Discrete Proteins Associated with Overwintering of Spruce and Douglas-fir Seedlings[1]

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Abstract.--Seasonal protein changes were followed in seedlings of interior spruce (a mixture of Picea glauca and P. engelmannii) and Douglas-fir (Pseudostuga menziessi) by SDS-PAGE. In seedlings of Douglas-fir a 30 kD protein and interior spruce a 30 and 27 kD protein that were not detected in the late summer, accumulated in seedling tissues during the fall. These proteins remained present throughout the winter, but declined rapidly in seedlings during the initial flush of spring growth. There was an increase in the total protein content of interior spruce seedling tissues during the fall, however, the accumulation of the 30 and 27 kD protein was tissue-specific since it increased in the apical bud, shoot and root tissue but not in the leaves. By late fall these proteins represented approximately 15% of the total seedling protein. These results suggest that conifer seedlings may utilize proteins as a storage reserve during overwintering. The potential of utilizing these "vegetative storage" proteins as biochemical markers of seedling quality is discussed.

INTRODUCTION

Seasonal changes in the nitrogen content of deciduous trees suggests that nitrogen is translocated from the leaves in the fall into the woody tissues, stored in these tissues during the winter and utilized for the first flush of growth in the spring (Kang and Titus 1980; Nelson et al. 1970). Specific proteins have been identified in the phloem tissue of several deciduous trees which accumulate in parallel with seasonal nitrogen fluctuations and are believed to be a storage form of nitrogen. These vegetative storage proteins can represent up to 30% of the total bark protein in overwintering trees and maybe an important source of nitrogen nutition (Wetzel et al. 1989). Soluble bark protein has been found to increase in the fall in some species of conifers and this has been associated with the development of

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frost hardiness (Pomeroy and Siminovitch, 1970). We report that conifer seedlings accumulate specific proteins in the fall and utilize these proteins during flushing.

MATERIALS AND METHODS

Plant Material

Interior spruce seedlings (seedlot 8534) used in this study were grown as 2-0 container stock by British Columbia Ministry of Forests Nursery at Surrey, British Columbia. For flushing experiments seedlings were considered "overwintered" in early February and brought into the laboratory. These seedlings were kept at room temperature under a 19 hr photoperiod at a light intensity of 70 einsteins/M2/sec. Douglas-fir seedlings were grown as 2-0 container stock by Peltons Reforestation, Maple Ridge, British Columbia. For the study of seasonal changes these seedlings were grown outdoors at B.C. Research Corporation. For protein analysis seedlings were divided into leaf, shoot, apical bud and root tissues and stored at -80°C.

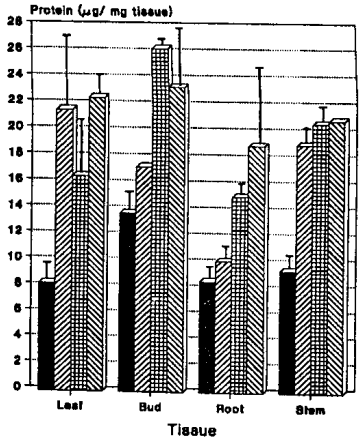
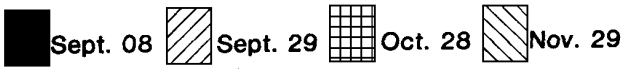


Figure 1. Changes in total protein for different tissues of interior spruce seedlings during the fall of 1988.



Protein Analysis

Seedling tissues (approx. 200 mg) were ground in a mortar and pestle with liquid nitrogen until a fine powder was achieved. Approximately 20-40 mg of tis-

