizada para sustentar el crecimiento. Se propone el uso de esta PDV de 26kD como un marcador de la capacidad de reactivación del crecimiento primaveral.*

EVIDÊNCIA DE UMA PROTEÍNA DE DEPÓSITO VEGETATIVO DE 26kDA NA SAVIA DO CAULE DE CARVALHO-ROBLE MADURO

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RESUMO

A distribuição de proteínas solúveis totais foi investigada na alburno de Quercus robur *L. de 20 anos, no momento da caída das folhas (outubro) e depois da expansão das folhas (junho), com o fim de avaliar as mudanças estacionais em conteúdo de proteína solúvel a nível da árvore intacta. O conteúdo de proteínas solúveis totais foi 10 vezes menor que o de amido. Em outubro, os segmentos superiores da alburno armazenaram significativamente mais proteínas solúveis que outros órgaõs. No caule sob o dossel, até 80% das proteínas presentes em outubro foi mobilizado para o crescimento de primavera, comparado a somente 50-60% do amido. Como o Carvalho-roble produz uma fração grande do crescimento anual antes da expansão foliar de primavera, as proteínas de depósito vegetativo (PDV) no caule são hidrolizadas e parte do pool de aminoácidos livres resultante deve de ser utilizado para sustentar a formação de madeira. Uma PDV de 26kD foi isolada por SDS-PAGE. Ao momento da caída das folhas, essa PDV representou até 50% das proteínas solúveis, enquanto que em junho esteve ausente. Isto sugere que esta proteína foi remobilizada para sustentar o crescimento. Propõe-se o uso desta PDV de 26kD como um marcador da capacidade de reativação do crescimento primaveral*

The humus layer was an eutrophic mull (pH 4.7) (Marçais and Bréda, 2006). Climate is oceanic with a continental influence. Average rainfall is ~744mm in Champenoux, and annual temperature 9.2°C.

Destructive sampling

Eight oaks with similar trunk circumference at 1.30m height were selected in October 2001. Four of those were felt at leaf fall (23rd October 2001). The remaining trees were felt the following spring after full expansion of leaves (13th June 2002). Tree samples were taken from various organs (stem, branches and coarse roots). Disk-like samples of stem (1-2cm in longitudinal thickness) were taken from heights of 0, 1.3 and 3meters, and below canopy. Additional samples were taken from three lateral branches at different heights in the canopy. One segment was cut from the middle of each branch. Furthermore, samples were collected from three lateral coarse roots (∅ >5mm).

Each tree component was quickly frozen in liquid nitrogen and brought in a Dewar container to the nearby laboratory (6km away) for storage at -80°C before freeze-drying for one week (bulk tray dryer Dura-Top™, FTS Systems™, Stone Ridge, New York, USA). Sapwood from the various organs was kept and cut into small pieces with a saw, then ground to a fine homogeneous powder in a laboratory mill (Cyclotec 1093 Tecator, Höganäs, Sweden).

Nitrogen contents

Total N content of the sapwood of each organ was measured after combustion of aliquots of freeze-dried powders in an elemental analyzer (NA 1500 NCS, Carlo Erba, Milan, Italy).

TNC extraction and analysis

Total non-structural carbohydrates (TNC) were extracted from 10mg of sapwood from each organ, ground with 650µl of cold methanol:water solution (7:3). The extracts were centrifuged at 17000g for 10min. The extraction on the pellet was repeated twice. The supernatants were combined and used to colorimetrically determine soluble sugar content. Starch was hydrolyzed to glucose from the pellet by α-myloglucosidase (EC 3.2.1.3, Boehringer Manheim Biochemicals, Mannheim, Germany) in $0.32M{\cdot}l^{-1}$ citrate buffer, pH 4.2 at 48°C for 30min. Glucose content was assessed colorimetrically at 620nm (spectrophotometer UV-visible DU 640B, Beckman Coulter, USA) as described by van Handel (1968), using glucose as standard.

