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# TOTAL SOLUBLE PROTEINS AMOUNT IN VEGETATIVE BUDS AND NEEDLES OF NORWAY SPRUCE DURING THE BURSTING TIME

V. CESAR, H. LEPEDUŠ and E. HAS-SCHÖN

*Department of Biology, Faculty of Education, University of J. J. Strossmayer L. Jägera 9, HR-31000 Osijek, Croatia*

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The vegetative buds and later on young needles as well as needles formed in spring previous year of *Picea abies* were used in the experimental work. Extracted proteins were determined spectrophotometrically and the amount of dry weight was measured. The data revealed that the dry weight of needles formed in spring previous year was about three times higher than in the vegetative buds at the beginning of investigations. During the sampling period the dry weight in those needles was keeping nearly the same level (39–54%). The increase in dry weight was noticed in the young needles 5–6 weeks after vegetative buds burst. The amount of total soluble proteins in the needles formed previous year was about 140 mg/g of dry weight and it was uniformed during the investigation period. In vegetative buds the amount of proteins was three times higher than in needles. But, the concentration starts to decrease just before bursting of the vegetative buds as well as during next 2 or 3 weeks when young shoot proliferated. After this decreasing period amount of proteins in newly formed needles briefly reached (in 2 weeks period) the level as it was in vegetative buds. In the following period the decrease of water amount as well as the protein amount in young needles was observed.

Key words: *Picea abies*, vegetative buds, needles, proteins

## INTRODUCTION

The rapidity of vegetative buds bursting into young shoots fascinates each spring, especially, in common species, as it is *Picea abies*, it recurs in great number of investigations. Although, the simple and clear description and explanation of embryonic shoot differentiation in completely formed needles and formation of new embryonic shoot is unknown. There is a number of authors dealing with those problems and trying, from different points, to give the best answers in this matter. The phenology of bud bursting, the influence of climate conditions and frost tolerance, perhaps, are the best known aspects of this issue (Benker 1994, Clapham *et al*. 1998, Hänninen 1990, 1996, Partanen *et al*. 1998, Qamaruddin *et al*. 1993, Worrall and Mergen 1967).

The investigations of cytokinin influence on bud development, its size and branch form (Bollmark *et al*. 1995, Chen *et al*. 1996) showed the positive correlation between the level of zeatin riboside and bud growth during the period of predetermination of next year's branch growth. Bilkova *et al*. (1999) found three types of polyphenols in buds: granular, vacuolar and droplike, all of them having tannin nature. The results of characterization of phenolic constituents, as the amount of polyphenols showed the differences between buds, young and older needles (Kraus and Spitteller 1997, Slimestad and Hostettmann 1996), indicating different metabolic activities. In addition, the seasonal accumulation of ultraviolet-B screening pigments (Fischbach *et al*. 1999) and the epicuticular wax production (Gordon *et al*. 1998) were investigated, as well as the starch metabolism (Egger and Hampp 1996, Egger *et al*. 1996, Hampp *et al*. 1994). In young needles there is a high amount of starch derived from imported sucrose originated in older needles. The study of spruce chloroplast structure (Senser *et al*. 1975) showed the presence of starch grain in needle primordia enveloped by bud scales, but also in plastids from 3–5 mm long needles that were exposed to light after the bud break.

The structure and development of vegetative buds of *Picea abies* was and still is fascinating issue investigated by several authors (Lewis and Dowding 1924, Korody 1937, Romberger 1966, Jansson and Bornman 1983). It was shown that the crown situated on basal part of embryonic shoot is acting as a transport barrier (Jansson *et al*. 1983). Hejnowicz and Obarska (1995) described initiation of vegetative buds in June and its development until April. The complex study of development and ultrastructure of spruce needles outer epidermal wall is given by Tenberge (1992).

Former investigations in our laboratory pointed out a short preparatory period of two weeks before the bursting when the rapid changes in anatomy and constitution of tissue of vegetative buds of *Picea abies* are taking place contemporary with the significant increase of mitotic activity and the enlargement of embryonic tissue. The remarkable occurrences were: lignified tracheary elements bursting into the embryonic shoot, disappearance of the callous from sieve areas in the wall of the cup-like structure, diffusion of the pectic substances from the middle lamellae in the wall of crown cells, and the different distribution of nuclei and their different size in leaf primordia (V. Cesar 1992. Theses, Univ. of Zagreb, Croatia, Cesar and Bornman 1996, Cesar 1997, Cesar *et al*. 1997).

