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# Distribution and partial characterization of seasonally expressed proteins in different aged shoots and roots of 'Loring' peach (*Prunus persica*)

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**Summary** During autumnal leaf senescence, leaf nitrogen in deciduous trees is translocated to storage sites, especially bark and xylem tissues. Proteins that accumulate in large amounts in bark and xylem in winter and are absent from these organs in summer are called storage proteins, and are believed to be vehicles for storing nitrogen reserves. These reserves are important for spring growth and help trees tolerate or recover from both abiotic and biotic stresses. Based on seasonal patterns of accumulation, we previously identified three storage proteins with molecular masses of 60, 19 and 16 kDa in bark tissues of 'Loring' peach (*Prunus persica* (L.) Batsch). To characterize the distribution of these proteins in different-aged tissues and to determine if they have any function other than nitrogen storage, we examined their seasonal distribution in bark tissues of current-year and 1-year-old shoots, scaffold branches, main trunks and 4–5-year-old roots of 'Loring' peach. Verification of protein identity was based on molecular mass and reactions with antibodies directed against each specific protein. Protein distribution was variable. For all three proteins, the greatest amount was present in mid-winter in current-year and 1-year-old shoots. These tissues also showed the greatest seasonal variation in the amount of protein present. The 16 kDa protein was present only in the youngest shoots, whereas the 19 kDa protein was present in all tissues examined. The 60 kDa protein was absent in root tissue. The amino acid composition and sequence of each protein were determined. The 60 kDa protein was identified as a dehydrin, and the 19 kDa protein appeared to be related to a family of allergen proteins in Rosaceous plants, some members of which are associated with pathogenesis-related proteins. The amino acid sequence of the 16 kDa protein appeared to have no homology with any proteins in the SwissProt database. Therefore, it is likely that the 16 kDa protein, in a strict sense, is a bark storage protein. Defining storage proteins solely by their pattern of accumulation and the extent to which they accumulate may not be a good functional definition. It is possible that storage proteins have functional roles in addition to nitrogen storage.

*Keywords: cold acclimation, dehydrins, dormancy, nitrogen metabolism, pathogenesis-related proteins, storage proteins, stress proteins.*

## Introduction

During autumnal leaf senescence, leaf proteins in deciduous trees are hydrolyzed and translocated in phloem sap to other organs for storage (O'Kennedy and Titus 1979, Kang and Titus 1987). Proteins that accumulate in large amounts in autumn and are degraded at the onset of spring growth are called storage proteins. These proteins represent the major component of the overwintering reserves of nitrogen and play a key role in supporting growth in early spring. Although storage proteins have been extensively studied in herbaceous plants, little information is available for woody plants (Stepien et al. 1994, Coleman 1997). Among woody plants, a bark storage protein in poplar has been the most extensively studied. The gene(s) encoding this protein has been isolated and control of expression by photoperiod has been demonstrated (Langheinrich and Tischner 1991, Coleman et al. 1992).

We previously identified three peach (*Prunus persica* (L.) Batsch) proteins with molecular masses of 16, 19 and 60 kDa, that accumulate in large amounts in bark and xylem tissues during autumn, and subsequently decrease and are no longer detectable after bud break (Arora et al. 1992, 1996, Arora and Wisniewski 1994). The 60 kDa protein has been identified as a dehydrin (a stress-related protein) and we have cloned the gene coding for this protein and characterized its induction by cold, drought and abscisic acid (Artlip et al. 1997, Artlip and Wisniewski 1997). No identity has yet been ascribed to the 16 and 19 kDa proteins of peach (Arora and Wisniewski 1994).

We have previously described seasonal fluctuations in these proteins in current-year shoots and in leaves, but little is known about their distribution in different-aged tissues or whether they have any function other than nitrogen storage. To gain a better understanding of the role(s) of the three proteins, we determined their seasonal distribution in bark tissues of

current-year and 1-year-old shoots, scaffold branches, main trunks and 4–5-year-old roots of 'Loring' peach. We also obtained information on the identity of these proteins, based on their sequence homology with other proteins in the SwissProt database.

