

Annual dynamics of the content of non-structural saccharides in the context of structural development of vegetative buds of Norway spruce

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Summary

Apical buds are very important organs as they determine further growth and development of tree species. Bud physiological state, including saccharide metabolism, determines their growth activity, and thus the development of the whole crown architecture. The present study focused on annual dynamics of the contents of non-structural saccharides (NSS) related to structural development in terminal vegetative buds of Norway spruce (*Picea abies* L. Karst.) during 1995. Two types of material were analysed: 1) four-year-old nursery-planted trees; and, 2) adult individuals from two mountain sites in the Czech Republic. Sugar and starch contents were determined, and starch was localised histochemically. Generally, the dynamics of the NSS content reflects the major morphogenetic and developmental changes occurring during the annual cycle. The highest content of sugars corresponds with a bud dormant state. Bud break is accompanied by the lowering of sugar content, reflecting the transition of buds to metabolically active sinks. During bud cold hardening in autumn, a massive gradual sugar accumulation takes place. The most pronounced change during the annual cycle is found in the content of raffinose, with the highest values observed during autumn and winter. The possible role of raffinose in bud frost tolerance is discussed. The results obtained from the buds sampled at the mountain sites are both qualitatively and quantitatively similar to the more detailed NSS-dynamics of young trees, thus, giving the justification to generalisation of the above described pattern for vegetative buds of Norway spruce.

Key words: Bud development – *Picea abies* L. Karst. – pinitol – raffinose – source–sink relationship.

Abbreviations: NSS non-structural saccharides. – PF pinitol fraction. – RFO raffinose family oligo-saccharides.

Introduction

Plant growth and development depend first upon the control of processes connected with production, allocation, trans-

port, and utilisation of photo-assimilates. At some stage, all growth processes and stress responses interfere with carbohydrate metabolism. This can occur at the level of carbon assimilation, at the level of transportation, where sink–source relationships can be altered, or at the level of utilisation (Hampp 1992).

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Non-structural saccharides (NSS) are those that can be accumulated and then made available for metabolism and translocation to other plant parts. They are the primary source of reserve energy stored in plants. Tracing shifts in saccharide pools have become an important tool for studying plant developmental processes. Valuable information was obtained for understanding phenological changes of total NSS in trees during the annual vegetative cycle (Fisher and Holl 1992, Rey and Jarvis 1998). Better understanding of saccharide metabolism and annual changes was achieved through other studies focused on the investigation of the enzymes of starch metabolism in needles of Norway spruce (Egger and Hampp 1993).

Since carbohydrate status of plant organs is known to be a sensitive indicator of the physiological responses to natural and anthropogenic stresses, the annual dynamics were followed in needles or buds of Norway spruce affected either by air pollution (Kainulainen et al. 1995, Peace et al. 1995, Svobodová et al. 1996) or by water and nutrient availability (Stockfors and Linder 1998).

Buds containing apical meristems belong to the most important plant sinks. All aspects of development – growth and differentiation, histogenesis, and organogenesis – may be investigated in relation to the activity of apical meristems. In conifers such as Norway spruce, the size and number of embryonal organs of the buds laid down the previous year (Owens et al. 1977) predetermine the regular shoot growth in the following growing season. The accessibility of these formative regions for tree growth is, besides others, dependent on the energetic status of a bud as an organ, it means on availability and partitioning of carbohydrates.

According to our knowledge, little is known about changes in the dynamics of NSS content in buds of coniferous species during their annual developmental cycle. For a part of the annual cycle, the content and partitioning of NSS in buds of *Pinus sylvestris* L. was described and discussed in connection with frost tolerance (Hansen et al. 1996). In the framework of their anatomical study, Hejnowicz and Obarska (1995) published data about starch grain histochemical localisation in Norway spruce buds, but without quantification.

The aim of this study was to follow the changes in contents of non-structural saccharides (NSS), i.e. sugars and starch, and to describe their annual dynamics in terminal vegetative buds of Norway spruce (*Picea abies*). In order to better understand the physiological background of the observed phenomena, these dynamics were related to bud structural development and starch histochemical localisation.