It was expected that the changes in the protein amount and the water content will appear in vegetative buds simultaneously with previously de-

scribed changes. So, in this paper the amount of total soluble proteins and the water content were investigated in about a year old needles (those that arose from vegetative buds during previous spring) and in vegetative buds, as well as in young shoots and young needles, consequently.

Abbreviations: DW = dry weight, FW = fresh weight, NS = not significant.

## MATERIAL AND METHODS

Three different, about 25-year-old trees of Norway spruce (*Picea abies* (L.) Karst.; Osijek, Croatia) were used in experimental work, signed as genotype A, B and C. For each genotype the sampling material were vegetative buds, later on young shoots and consequently, young needles, as well as needles formed in spring previous year (in 1997), respectively. Sampling was done once a week from 24 March to 2 July, 1998 (weeks 1–15). Vegetative buds were dissected from the branch and the scales were removed. Needles were removed from the branch and cut in small pieces.

Proteins were extracted in 1 M sucrose and 0.056 M 2-mercaptoethanol in 0.2 M tris-HCl buffer, pH 8.5 (Wetter and Dyck 1983). After precipitation (Bensadoun and Weinstein 1976) proteins were determined spectrophotometrically (Lowry *et al*. 1951, McDonald and Chew 1965). The amount of dry weight was determined by drying on 105 °C during 24 h in each sample. All measurements were done in triplicate and averaged.

The achieved data were statistically worked out by t-test and correlation coefficient (Pavlić 1977).

## RESULTS AND DISCUSSION

The amount of total soluble proteins in about a year old needles (formed in spring 1997) was from 103 to 217 in genotype A, from 86 to 168 in B and 94 to 181 mg/g of dry weight in C (Table 1). The average amount of proteins was 154, 115 and 142 mg/g of dry weight in A, B and C, respectively. The portion of dry weight in those needles could be considered as uniformed from 54.7 to 47.1% in A, 54.2 to 42.4% in B and 53.6% to 38.5% in C, although the water content was about 10% lower in March and April in each genotype (Table 1, Fig. 1).

*Table 1*
The amount of proteins and dry weight in about one-year-old needles of genotypes A, B and C

| Week | % DW | | | Proteins, mg/g DW | | | Statistical sample |
|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | |
| 1 | 52.84 | 54.22 | 52.87 | 150.59 | 99.34 | 148.46 | |
| 2 | 54.66 | 52.54 | 51.68 | 103.97 | 86.22 | 94.33 | |
| 3 | 49.93 | 52.55 | 53.50 | 138.99 | 99.71 | 137.96 | |
| 4 | 52.96 | 50.71 | 53.66 | 115.99 | 98.92 | 140.91 | |
| 5 | 46.49 | 49.22 | 53.81 | 142.87 | 102.60 | 134.57 | |
| 6 | 50.09 | 48.93 | 53.09 | 121.20 | 137.38 | 150.14 | 4 |
| 7 | 43.08 | 46.32 | 46.15 | 206.31 | 86.90 | 126.15 | |
| 8 | 46.55 | 44.34 | 42.17 | 149.26 | 114.57 | 157.53 | |
| 9 | 44.12 | 46.36 | 44.15 | 168.00 | 100.88 | 176.35 | |
| 10 | 45.74 | 44.25 | 44.45 | 217.01 | 132.07 | 117.48 | |
| 11 | 46.55 | 46.12 | 41.85 | 151.64 | 92.56 | 133.09 | |
| 12 | 46.95 | 45.30 | 38.47 | 161.02 | 123.80 | 181.21 | |
| 13 | 45.93 | 42.39 | 42.98 | 137.06 | 168.01 | 141.11 | 5 |
| 14 | 46.57 | 43.27 | 41.43 | 155.49 | 136.58 | 144.15 | |
| 15 | 48.22 | 43.40 | 41.28 | 202.49 | 154.29 | 150.82 | |

In vegetative buds and young shoots the average amount of proteins was 284 in A, 232 in B and 254 mg/g of dry weight in C and the water content was about 80% in each (Fig. 1). The proliferation of vegetative buds into young shoots and needles caused the changes in water content as well as in the protein amount. The water portion was slowly decreasing and approaching the values as they were in a year old needles (Fig. 1). In young shoots and young needles, respectively, the amount of proteins was falling down (167 in A, 96 in B and 108 mg/g of dry weight in C) and going up (625, 641 and 389 mg/g of dry weight in A, B and C) in a period of about one month and than the concentration of proteins took the level as it was in a year old needles (Table 2, Fig. 2).