## Materials and methods

### *Plant material and tissue collection*

For 1 year, beginning in August, tissue samples were collected monthly from a block of 14-year-old 'Loring' peach (*Prunus persica* L. Batsch) trees growing at the USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV. Bark samples were taken from current-year and 1-year-old shoots, scaffold limbs, trunks and 4–5-year-old roots. New roots were exposed by excavation at each sampling period. Bark tissues were scraped from collected shoots and roots or obtained with a cork borer from scaffold limbs and trunks. After collection, tissues were immediately ground with a mortar and pestle in liquid nitrogen and stored at –80 °C until further processing.

### *Protein extraction, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting*

Proteins were extracted in borate buffer (50 mM sodium borate, 50 mM ascorbic acid, 1 mM PMSF) at 4 °C and separated by SDS-PAGE as previously described (Wetzel et al. 1989, Arora et al. 1992). Briefly, bark tissue was homogenized at 4 °C in borate buffer for 90 s with a Kinematica Polytron (Kriens-Luzern, Switzerland), and then centrifuged at 26,000 *g* for 1 h at 4 °C. The resulting supernatant was collected, filtered successively through 0.4 and 0.2 μm filters, and assayed for protein content by a modified Bradford assay (Ramagl and Rodriguez 1985). The appropriate volume of extraction buffer containing 200 μg of protein was diluted to 1.0 ml with deionized water. Protein was precipitated from the diluted sample by adding 0.1 ml of trichloroacetic acid (final concentration about 10% (v/v)). The precipitate was collected by centrifugation at 16,000 *g* for 15 min at 4 °C, washed with acetone, and dried. The pellet was resuspended in SDS-PAGE loading buffer, boiled for 3 min, and cooled to room temperature. Discontinuous SDS-PAGE was performed with a PROTEAN II electrophoresis unit (Bio-Rad, Hercules, CA) using a 4% stacking gel and a 12.5% running gel. Gels were stained with the fluorescent dye, SYBR Orange (Molecular Probes, Eugene, OR). Seasonal patterns of protein expression and quantification of specific proteins were observed and documented with a STORM gel and blot imaging and analysis system (Molecular Dynamics, Sunnyvale, CA).

The immunoblotting procedures followed are described in Arora and Wisniewski (1994). Immunoblots were probed with polyclonal antibodies directed against one of the three proteins of interest. Details on the preparation of the anti-19 kDa and anti-16 kDa antiserums are provided in Arora et al. (1996). Details on the anti-dehydrin antiserum are provided in Arora and Wisniewski (1994). Separated proteins (~3 μg) were electroblotted onto 0.45 μm nitrocellulose membranes with a Mini Trans-Blot Electrophoresis Transfer Cell (Bio-Rad). Electroblotting was carried out for 1.25 h at 100 V of field intensity in Towbin buffer (25 mM Tris-HCl, pH 8.3, containing 192 mM glycine and 20% (v/v) methanol) at 4 °C. Immunoblots probed with the 16 and 19 kDa antibodies (diluted 1:2000) were blocked with 1% bovine serum albumin in Tris-buffered saline containing Tween 20 (10 mM Tris-HCl, pH 8.0, 150 mM NaCl and 0.05% Tween 20). Immunoblots probed with the dehydrin antibody (diluted 1:1000) were blocked with gelatin. Immunoreactive bands were detected by alkaline phosphatase assay (ProtoBlot Western AP kit, Promega, Madison, WI).

### *Protein composition, sequencing and sequence comparison*

To analyze the proteins, bands containing the proteins of interest were excised from SDS-PAGE gels and hydrolyzed in 6 *N* HCl. Liberated amino acids were derivatized by reaction with phenylisothiocyanate, and the resulting phenylthiocarbamyl amino acids were quantitatively determined by reverse-phase high performance liquid chromatography (HPLC) at the Protein and Carbohydrate Structure Facility, University of Michigan, Ann Arbor, MI.