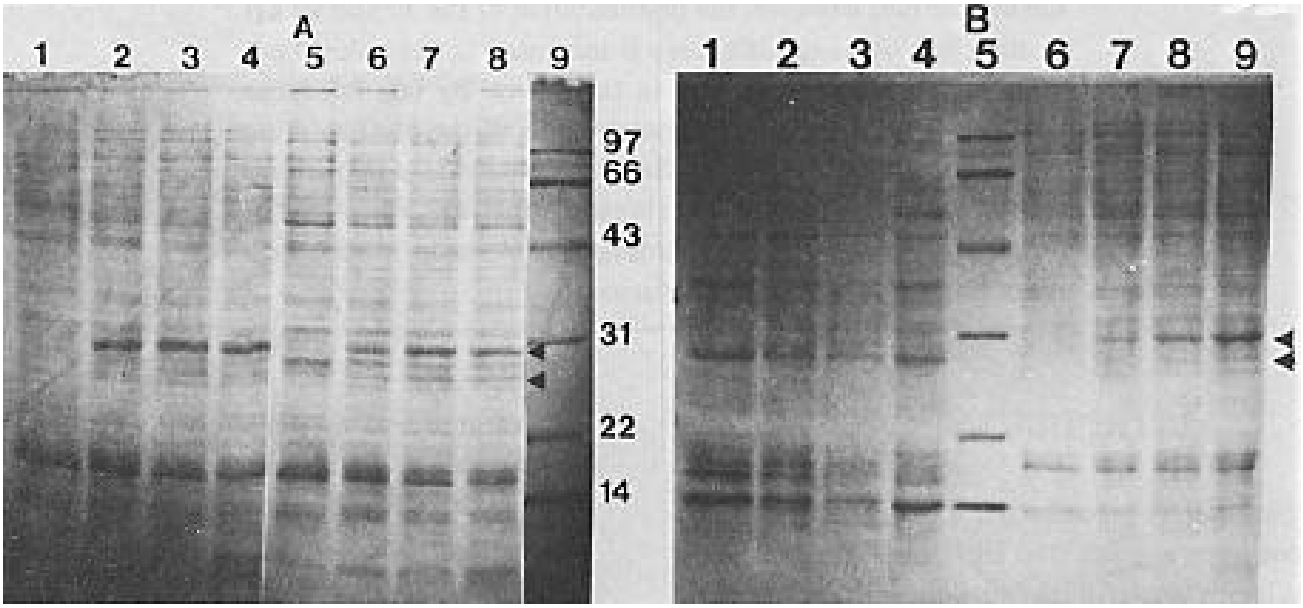


Figure 2. Changes in the protein profile for different tissues of interior spruce seedlings during the fall of 1988. A) lanes 1-4 apical bud; lanes 5-8 shoot; lane 9 mw standards. Lanes 1,5 Sept 8; lanes 2,6 Sept 29; lanes 3,7 Oct 28; lanes 4,8 Nov 29. B) lanes 1-4 leaves; lane 5 mw standards; lanes 6-9 roots. Lanes 1,6 Sept 8; lanes 2,7 Sept 29; lanes 3,8 Oct 28; lanes 4,9 Nov 29. (arrows denote discrete proteins).

sue was placed in a pre-weighed microfuge tube, 51/mg tissue of solubilizing buffer (0.125 M Tris-HCl pH 6.8 containing 22.5% mercaptoethanol, 9% sodium dodecylsulfate and 22.5% glycerol) was added and the sample was boiled for 6 - 7 minutes. The homogenate was centrifuged at 16000 x g for 10 mm, the supernatant removed and the sample was stored at -7OoC. Sample protein was determined using the method of Ghosh et al. (1988). For analysis of specific protein changes, samples were fractionated by SDS-PAGE on 12% polyacrylamide gels with a 5% stacking gel (Laemmli, 1970).

RESULTS

Interior Spruce

The protein content of the apical bud, shoot, leaf and root tissue of interior spruce seedlings increased during the fall (fig. 1). SDS-PAGE analysis revealed that two specific proteins of apparent molecular weights 30 and 27 kD accumulated during this period (fig. 2). These proteins were not detected in seedlings sampled in early September, began to accumulate in late September and reached a maximum level by the end of October. Apical bud, shoot and root tissue contained equivalent levels of the proteins but they were not detected in the leaves. The temporal appearance of these proteins in the fall was similar among all the individuals tested although there was some variation in the relative amounts of the two proteins in different seedlings. The level of the 30 and 27 kD proteins relative to total protein increased throughout the fall and by late October represented approximately 15% of the tissue protein (table 1).

Table 1. The percentage of total protein represented by vegetative storage proteins of interior spruce during the fall of 1988.

Collection Date	Protein Concentration (% of total protein)
September 8	n.d.[1]
September 29	7.92±3.58[2]
October 8	11.87±6.91
November 29	15.65±5.78

[1]n.d. = not detectable

[2]mean S.D.

When overwintered seedlings were placed in a favorable environment for growth (day 0) the buds expanded by day 7 and a flush of new growth occurred by the end of the three week sampling period. The levels of 30 and 27 kD protein in these seedlings declined to undetectable levels by day 7 in the apical buds, day 12 in the shoot tissue and day 21 in the roots (fig. 3)

Douglas-fir

Seasonal changes in the morphology and protein profiles of the apical bud were followed in seedlings of Douglas-fir (fig. 4). Bud scales began to develop on seedlings in early September and by late September visual inspection suggested that bud development was complete since no further morphological changes were noted until spring. A 30 kD protein began to accumulate in the bud tissue in early November and by late November this protein had reached its maximum level and remained at this level throughout the winter. The apical bud began to swell in early April and by the middle of the month, needles were protruding from the bud scales. The levels of the 30 kD protein had declined to undetectable levels in seedlings by early April.