Materials and Methods

Plants

Two types of plant material were chosen for the analyses: buds of Norway spruce *Picea abies* L. Karst. sampled either from: A) young four-year-old, nursery-grown; or, B) adult (40–60 years old) individuals from natural sites located in mountain areas.

Young trees

In October 1993, one hundred fifty 3-year-old trees of Norway spruce from the forest nursery Zbiroh-Vlastec (Central Bohemia) were potted and transported to the field station in Lužnice by Treboň (South Bohemia; longitude 14° 44' E, latitude 49° 05' N, altitude 420 m above sea level). Trees were left outdoors and pots buried in laminate basins filled with soil. To prevent natural water stress, especially during summer months, trees were regularly watered three times per week during 1994 and 1995. Average daily temperature and precipitation were recorded for the year, and some important data are given in Fig. 1.

During the 1995 growing season, vegetative buds of 4-year-old trees were regularly sampled at 1-week intervals, from the beginning of April until the middle of June, and then at 2-week intervals until the end of October. The dormant state of buds was sampled on 16 January 1995 (Late Dormancy), and on 23 October and 20 November 1995 (Early Dormancy). In 1995, 20 collections in total were made. The terminal buds of branches of the first or the second orders were analysed.

Adult trees

Two natural stands of Norway spruce were selected as sampling sites in two Czech mountainous regions. Both sites had the character of natural climatic spruce stands.

Sampling site K (medium-damaged) was selected in the Krkonoše Mts. National Park (NP) in North Bohemia (longitude 15° 40' E, latitude 50° 40' N), near the top of the Medvědin Mt. (1130 m). Sampling site S (moderately damaged) was selected in the Šumava NP in South Bohemia (longitude 13° 30' E, latitude 49° 05' N), near the top of the Malá Mokrůvka Mt. (1250 m). Four representative trees per site, age 40–60 years, were selected for sampling and analyses (site K: trees K1–K4; site S: trees S1–S4).

Starting in February 1995, buds were sampled at regular 1-month intervals until November 1995, from second or third order branches approx. 8 m above ground level. Ten collections were made in 1995.

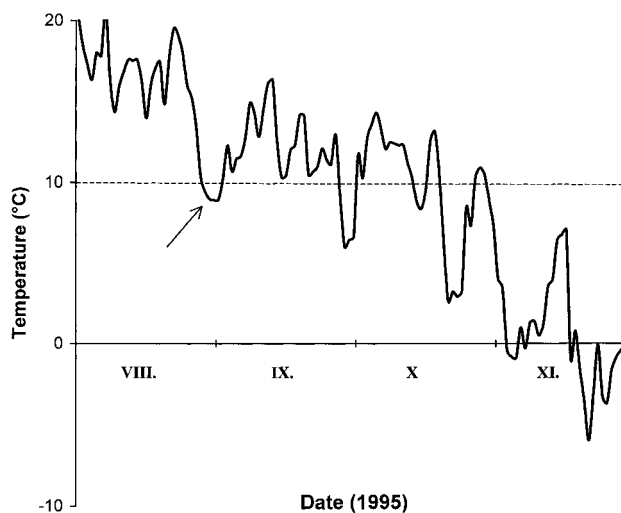


Figure 1. Average daily air temperatures recorded in the Lužnice station where young trees were cultivated. 1 August–30 November 1995. Arrow indicates 5 days at the end of August when night temperatures decreased below 8 °C.

Anatomical study and starch localisation

The anatomical description and histochemical detection of starch was done on paraffin longitudinal median sections (thickness 12 μm) prepared essentially according to Johansen (1940). Samples were fixed with 70% FAA (formaldehyde/acetic acid/ethanol/water 1/1/9/9, v/v/v/v), and infiltration was improved by application of lowered pressure. Lugol solution (iodine/potassium iodide) was used to localise starch grains. This localisation was verified using polarised microscopy (Johansen 1940). Twenty-five and fifteen buds per sampling date were sectioned during dormant states and vegetative growth season in 1995, respectively.