Partial amino acid sequence information for the 60 kDa protein had been obtained previously (Arora and Wisniewski 1994), and the full amino acid sequence was obtained from translation of the gene sequence for peach dehydrin as reported by Artlip et al. (1997). The 16 and 19 kDa proteins were partially purified by free-solution isoelectric focusing using a Rotofor (Bio-Rad), followed by SDS-PAGE separation as described by Arora and Wisniewski (1994). Bands representing the 16 and 19 kDa proteins were identified by immunoblotting and then excised from the SDS-PAGE gels for sequence analysis. This also allowed us to obtain the isolectric focusing point (pI) of the 16, 19 and 60 kDa proteins. Sequencing of the 16 and 19 kDa proteins was carried out by partial digestion at the Harvard Microchemistry Facility (Cambridge, MA), followed by partial purification by reverse-phase HPLC, and then N-terminal sequencing of individual peptides by Edman degradation. In a second approach, sequencing of peptide fragments was determined by NMR spectroscopy. Sequence comparisons were conducted with BLAST (National Center for Biotechnology Information) software.

## Results

Seasonal patterns of protein expression in current-year and 1-year-old shoots, scaffold, trunk and 4–5-year-old root bark tissues of 'Loring' peach trees are presented in Figure 1. Several major proteins in bark tissues exhibited seasonal patterns of regulation. Three of these proteins, with molecular masses of 60, 19 and 16 kDa, accumulated in bark tissue of current-year and 1-year-old shoots, scaffold, trunk and 4–5-year-old roots in fall, peaked in late fall to mid-winter, and were absent from bark tissue in spring after bud break. This seasonal pattern was most evident in current-year shoots (Figure 1A). The identity of these proteins in the various plant organs was

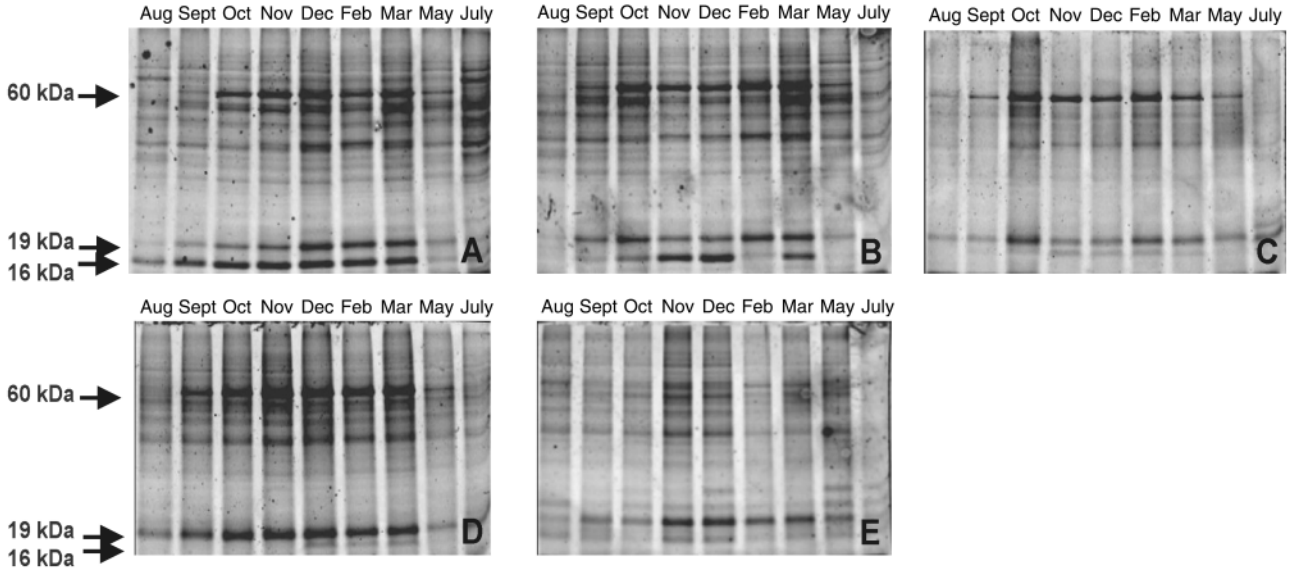


Figure 1. One-dimensional SDS-PAGE of soluble proteins obtained from peach bark tissues sampled over the course of a year. (A) Current-year shoots, (B) 1-year-old shoots, (C) scaffold limbs, (D) trunk and (E) roots. Three major proteins (60, 19 and 16 kDa) exhibiting a seasonal pattern of accumulation are identified by arrows.

confirmed by immunoblotting with separate antibodies specifically directed against each protein (Figure 2). This confirmation indicates that each of the specific proteins in the various tissues was immunologically related and most likely identical.