Protein extraction for quantification and SDS-PAGE

Sapwood samples (10mg of dry matter) were ground at 4°C in 2ml of extraction buffer (0.1M phosphate buffer pH 7.4, 5mM DTT, 0.3% (m/v) PEG 6,000, 1.3% (m/v) PVP 20000, 1µM leupeptin, and 1µM pepstatin) by using a balk crusher

(Retsch MM 301, Gmbh & Co, Germany) during 45s, twice. Homogenates were centrifuged twice at 12000g at 4°C for 15min and the supernatants were used for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) after boiling for 5min with an equal volume of 2× Laemmli sample buffer (Laemmli, 1970). Protein content from the supernatants was determined colorimetrically at 595nm according to Bradford (1976) using bovine 1 serum albumin as standard.

SDS-PAGE.

Electrophoresis was performed using 14% SDS-polyacrylamide gels; 0.025M Tris base, 0.192M Glycine, and 0.1% SDS for running buffer (pH 8.3) at 20mA/gel. Gels were stained with silver nitrate according to Yan *et al.* (2000).

Image analysis

Gels were digitalized with a Bio-Rad model GS 690 Imaging densitometer scanner. The integrated intensity of protein bands was measured manually using Bio-Rad Multi Analyst software and then plotted relative to the total intensity of the proteins in the lane. Bands with the annual variation (accumulation in autumn and winter, marked decrease in spring and very low content until the end of the summer) were defined as VSP. The intensity of a band being correlated to its protein content (Langheinrich and Tischner, 1991; Noquet *et al.*, 2001), it was possible to plot the contribution of each band to the total protein content.

Total amino acid analysis

Free amino acids were extracted at 4°C by grinding 10mg of ground dry matter of each organ with 650µl of methanol/water solution (7/3) during 10min. The extract was centrifuged 5min at 17000g. The supernatant was kept, the pellet was rinsed twice with 650µl of MW solution and each time centrifuged 5min at 17000g. The supernatants were kept and combined with the first one for amino acid quantification. Free amino acid content was assessed colorimetrically at 570nm as described in the ninhydrin method of Yemm and Cocking (1955), with leucine as standard.

Nitrate analysis

Ten mg of lyophilised tissues were incubated for 1h at 45°C in 1ml of distilled water. The suspension was centrifuged for 15min at 5000g and nitrate content from the supernatant was measured colorimetrically at 410nm according to Cataldo *et al.* (1975), using NO_3 as standard.

Statistical analyses

Data were analyzed by one, two or three ways analysis of variance (ANOVA), using the SAS software package, version 6.12 (SAS, Cary, NC, USA). Unless otherwise mentioned, dates, organs or species differences were considered significant if $P<0.05$.

TABLE I
TOTAL NITROGEN IN THE SAPWOOD OF STEM, BRANCHES AND COARSE ROOTS OF MATURE *Quercus robur* TREES

	October 2001	June 2002	P-values
Total N (g·100g^{-1} DW)			
Branches	0.26 ±0.02 a	0.22 ±0.01 a	0.1513
Below canopy stem	0.17 ±0.02 b	0.13 ±0.02 b	0.2276
Stem 3	0.15 ±0.03 b	0.11 ±0.02 b	0.3175
Stem 130	0.16 ±0.03 b	0.14 ±0.02 b	0.5633
Stem 0	0.13 ±0.01 b	0.14 ±0.01 b	0.6880
Coarse roots	0.19 ±0.02 b	0.26 ±0.03 a	0.1679

For a given sampling date, similar letters indicate no significant difference among compartments (Anova, $p>0.05$). The significance of sampling date effects are given by P-values. Means ±SE (n=4). Stem 0: 0-1.30m height, Stem 130: 1.30m height, Stem 3: 3m height.