The data for protein concentration were differently arranged and *t*-test was used to find out when there were significant differences during the bud-bursting period. The previous investigations (Cesar and Bornman 1996) showed that the moment of bud flush is the central moment in this event, so, this was the central point for making the statistical groups. Second criteria were the time when the stem lignification occurred (Fig. 2). The chosen statistical groups, making statistical samples 1–5 are signed in Tables 1 and 2, and the *t*-test results are given in Table 3. There was no sig-

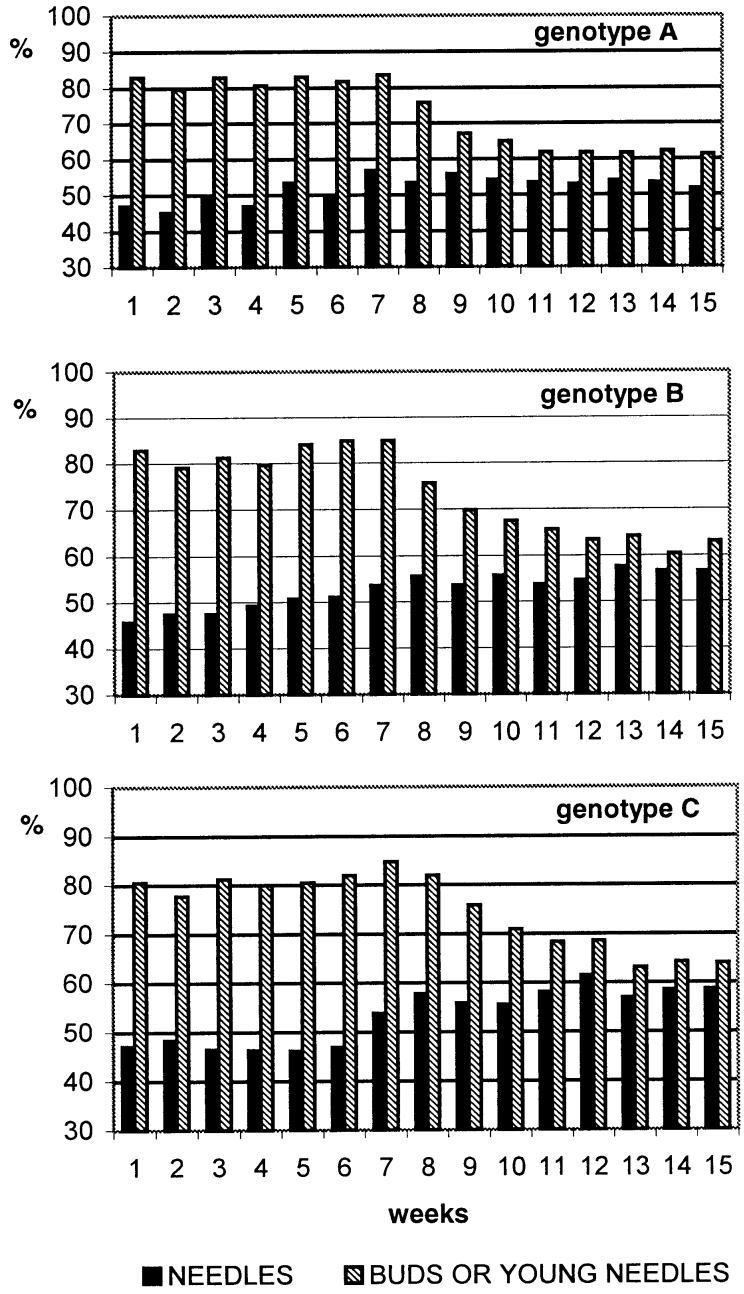


*Fig. 1.* The percent of water content in vegetative buds, young shoots or young needles, consequently, and needles formed in spring previous year during the sampling period from 24 March to 2 July, 1998