The extent of accumulation and the pattern of seasonal accumulation of the 16, 19 and 60 kDa proteins differed among tissue types (Figures 1–3). The 60 kDa protein was evident in current-year shoots, 1-year-old shoots, scaffold and trunk bark tissues, but was present in relatively low amounts in root bark tissues (Figures 1, 2 and 3A). The 19 kDa protein was present in all tissue types (Figures 1, 2 and 3B); it was most evident in current-year and 1-year-old shoots, but was present in relatively low amounts in other tissues (Figures 1, 2 and 3C). The 16 kDa protein was also the only protein that did not display a distinct seasonal pattern of accumulation in scaffold and trunk tissues.

Although general patterns of accumulation were evident, transient changes in the amounts of these proteins were also observed. These transient changes are most clearly illustrated in Figure 3 where the relative abundance of each of the proteins in each of the tissue types is presented. Densitometry plots of the 16 kDa protein revealed large amounts of protein

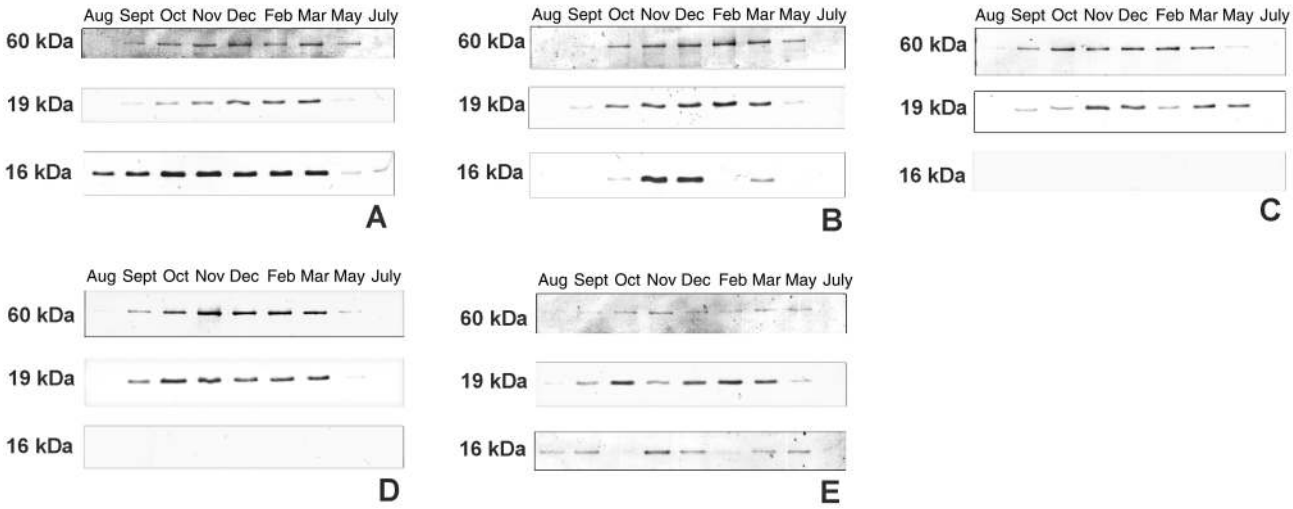


Figure 2. Immunoblots of soluble proteins obtained from peach bark tissues sampled over the course of a year. Each protein was identified based on reaction with polyclonal antibodies as described in the Materials and methods. (A) Current-year shoots, (B) 1-year-old shoots, (C) scaffold limbs, (D) trunk and (E) roots.