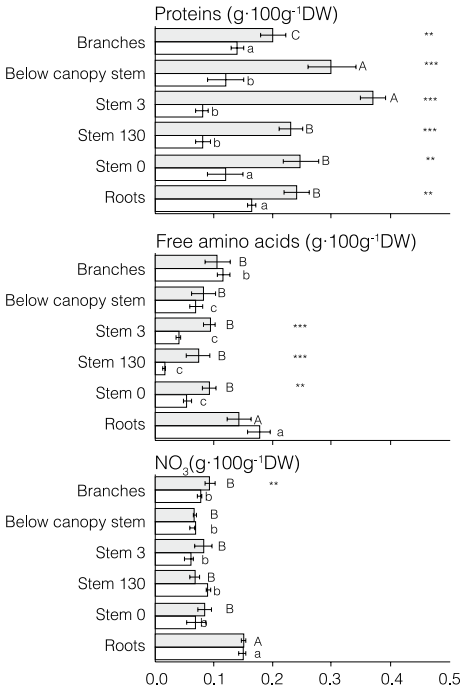

Figure 1. Soluble protein, amino acid and nitrate contents (g·100g^{-1} DW) in the sapwood of various perennial compartments of 20-year-old *Quercus robur* trees sampled during October 2001 (grey bars) and June 2002 (white bars). Means ±SE (n= 4). Stem 0: 0-1.30m height, Stem 130: 1.30m height, Stem 3: 3m height. Stars indicate significant differences between dates (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Different letters for the same sampling date (capital letters for October and small letters for June) indicate significant differences to the two-way ANOVA followed by the Tukey test ($P<0.05$).

Results

Seasonal variations of total N and soluble nitrogenous compounds

Nitrogen content of sapwood was significantly higher in branches than in stem and did not change with date except in roots, where N content increased in June, in branches (Table I). Soluble proteins of sapwood represented less than 0.35g·100g^{-1} DW (Figure 1). In October, the highest content was observed in the stem segment close to the canopy (Stem 3, below canopy stem) whereas sapwood of branches, stem-collar and roots displayed the highest contents during June. Between October and June, soluble proteins decreased dramatically (-80%) in the stem close to canopy, and less so in roots and branches (-30%). As a consequence, branches and roots exhibited the highest soluble protein contents within the tree during June. Free amino acid content represented less than 0.2g·100g^{-1} DW in the sapwood and changed in the lower stem segments, where a significant decrease (-75%) was observed between October and June (Figure 1). In June, the highest amino acid contents were found in roots, stem under the canopy and branches. Nitrate content was larger in the sapwood of roots compared to other organs (0.15g·100g^{-1} DW; Figure 1). Nitrate content remained stable from October to June, except in the branches, where it increased significantly in June.

Seasonal variation of TNC

Starch never exceeded 25g·100g^{-1} DW in the sapwood (Figure 2). The highest content was found in the coarse roots and in stem collar (stem 0m). Starch decreased significantly between October and June (about -50%) in all compartments except in the below canopy stem (non significant). Soluble sugar content was ten times smaller (Figure 2). While in October there were no differences between organs, during June soluble sugar content was significantly higher in branches and roots than in the stem. From October to June, soluble sugars decreased slightly but significantly in the stem (0m, 1.30m and below canopy).

Isolation of a vegetative storage protein

As the major decrease in soluble proteins was detected in the stem, proteins from the sapwood of this organ were