Non-structural saccharide content determination

Ten samples, each prepared from 5–15 buds, and fifteen samples, each prepared from 15–25 buds, were analysed per each sampling date during vegetative and dormant periods, respectively.

Soluble NSS-sugars

For sugar analysis, samples of dissected meristematic parts (during dormant stage), or whole developing buds with removed young needles (during bud break and early shoot growth) were used (Fig. 2). Samples were frozen in liquid nitrogen, freeze-dried, and dry weight determined. Specimens were homogenised by shaking with glass balls, and boiling with 80% methanol (0.5 mL) at 75 °C for 10 min, and then evaporating the methanol. The residue was dissolved in re-distilled water in an ultrasonic bath for 10 min. The samples prepared for sugar determination were stored after filtration (using membrane filters Whatman, 0.45 μm) at –18 °C.

Content of extracted soluble NSS was determined using high-pressure liquid chromatography – HPLC with refractometric detection (temperature 80 °C; column: Oston LGKS Ca²⁺; Watrex, Czech Republic; eluent: re-distilled H₂O). Preliminary identification of raffinose and pinitol by the above described system was further verified by

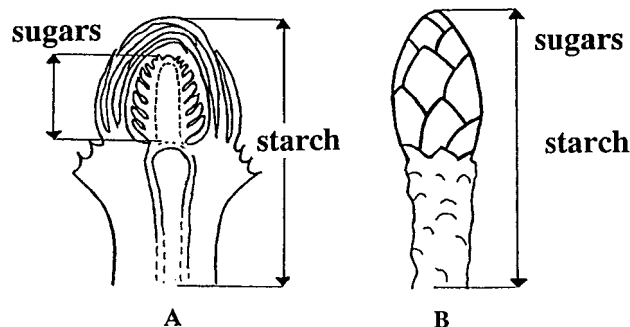


Figure 2. The system of bud sampling for HPLC (sugars) and anthrone (starch) methods during annual bud developmental cycle 1995. A) Dissection of embryonic shoot for sugars and the whole bud for starch; young trees: January–5 May, 29 September–November; adult trees from mountain stands: February–June; B) for both analyses the whole bud with removing of young needles; young trees: 19 May–14 August, adult trees: July–August.

using high pH anion exchange chromatography with pulse amperometric detection (Dionex, Sunnyvale, USA; column: CarboPac PA1 (4 × 250 mm) with companion guard column (4 × 50 mm); eluent: 100 mmol/L NaOH).

Non-soluble NSS-starch

Whole terminal buds were analysed with removal of young needles (Fig. 2). Three parallel samples were taken. Two samples were homogenised and purified with 70% ethanol. A third sample was taken to determine dry weight. Further steps used were modified from the protocols of starch determination using the colorimetric anthrone method (Viles and Silverman 1949, Yemm and Willis 1954), with the following modifications leading to a higher method reproducibility: 1) enclosure of two acetone steps during specimen purification before starch extraction to remove high content of compounds of secondary metabolism (mainly phenolic compounds); and 2) including of specimen incubation with chloralhydrate during starch extraction to achieve better starch evolution into solution before filtration (Bourne and Weigel 1965). Plant material was ground with 4.5 mL of 80% acetone. Obtained mixtures were centrifuged for 5 min at 200 G. The supernatant was removed and the pellet was shaken in 6 mL of 80% acetone, centrifuged, supernatant removed, and pellet shaken in 50% ethanol. After centrifugation, the supernatant was again removed.

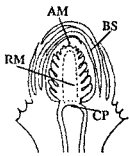

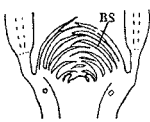
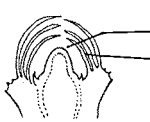


Results and Discussion

Anatomical changes during vegetative bud and shoot development

Structural analysis served as a background for more thorough interpretation of bud carbohydrate status as it enabled the precise adjoining of the changes in the structure to the changes in carbohydrate status of the buds during the whole annual cycle.