DISCUSSION

Recently, specific storage proteins have been identified in vegetative tissues of deciduous trees such as elderberry and poplar (Greenwood et al. 1986; Sauter et al. 1988; Wetzel et al. 1989). These vegetative storage proteins can represent up to 30% of the tissue protein in the overwintering trees and it is believed that they contribute significantly towards nitrogen nutrition during spring flush (J. Greenwood, Univ. of Guelph, Ontario, Personal communication). Proteins are classified as storage molecules based on their accumulation during the fall in preparation for overwintering, their high concentration in dormant seedlings, and their rapid decline during flushing of overwintered seedlings. Based on the

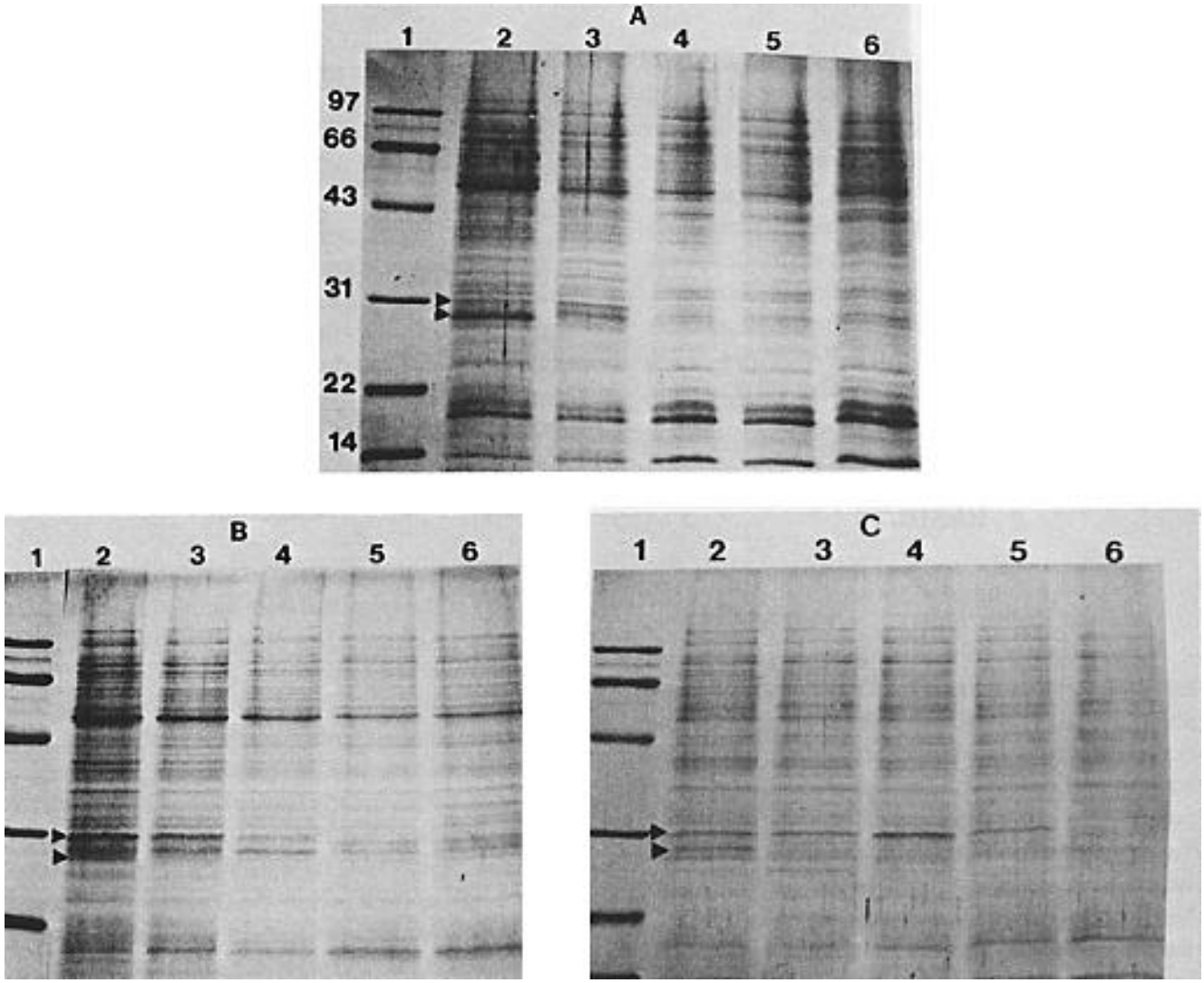


Figure 3. Changes in the protein profile for different tissues of interior spruce seedlings during flushing of overwintered seedlings. A) apical bud; B) shoot; C) roots; For A, B & C lane 1 mw standards; lane 2day 0; lane 3 day 3; lane 4day 7; lane 5 day 12; lane 6 day 21. (arrows denote discrete proteins).