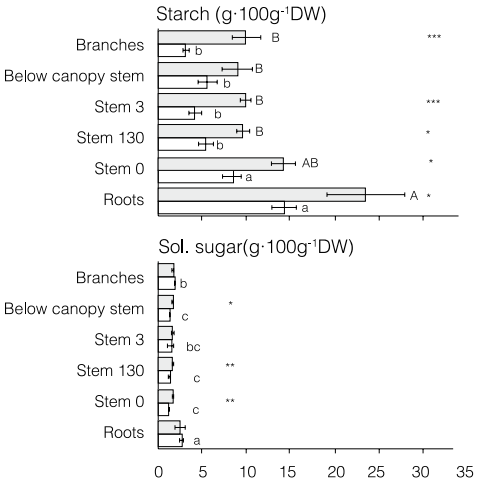

Figure 2. Starch and soluble sugar contents (g·100g^{-1} DW) in the sapwood of various perennial compartments of 20-year-old *Quercus robur* trees sampled during October 2001 (grey bars) and June 2002 (white bars). Means ±SE (n= 4). Stem 0: 0-1.30m height, Stem 130: 1.30m height, Stem 3: 3m height. Stars indicate significant differences between dates (* p<0.05, ** p<0.01, *** p<0.001). Different letters for the same sampling date (capital letters for October and small letters for June) indicate significant differences to the two-way ANOVA followed by the Tukey test (P<0.05).

separated by SDS-PAGE. The protein patterns in the presence of DTT are shown for the sapwood of the stem below the canopy (Figure 3). Nearly 20 protein bands were separated in each lane. One VSP, at 26kDa (arrow), exhibited the largest decrease between October and June. It was not possible to isolate the 26kDa VSP when the DTT was absent from the sample buffer. During October, the relative intensity of the 26kDa band varied with height in the stem; the highest intensity was observed in the sapwood just below the canopy (Figure 4). The 26Da VSP accounted for 20% of the total soluble proteins in the sapwood of the stem at the collar and for about 50% in the sapwood stem below the canopy (Figure 4).

Discussion

In mature oak trees, TNC storage in the sapwood of perennial organs corresponded with well documented figures for *Quercus* species (Barbaroux *et al*., 2003; Hoch *et al*., 2003). On a dry weight basis, starch was the major storage compound. The highest starch content was found in coarse roots, similarly to the reports by Barbaroux *et al*. (2003), who observed the same distribution of TNC among the organs of 45 year-old *Q. petraea* (Matt.) Liebl. This starch distribution was related to a higher parenchyma/xylem ratio in roots than in the stem, as a result of less ageing processes in roots (Helinska-Raczkowska, 1994). The storage carbon pool probably increased in the vicinity of highest priority sinks, enabling spring fine root growth and efficient nutrient acquisition (Barbaroux *et al*., 2003). As already reported in *Q. petraea*, the utilization of TNC between October and June occurred in all organs in *Q. robur* proportionally to the available content, so that the distribution pattern remained unchanged within the trees between the two dates (Figure 2).

Limiting nitrogen resources are known to constrain growth of trees (Dyckmans and Flessa, 2001). The ability to store and to redistribute internal N resources is a fundamental element of the C and N balance of forest trees and other perennials. On a content basis, sapwood is considered as the main N-storage tissue in deciduous trees (Millard and Proe, 1991). The present results show that the N content of oak sapwood was low (about 0.3% DW) compared to that of leaves (2.30 ±0.06% DW), and stable between October and June. In spring, before leaf development is achieved, trees are expected to remobilize available N from perennial tissues such as bark and mature xylem (Bao *et al*., 1992). In sapwood of mature oaks, soluble N was mostly stored as soluble proteins, rather than free amino acids. Considering the nitrogen to protein conversion factor (Yeoh and Wee, 1994) and the total N and protein contents in October, 64 and 45% of total N was present as N-proteins in the stem sapwood at 3m height and below the canopy, respectively.