In February, the buds were dormant (Table 1). The embryonic shoot containing pre-formed leaf primordia, apical, and rib meristems was enclosed in a thick cover of bud scales. The embryonic shoot was separated from the subjacent shoot by a collenchymatic plate, and below that, a central bud cavity. In the basal part of the embryonic shoot was a cylinder of anastomosing procambial strands that differentiated into vascular bundles that joined with those of the subjacent shoot. Before bud break in spring, the apical meristem renewed its metabolic and mitotic activities, and started to form new bud scale primordia. Thus, this stage of development was called early scale initiation. Simultaneously, leaf primordia and rib meristem started to grow and differentiate, causing shoot growth during late spring and early summer. This phenological event occurred during the stage of late scale initiation. The apical meristem continued to initiate bud scales, which differentiated and covered the bud apical meristem. Then the apical meristem started to differentiate into a new, pre-formed bud by early leaf primordia initiation. In the mid-summer,

Table 1. Timing of both developmental and phenological events of bud development and types and localisation of starch grains during annual cycle 1995 of buds of Norway spruce. AM: apical meristem; CP: collenchymatic plate. Semi-quantitative evaluation of starch deposition: - non-present; + little amount; ++ high amount; none record: structure is not present during bud developmental stage.

Bud developmental stage	Dormant bud		Early scale initiation		Late scale initiation		Early leaf initiation		Late leaf initiation	
										
Timing for young trees	January – March		April – mid of May		mid of May – end of June		July		August – end of September	
Timing for adult trees	January – April		May – mid of June		mid of June – mid of July		mid of July – mid of August		mid of August – end of September	
Type of starch grains	big	small	big	small	big	small	big	small	big	small
Bud rib meristem (RM)	-	-/+	-	+	-	+	-	+	-	-
Leaf primordia (LP)	-	-	-	++	-	++	-	++	-	-
Basal part of old bud scales (BS)	++	-	++	-	++	-	++	-	++	-
Cortex of subjacent last year shoot (SC)	++	-	++	-	++	-	++	-	++	-

shoot growth ceased, and formation of leaf primordia continued in the stage of late scale initiation. In the autumn, buds became fully developed, then entered the early dormant state. The anatomical terminology used is based on the study of Owens et al. (1977). Hejnowicz and Obarska (1995) already described anatomical structure of buds of Norway spruce and their ontogenetic changes during annual developmental changes. Our results verified their structural findings.

The timing of the phenology of bud and shoot developmental events differed in both materials studied (Table 1) based on shifts in entering different developmental and phenological phases. It is known that timing of developmental events during the annual cycle differs, depending on either inner or external conditions. Quite often, the timing is altitude-dependent on implying environmental conditions for different altitudes (Oleksyn et al. 1998). As our results showed, the adult trees located in two different geographic positions but in comparable altitudes, exhibited similar timing of developmental events.

Starch content and histochemical localisation

Two types of starch grains were recognised during the annual cycle of spruce buds (Table 1): 1) Larger grains (reserve

starch) darkly stained, which were generally present during dormant state, and in the beginning of bud break in cells of primary cortex of subtending last year increment, and in basal parts of bud scales; 2) Smaller grains (transitory starch, as described by Sitt and Steup 1985) were present during the whole year in young bud tissues, i.e. leaf primordia or young developing bud scales, but rarely in the pith meristem. This pattern was the same for young and adult trees. Only timing of starch grain deposition differed, based on the above-mentioned time-shifts in the developmental changes (Table 1).