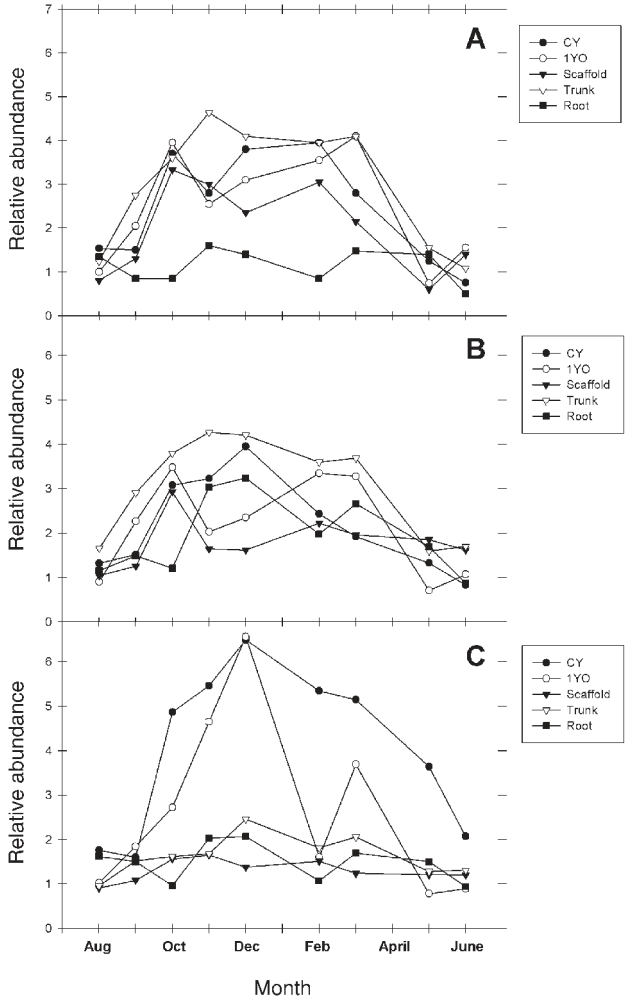


Figure 3. Quantification of 60 (A), 19 (B) and 16 kDa (C) proteins obtained from peach bark tissues sampled over the course of a year. Relative abundance of each protein, based on intensity of staining with SYBR Orange, was quantified with a STORM gel and blot imaging analysis system. Abbreviations: CY = current-year bark; 1YO = 1-year-old bark; Scaffold = scaffold bark; Trunk = trunk bark; and Root = 4–5-year-old roots.

present in bark tissues of current-year and 1-year-old shoots compared with other tissue types (Figure 3C). A sharp drop in the amount of 16 kDa protein in bark tissue of 1-year-old shoots was evident in February and was followed by a subsequent increase in March. Although the 19 kDa protein was present in all tissues, accumulation was greatest in trunk tissues, and 1-year-old shoot and scaffold tissues showed a transitory decrease in the amount of 19 kDa protein in late fall to mid-winter (Figure 3B). The 60 kDa peach dehydrin exhibited a distinct seasonal pattern of accumulation in all tissues except roots (Figure 3A). The greatest accumulation occurred in bark tissue of 1-year-old shoots, and again some transient decreases were observed. Overall, the densitometry plots demonstrated both the general seasonal pattern of protein abundance and the differences in accumulation among the various aged stem and root tissues of peach.

Protein extracts from current-year shoots were fractionated by free-solution isoelectric focusing in a Rotofor apparatus, and each fraction, ranging from pH 3.1 to 9.3, was further separated by SDS-PAGE. The isoelectric points obtained were 7.3, 6.6 and 7.4 for the 60, 19 and 16 kDa proteins, respectively. The amino acid composition of each of the three proteins is presented in Table 1. The 60 kDa protein was rich in glycine and hydrophilic amino acids and low in aromatic amino acids. The 60 kDa protein was also rich in Glu/Gln, Asp/Asn and Thr. The amino acid composition of the 19 kDa protein was more evenly distributed, with Glu/Gln and Gly being the predominant amino acids (10.7 and 11.2%, respectively). The 16 kDa protein was glycine-rich (24%).