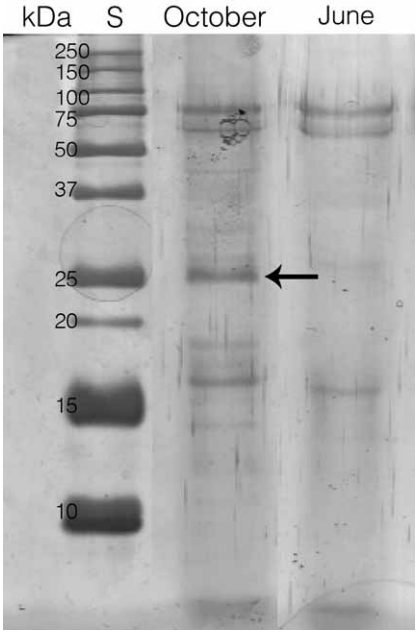

Figure 3. SDS-PAGE profiles of soluble proteins of stem sapwood sampled below the canopy of 20-year-old *Quercus robur* trees in October 2001 (leaf absence, lane 1) and June 2002 (leaf maturity, lane 2), showing changes in the prominent 26kDa polypeptide (arrow) between the two dates. Lane S, standard proteins. Two µg of protein was loaded per 20 lane.

Soluble protein distribution within the tree changed with the seasons. During October, the upper part of the stem sapwood (at 3m height and below the canopy) stored significantly more soluble proteins than other organs. By contrast, in June, branches and roots stored more proteins than the stem. These different distributions reflect a chronological sequence for the remobilization of soluble proteins in distinct organs. From October to June, the sapwood of the youngest stem parts (3m height and below the canopy) exhibited the strongest decrease in soluble protein content. These results suggest a preferential accumulation of vegetative storage proteins (VSP) in the sapwood of the youngest parts of the stem. Stem sapwood presenting both the highest soluble protein content in October and the largest biomass at whole tree level, the study was focused on the isolation of VSP from the stem sapwood. Soluble proteins were separated by SDS-PAGE and about 20 bands were detected. Among them, a 26kDa polypeptide, only present in October, and representing a major band of the soluble proteins pattern, corresponded to the definition of a vegetative storage protein (VSP).

Among tree species, the molecular mass of VSPs varies usually from 15 to 45kDa in SDS conditions (Stepien *et al*., 1994), 16.5-19kDa for peach tree (Gomez and Faurobert, 2002), 32-36kDa in bark poplar (Coleman *et al.,* 1993). In *Populus euramericana*, native VSP molecular mass was 110-230kDa (Stepien and Martin, 1992). Some VSP were

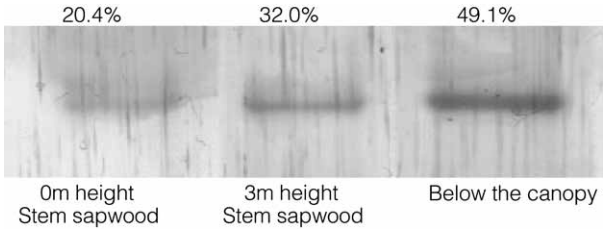

Figure 4. Intensity of the 26kDa polypeptide from the SDS-PAGE profiles of stem sapwood of 20-year-old *Quercus robur* trees sampled in October 2001 (leaf absence) at the collar (lane 1), 3m above the collar (lane 2) and below the canopy (lane 3). The same amount of total soluble proteins (2µg) was loaded per lane. Numbers on the last line correspond to the amount of the 26kDa polypeptide expressed as percent of the amount of total soluble proteins.

identified as lectins or glycoproteins in apple (O'Kennedy and Titus, 1979) and in poplar (Stepien *et al.,* 1994). Glycosyl residues can represent 10-22% of the sugars present in bark poplar (Stepien *et al*., 1994) which confer a high cellular thermostability. A VSP is a polypeptide present in an important rate than other polypeptides; furthermore, it is absent in the vegetative period and present in the dormancy period (Chapin *et al*, 1990). The biochemical characteristics of the 26kDa VSP identified in the present experiment remain to be revealed. The absence of polypeptides for non-reducing SDS-PAGE analysis should indicate that denaturing in the presence of DTT can convert an oxidized protein with high molecular mass in reduced monomeric subunits. The presence of cysteine residues in the protein, allowing reduction of disulphide bridges, could explain this particularity. A cysteine rich VSP present in wheat seeds was identified as an endoprotease (Callis, 1995).