Our results of starch localisation during annual developmental cycle are in accordance with those of Hejnowicz and Obarska (1995). The only exception is that during transition to dormant state, when using PAS reaction, they found a large amount of starch grains in rib meristem. They also found no starch in winter buds, but a high occurrence of accumulated tannins in vacuoles of pith cells. On the basis of other simultaneous analyses, we may confirm a frequent simultaneous localisation for both types of compounds (Bilková et al. 1999). Our study proved that polarised microscopy is an indispensable complement for the study of starch localisation due to non-specific reactions of either PAS or Lugol reagents with other groups of compounds. In our case, a massive accumulation of phenolic compounds was observed during certain developmental stages. Their strong dark-brown reaction opti-

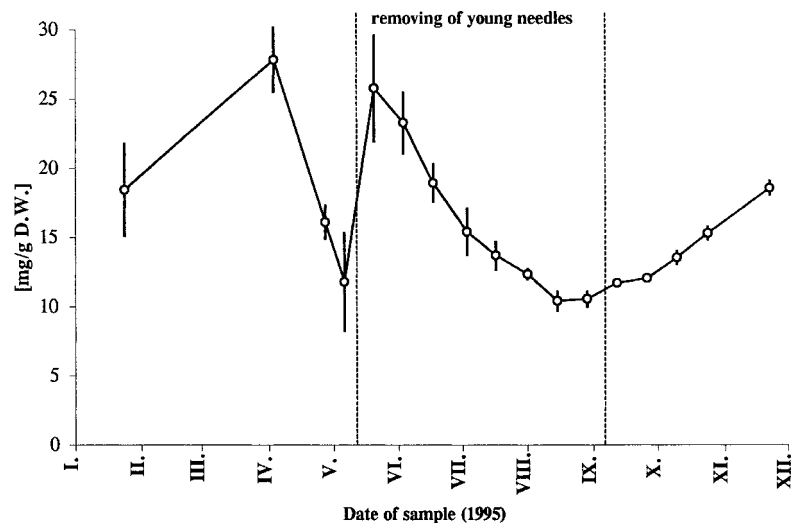


Figure 3. Annual dynamics of starch content in the buds of Norway spruce. Young 4-year-old trees. D.W. – dry weight. Bars: STD ($n = 10$). Removing of young needles corresponds to the sampling system described in Fig. 2.

cally interfered with evaluation of black to dark-blue reaction of starch, which could lead to overestimation of starch occurrence/content.

Seasonal accumulation pattern of starch in buds was as follows (Fig. 3): before phenologically detectable bud break but after metabolic activity had begun, starch gradually accumulated and reached its maximum value (approx. $27 \text{ mg} \cdot \text{g}^{-1}$ of dry weight). During bud break, stored starch was partially mobilised (April). During this period, the bud underwent remarkable structural and developmental changes that demanded high energy supply, and starch content reached its minimum value (app. $12 \text{ mg} \cdot \text{g}^{-1}$ of dry weight). Immediately afterwards, starch accumulated again (beginning of May) to the maximum value, corresponding to the assumed beginning of photosynthetic activity of current-year needles. Slow, continuous mobilisation took place from the end of May until the beginning of August, then accumulation to the amount characteristic for a dormant state occurred. During transition to dormancy and the dormant state, the absolute values indicated a small amount of starch. This finding was also supported by our quantitative starch measurements and by microscopy with polarised light. Relatively stable values were maintained during late dormancy (app. $18 \text{ mg} \cdot \text{g}^{-1}$ of dry weight).

There were no remarkable changes identified in the pattern of starch storage between plant material taken from young and adult trees. The only exception was less accumulation of starch before bud break in buds of adult trees (Fig. 4 A, B). Thus, we consider the above pattern as general for buds of Norway spruce.

Sugars

Seasonal pattern of total sugar content in meristematic tissues of buds taken from young trees is given in Fig. 5. The

total sugar content was at its maximum (app. $210 \text{ mg} \cdot \text{g}^{-1}$ of dry weight) in late dormant state. Before bud break, a rapid lowering of reserves was recorded to almost one third of the initial value recorded in dormancy, indicating a rapid turnover of metabolites during this period of rapid growth and differentiation. The sugar content was at its minimum in late July/early August ($25\text{--}30 \text{ mg} \cdot \text{g}^{-1}$ of dry weight). During that time, the apical meristem produced new leaf primordia, thus it could be implied that at that period carbohydrates were utilised for this differentiation process. From the end of August, the accumulation slowly increased to the values characteristic for the beginning of winter dormancy (app. $165 \text{ mg} \cdot \text{g}^{-1}$ of dry weight). We detected the same pattern of sugar content and composition in buds sampled from adult trees with similar time shift as in case of structural changes and starch content, only the total values were higher during the whole growing season (Fig. 6 A, B). We consider the above pattern as general for buds of Norway spruce.