*Table 2*
The amount of proteins and dry weight in vegetative buds, consequently, young shoots and, later on, after bursting, young needles of genotypes A, B and C

| Week | % DW | | | Proteins, mg/g DW | | | Statistical sample |
|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | |
| 1 | 16.94 | 17.03 | 19.42 | 490.44 | 288.73 | 383.26 | |
| 2 | 20.38 | 20.80 | 22.21 | 358.34 | 197.02 | 338.88 | |
| 3 | 17.11 | 18.69 | 18.74 | 286.32 | 172.50 | 403.68 | 1 |
| 4 | 19.28 | 20.35 | 19.98 | 167.53 | 96.41 | 375.93 | |
| 5 | 17.00 | 15.87 | 19.51 | 222.18 | 278.81 | 292.31 | |
| 6 | 18.21 | 15.03 | 18.03 | 528.94 | 641.18 | 218.30 | |
| 7 | 16.37 | 15.01 | 15.20 | 625.47 | 394.40 | 107.96 | |
| 8 | 24.15 | 24.31 | 18.04 | 258.88 | 257.55 | 299.39 | 2 |
| 9 | 32.87 | 30.13 | 24.05 | 194.55 | 173.42 | 298.13 | |
| 10 | 35.01 | 32.50 | 29.04 | 223.39 | 191.42 | 389.43 | |
| 11 | 38.09 | 34.37 | 31.60 | 157.31 | 138.38 | 119.87 | |
| 12 | 38.20 | 36.58 | 30.33 | 165.16 | 171.41 | 142.61 | |
| 13 | 38.33 | 35.78 | 36.88 | 168.49 | 162.10 | 161.50 | 3 |
| 14 | 37.73 | 39.61 | 35.74 | 197.59 | 146.30 | 133.83 | |
| 15 | 38.63 | 36.92 | 35.97 | 192.47 | 167.74 | 141.17 | |

nificant difference in protein concentration before (sample 1) and 5–6 weeks after bud bursting (sample 2). The significant difference occurred on protein concentrations in young shoots (sample 2) and well-formed young needles (sample 3). In the same period, young needles (sample 3) did not differ from the year older needles (sample 5). Observing the protein concentrations in about one year old needles, the arising trend in the second half of investigated period can be seen, but according the *t*-test, that was not significant (samples 4 and 5). Genotypes A, B and C showed similar attitude (Table 3).

Also, the correlation coefficient (r) has been worked out. There was a low correlation between protein concentrations in about a year old needles in investigated genotypes ($r_{A/B} = 0.20$, NS; $r_{B/C} = 0.27$, NS; $r_{A/C} = 0.11$, NS), what implicates that the amount of proteins is genotype dependent. The low, even none correlation was shown between protein concentration in buds, young shoots and young needles, on one side, and about one-year-old needles, on the other, for each genotype ($r_A = 0.03$, NS; $r_B = 0.02$, NS; $r_C = -0.23$, NS). The bud break happened in different moments for these three genotypes, for A it was 2 weeks after investigations started, for B after 3 weeks,

and for C after 5 weeks. Although, there was higher correlation in protein concentration in buds and young shoots during the bursting period among those genotypes ($r_{A/B} = 0.79$, $p < 0.001$; $r_{B/C} = 0.61$, $p < 0.01$; $r_{A/C} = 0.86$, $p < 0.001$) what is underlining the same behaving in that time independently of genotype.