seasonal changes in the 30 kD protein of Douglas-fir and the 30 kD and 27 kD proteins of interior spruce, it is possible that these proteins are accumulated for over-wintering and used as a source of nutrition during early spring growth. Furthermore, that fact that the same protein (based on molecular weight) shows similar seasonal changes in two different species suggests an important role for this protein during the overwintering process.

Conifer seedlings utilized for forest regeneration are generally grown in the nursery, lifted in the fall, overwintered in cold storage and planted in the spring. Perhaps the most crucial time to evaluate seedling quality is to determine the lifting date for cold storage, since lifting date can have a dramatic effect on seedling quality (Burdett and Simpson, 1984; Cannell et al. 1990). However, this is also a difficult time to evaluate seedling quality since the seedling is in various stages of quiescence and dormancy.

To date it has proven difficult to identify morphological or biochemical attributes of forest seedlings that can be used to evaluate their potential performance.

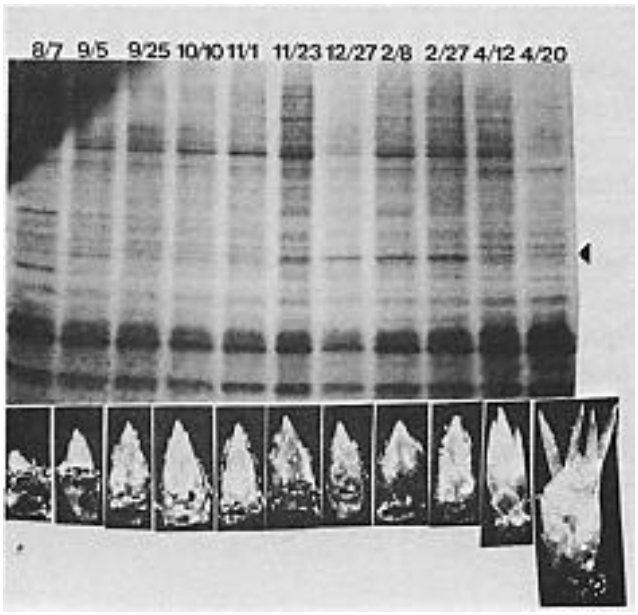


Figure 4. Seasonal changes in apical bud morphology and protein profiles of Douglas-fir seedlings. Numbers above each lane denote the collection date and photographs below each lane show the bud morphology of that sample.

The nutritional status is one attribute that can be intrinsically related to seedling growth potential. The use of macro/micro nutrients and carbohydrate reserves to evaluate seedling quality appears to be limited by fluctuations of these compounds that occur throughout the growth season due to stress and diurnal changes (Marshall, 1985; Landis, 1985). In contrast, the vegetative storage proteins only accumulate during the stage of seedling development associated with bud formation and acquisition of dormancy. Studies are underway to determine the relationship between the accumulation of the vegetative storage proteins, dormancy and seedling quality of interior spruce seedlings.

There also appears to be a relationship between the development of frost hardiness during the fall, lifting date and seedling quality (Burr et al. 1989). Currently, nursery growers rely on a frost hardiness test to determine the time to lift seedlings, but this technique can take up to two weeks. Pomeroy and Siminovitch (1970) found that soluble protein increased in bark and needles of mature red pine during the winter and that this increase was associated with the acquisition of frost hardiness. The accumulation of 30 kD proteins during the fall may also be associated with the development of frost hardiness and preparation for overwintering.

We believe that the possible role of vegetative storage proteins as storage reserves and in frost hardiness make them potential biochemical markers for seedling quality. Their use as biochemical markers is facilitated by the fact that they only accumulate during the stage of seedling development associated with dormancy, frost hardiness and the preparation for overwintering. If we can establish the relationship between the vegetative storage proteins and seedling quality an enzyme-linked immunosorbant assay (ELISA) can be developed so that nursery growers can perform a simple and rapid colorometric assay to determine the amount of protein present in nursery grown seedlings.

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