Peptide sequences of partial digests of each protein and comparisons with other reported protein sequences are presented in Table 2. We confirmed that the 60 kDa protein is a dehydrin (a subclass of late embryogenesis abundant proteins), as previously reported (Arora and Wisniewski 1994). The 19 kDa protein had a high degree of homology to proteins present in Rosaceous fruit trees and proteins in other species that are variously classified as allergens, pathogenesis-related proteins, fruit-ripening-related proteins and ABA-induced proteins in protein and gene sequence databanks. The identification of the 19 kDa protein as an allergen (MalD1) in the protein database is mainly based on proteins that have been isolated from pollen, whereas other sequences, in which the MalD1 protein is associated with other functions, are derived from other tissues. The 16 kDa protein sequence appeared to have no homology with any protein in the SwissProt database.

## Discussion

Storage proteins in woody plants are believed to act as an overwintering reserve of nitrogen that is used to support spring

Table 1. Amino acid composition (mol %) of three (60, 19 and 16 kDa) seasonally regulated peach bark proteins.

| Amino acid | 60 kDa | 19 kDa | 16 kDa |
|---|---|---|---|
| Ala | 5.7 | 7.9 | 8.4 |
| Arg | 1.8 | 0.6 | 3.0 |
| Asp | 10.0 | 8.9 | 7.2 |
| Cys | 0.0 | 1.6 | 2.4 |
| Glu | 11.4 | 10.7 | 9.6 |
| Gly | 24.0 | 11.2 | 24.1 |
| His | 6.2 | 3.9 | 3.0 |
| Ile | 1.2 | 7.3 | 6.6 |
| Leu | 4.2 | 7.9 | 6.0 |
| Lys | 8.0 | 7.9 | 1.8 |
| Met | 0.7 | 0.0 | 0.0 |
| Phe | 0.5 | 3.4 | 2.4 |
| Pro | 5.9 | 5.6 | 4.2 |
| Ser | 2.3 | 8.9 | 8.4 |
| Thr | 9.6 | 5.1 | 3.0 |
| Tyr | 4.7 | 5.1 | 1.8 |
| Val | 3.9 | 3.9 | 7.8 |

Table 2. Sequence comparison of digest fragments of seasonally regulated peach bark proteins with other reported protein sequences. Underlined amino acids indicate where related sequences differ from the obtained peach sequence.

| Plant | Polypeptide | | Sequence | Identification |
|---|---|---|---|---|
| **Peach** | **60 kDa** | **1** | **RLPGGQKDDQYL** | Dehydrins, cold-acclimation and drought-induced proteins |
| | **60 kDa** | **2** | **RLPGGQNVDPTTGPYGGGGAAG** | |
| | **60 kDa** | **3** | **RLPIGQKVD** | |
| Barley | B8 | | KLPGGAH | |
| | B9 | | KLPGGAH | |
| Spinach | CAP 85 | | KLPG-QH | |
| Wheat | WCS 120 | | KLPGGHGDHQQTGGT | |
| **Peach** | **19 kDa** | **1** | **ITFGEGSQYGYVK** | Allergens, pathogenesis-related proteins |
| Apricot | — | | ITFGEGSQYGYVK | |
| Cherry | — | | ITFGEGSQYGYVK | |
| Apple | MalD1 | | ITFGEGSQYGYVK | |
| Pear | — | | ITFGEGSQYGYVK | |
| Hazel | — | | ITFGEGSRYKYVK | |
| **Peach** | **19 kDa** | **2** | **HSEILEGDGGPGTIK** | Allergens, pathogenesis-related proteins, ABA-responsive proteins, fruit ripening-related proteins |
| Apricot | — | | HSEILEGDGGPGTIK | |
| Cherry | — | | HSEILEGDGGPGTIK | |
| Pear | — | | HAEILEGNGGPGTIK | |
| Pea | — | | EILEGNGGPGTVK | |
| Bean | — | | EIVEGNGGPGTIK | |
| Birch | — | | ENIEGNGGPGTIK | |
| **Peach** | **19 kDa** | **3** | **AFVLDADNLVPK** | Allergens |
| Cherry | — | | AFVLDADNLVPK | |
| Apple | MalD1 | | AFVLDADNLIPK | |
| Pear | — | | AFVLDADNLIPK | |
| Apricot | — | | AFILDADNLIPK | |
| Birch | — | | SFVLDADNLIPK | |
| **Peach** | **16 kDa** | **1** | **GQAHLIPNVSSGHIK** | No known sequence homology with other proteins |
| | **16 kDa** | **2** | **EKVEYDDENKVATLIGLDGEVFK** | |