The highest decrease in soluble protein content between October and June was observed in the upper part of the stem and coincided in October with the highest contribution of the 26kDa to the soluble protein fraction (up to 49%). This supports the idea of a large remobilization of the 26kDa VSP for stem growth. Oak trees achieve at early wood growth before leaf expansion in spring (Dougherty *et al*., 1979; Bréda and Granier, 1996; Barbaroux and Bréda, 2002) to recover from winter embolism and regain functionality for water flow before the onset of transpiration. In June, the decreased soluble protein contents in perennial organs of mature oak could reflect their use, in part, for early wood growth of both stem and canopy. Tian and Hu (2003) suggested that proteinaceous materials are absent from the vacuoles of bark tissues and new shoot growths; nevertheless, the study was carried out in whole sapwood of mature trees, where VSP isolation was not possible due to high contents in contaminants (polyphenols and other substances). Furthermore, plants are unique in their ability to store proteins in specialized protein storage vacuoles (PSVs) within seeds and vegetative tissues. It is well known that accumulation of proteins into PSVs and later degradation for renewed growth is important physiologically for many plant cells and organs, and vacuolar accumulation of proteins is not only as VSP but also soluble proteins (Greenwood *et al*., 1986; Sauter *et al*., 1988; Sauter y Wellenkamp, 1988; Wetzel *et al*., 1989, 1991).

The first steps of wood growth (reviewed by Plomion *et al*., 2001) consist in high cell division and cell expansion initiated by cambial activity. The following steps correspond to wood biosynthesis with ordered deposition of a thick secondary cell wall mainly made up of polysaccharides (cellulose, hemicellulose), lignin and cell wall protein. Cell walls are a place of lively metabolic activity, containing a wide range of proteins with different functions beside structural proteins (Carpita and Gibeaut, 1993; Cassab, 1998). All these steps are influenced by the presence of numerous proteins driving many properties: i) enzymes, for example: pectin methyl esterase, pectinase, cellulose synthase (Vander Mijnsbrugge *et al*., 2000; Plomion *et al*., 2001); and ii) cell wall structural proteins, for example: extensin and proline rich proteins, which are sequestrated in the cell wall matrix (Cassab, 1998).

Rowland and Roberts (1999) reported that different lignin preparations from leaves of a range of perennials, grass, shrubs and trees contained up to 30% of the foliar N. In poplar stem, numerous xylem-linked proteins appeared during summer and most of them were identified as enzymes involved in the phenyl propanoid pathway linked to xylogenesis at the expense of 32kDa and 36kDa VSP, which was abundant in January and disappeared between April and July (Vander Mijnsbrugge *et al*., 2000). In oak, between October and June, VSPs were hydrolysed and a part of the resulting free amino acids pool should be used to sustain wood growth. The other part of this pool should be exported to other sinks. Decrease of soluble protein content between October and June was not accompanied by an accumulation of free amino acids in June (Figure 1). This may probably be linked to either rapid utilization or amino acids export. Even during winter, specific protein turnover and maintenance metabolism have to be considered, and a more detailed kinetic coupled with ^{15}N experiments would be of interest, although such experiments remain difficult to realize on mature trees.

Soluble protein contents were ten times lower than that of starch, in agreement with high C/N ratio usually found in wood. However, even if soluble protein contents were lower than TNC, the role of these compounds seems not to be negligible, if it is considered that up to 80% of the protein content present in October was mobilized between October and June. In comparison, in the stem ~50-60% of the starch content present in October was mobilized (Figure 2). Thus, in mature pedunculate oak trees, stored proteins should be a growth limiting factor for new growth. On a pluriannual scale, any perturbation in protein storage could have a detrimental effect on the ability for regrowth. Moreover, as the 26kDa VSP corresponded to about 50% of the soluble protein content in mature oak trees, this protein may serve, in autumn, as an indicator of the reactivation capacity in the following spring.

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