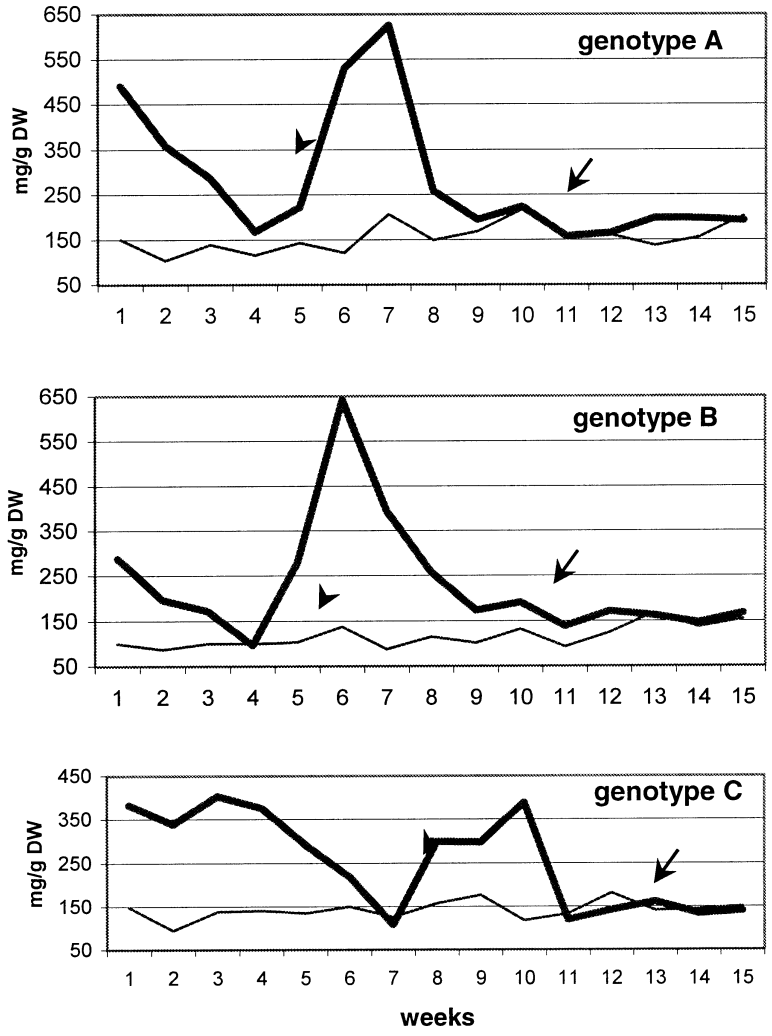


*Fig. 2.* The amount of proteins in vegetative buds, young shoots or young needles, consequently, and needles formed in spring previous year expressed on mg/g of dry weight during the sampling period from 24 March to 2 July, 1998. Arrowhead indicates the bud break. Arrow indicates occurred lignification in stem

*Table 3*
The differences in protein amount between chosen statistical groups in genotypes A, B and C

| Statistical samples | P (t) | | |
|---|---|---|---|
| | A | B | C |
| 1 vs. 2 | NS | NS | NS |
| 2 vs. 3 | < 5% | < 5% | < 5% |
| 3 vs. 5 | NS | NS | NS |
| 4 vs. 5 | NS | NS | NS |

NS = not significant

Those results led us to conclusion that young needles on recently burst young shoots needed a period of about one month to 40 days to develop on the same level as the needles from the previous year, regarding to the protein amount. Also, the protein amount in vegetative buds as well as in young shoots is higher than in the needles from previous year.

The data given by Pitel and Cheliak (1985), showing the significantly higher protein amount in buds than in needles during the winter period for *Picea glauca* coincided. In early spring, multiplied mitotic activity in embryonic shoot caused the significant enlargement of young shoot (Cesar and Bornman 1996, Cesar *et al.* 1997) not followed with equally fast protein synthesis what resulted in decrease of the protein content. New flush was suddenly exposed on light conditions and protein synthesis increased (Schmitz *et al.* 1993). Senser *et al.* (1975) demonstrated that the development of thylakoid system in needle plastids is taking place few weeks after the bud break being complete a month later.

The lignification in young needles is taking place immediately after the bud break and it is terminated about a month later. In that period the amount of lignin is increasing 10–20 times. This phase is correlated with a transient increase in apoplastic guaiacol and coniferyl alcohol peroxidase activity (Polle *et al.* 1994). In addition, the low activity of cinnamyl alcohol dehydrogenase during the bud break, in a few weeks later attains the maximum, and after a while it is taking a low constant activity (Galliano *et al.* 1993). Tenberge (1992) reports that at the starting point outer epidermal wall of spruce needles is thin but already layered. It grows 30 to 40 times in thickness and the complexity of its fine structure increases as well. During ontogeny, new layers and sublayers arise by apposition and interposition of cell wall compounds to the inner wall surface as well as between existing lamellae. The wall that is cuticularized from the very beginning becomes cutinised and lignified successively. Lignification starts before the cell wall is completed when cells achieve their final size in June, what coincides

with decrease of protein amount we have got. According to Tenberge (1992) lignification lasts until the end of the growing season, since the finally deposited polysaccharide lamellae usually become lignified, too.

Considering all these events happening immediately after the bud break, they should be correlated with quite a high amount of total soluble proteins, which is decreasing when needles became more differentiated. Also, during the levelling off period, the water content went to the same level as in a year older needles and the mitotic activity seems to decrease.

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