growth. They are classified on the basis of two criteria: (1) they accumulate in high amounts in dormant shoots; and (2) they are nearly or completely absent during growth (O'Kennedy and Titus 1979, Wetzel et al. 1989). The identification of such proteins in woody plants has been well documented (Stepien et al. 1994, Coleman 1997). The three proteins we characterized fit the definition of storage proteins; however, there is strong evidence that at least two of the proteins (60 and 19 kDa) may have other functions in addition to nitrogen storage. The 60 kDa protein is a dehydrin (Wisniewski and Arora 1994) and the 19 kDa has homology with proteins classified as allergens, pathogenesis-related (PR) proteins and ABA-induced proteins, suggesting that it may also play a role in the physiology of woody plants in addition to nitrogen storage. The 19 kDa protein was the only one of the studied proteins to show a distinct seasonal pattern of accumulation in roots (Figure 3B), perhaps indicating a functional role of this protein in roots relating to its identification with PR or ABA-inducible proteins. This putative PR protein may accumulate in all tissues during the dormant period as a defense mechanism against pathogens at a time when many cellular processes are inactive. A similar pattern of accumulation and putative function has been reported for a defensin in current-year peach bark tissues (Wisniewski et al. 2003). Defensins are a family of low molecular mass, extracellular proteins that exhibit potent antimicrobial activity (Broekaert et al. 1995). The 16 and 60 kDa proteins showed little accumulation or seasonal pattern in roots.

Although we observed qualitative changes in amounts of the 16, 19 and 60 kDa proteins, quantitative interpretations of the data should be made with caution. Densitometric analysis of any specific protein in a total soluble protein blot relies on equivalent amounts of protein being loaded from each sample. We attempted to adjust for variations in protein concentration among samples, but some sample-to-sample variation occurred (Figure 1). Nevertheless, the data strongly support the conclusion that these proteins exhibit seasonal fluctuation.

Analysis of the amino acid composition of the proteins (Table 1) indicated that the 60 kDa dehydrin, like other dehydrin proteins, is rich in glycine and hydrophilic amino acids and low in aromatic amino acids (Close et al. 1989, Arora and Wisniewski 1994). The 19 kDa protein comprised similar amounts of hydrophilic and hydrophobic amino acids, suggesting that it may have some membrane spanning regions, al-

though complete sequence data are needed to verify this assumption. The 16 kDa protein was glycine-rich (24%), a characteristic that is shared with the 60 kDa protein.

The 16 kDa protein was the only one of the studied proteins for which we were unable to assign a function based on amino acid sequence. In this respect, it is similar to a poplar (*Populus deltoides* Bartr. ex Marsh.) bark storage protein of 32 kDa that also lacks homology with other known proteins (Coleman et al. 1992). Bark storage proteins do not accumulate uniformly throughout the tree (Coleman 1997), but are present in greatest amounts in tissues adjacent to sites of previous and subsequent growth. This reduces the distance needed to transport nitrogen from senescing leaves (source) to the storage site (sink) during autumn, and to relocate nitrogen to growing shoots in the spring, therefore increasing efficiency. Among plant organs, the 16 kDa protein exhibited the most distinct seasonal pattern in bark tissues of current-year and 1-year-old shoots. Bark tissues of older stems (scaffold and trunk) and roots showed no seasonal pattern of distribution, indicating that the 16 kDa peach bark protein may serve as a vehicle for nitrogen storage on a seasonal basis, similar to the poplar bark storage protein. These results also highlight the importance of surveying seasonal patterns of protein accumulation in tissues of different ages when studying storage proteins.

The sharp transient decrease in the amount of the 16 kDa protein in February and the subsequent increase in March may not be anomolous. This fluctuation was considered to be real and not an artifact caused by uneven loading of samples, because the intensities of other proteins in the gel (Figure 1B) were comparable with the intensities of the corresponding proteins in other months. Additionally, proteins were re-extracted in February and March and the resulting gels gave similar results (data not shown). Sauter and van Cleve (1990) also reported transient changes in bark storage protein content in poplar (*Populus* × *canadensis* Moench '*robusta*') during fall and winter that coincided with changes in protein-body structure and with changes in the population of vesicles or tubular membrane cisternae of cells. They suggested that these transient changes indicate an exchange of nitrogen compounds between the storage pool and structural proteins during these periods. These transient changes also reinforce the concept that the expression and turnover of storage proteins is a dynamic process and may be regulated by environmental cues. Although induction of bark storage protein in poplar is reported to be regulated primarily by a short photoperiod (Coleman et al. 1992), induction can be augmented by low temperatures and nitrogen fertilization (Coleman et al. 1993, van Cleve and Apfel 1993) and can even occur in a long photoperiod. These observations suggest that the synthesis of bark storage proteins occurs in response to changes in nitrogen availability, such as might occur during autumnal leaf senescence. Fluctuations in amounts of amino acids and nitrogen have been extensively studied in fruit trees, but the effects of nitrogen management practices on the induction and accumulation of specific proteins (storage and otherwise) have not been documented.

The seasonal pattern of expression of the 60 kDa peach dehydrin confirms our previous findings (Arora and Wisniewski 1994, 1996). The low accumulation of the dehydrin in root tissue is of interest because root tissue is also the least cold hardy of plant tissues and further supports the hypothesis that dehydrins play a role in cold acclimation. The lack of seasonality in the accumulation of the 60 kDa peach dehydrin in root tissue may be associated with the relatively minimal seasonal change in cold hardiness of roots compared with aboveground tissues. Upregulation and accumulation of the 60 kDa peach dehydrin gene and protein are closely linked to the timing and extent of cold acclimation in peach bark and xylem tissues (Arora and Wisniewski 1994, 1996, Artlip et al. 1997). Dehydrins have also been associated with cold acclimation in birch (Welling et al. 1997, Rinne et al. 1998), hybrid poplar and willow (Sauter et al. 1999), blueberry (Muthalif and Rowland 1994) and *Rhododendron* (Lim et al. 1999), and with osmotic status in Scots pine (Kontunen-Soppela and Laine 2001). Our finding that fluctuations in the seasonal pattern of dehydrin also occur in bark tissues of current-year and 1-year-old shoots and scaffolds indicates that the expression and turnover of this protein is more dynamic than previously expected.

We conclude that one must be cautious when classifying proteins in woody plants based only on abundance and seasonal patterns of appearance. Additionally, the differences in distribution of these proteins among plant organs need to be considered when attempting to assign function to a protein. For instance, if the 16 kDa protein is a storage protein, then the major reservoir for nitrogen storage as protein is in bark tissues of current-year and 1-year-old shoots. This may have important implications for pruning practices. Our results may have additional relevance given that different tissues and organs within a tree can vary significantly in their seasonal patterns of cold hardiness or their freezing tolerance at any specific point in time (Sakai and Larcher 1987). Differences in the pattern and extent of accumulation of some proteins in specific tissues and organs may help to explain the observed differences in degree of cold hardiness. Lastly, we speculate that the accumulation of the 60 and 16 kDa proteins in the aboveground portions of the tree and their absence in roots indicates that their expression is regulated by photoperiod. In contrast, the 19 kDa protein accumulated in all tissues, perhaps reflecting some other mode of induction and regulation. The genes coding for the 16 and 19 kDa proteins are currently being cloned, and we are also attempting to characterize the cellular location of these proteins and identify their functional roles.

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