Generally, we observed the accumulation of sugars in buds before winter period, which has, by many authors, been reported in needles of conifers (e.g. Kainulainen et al. 1995, Peace et al. 1995). The increased intracellular sugar concentration is known to be involved in frost hardening, and to be an important contributor to the increased cold tolerance of conifers in winter when sugars can act as cryoprotectants (Hampp 1992, Ogren 1997, Ogren et al. 1997). Our results on buds are also in good accordance with those conclusions. The increase in total sugar content during cold hardening, however, need not be of primary importance as the composition of individual sugars.

The total sugar content of bud tissues was composed of a combination of five main sugar components: content of each was greater than 2% of total soluble saccharide content, at least during a part of the annual cycle. Those sugars were identified as sucrose, glucose, fructose, raffinose (with a small amount of stachyose), and a fraction with identified pi-

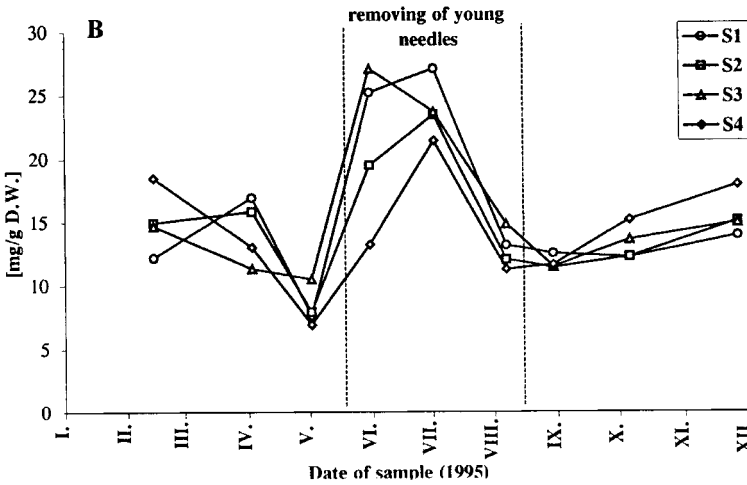
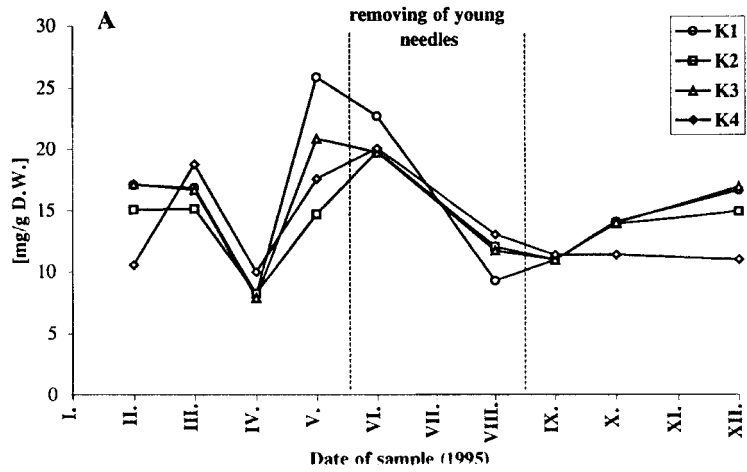


Figure 4. Annual dynamics of starch content in buds of Norway spruce. Adult trees from mountain stands. A) K1–K4: trees from the sampling site in the Krkonoše Mts. B) S1–S4: trees from the sampling site in the Šumava Mts. D.W.: dry weight. Removing of young needles corresponds to the sampling system described in Fig. 2.

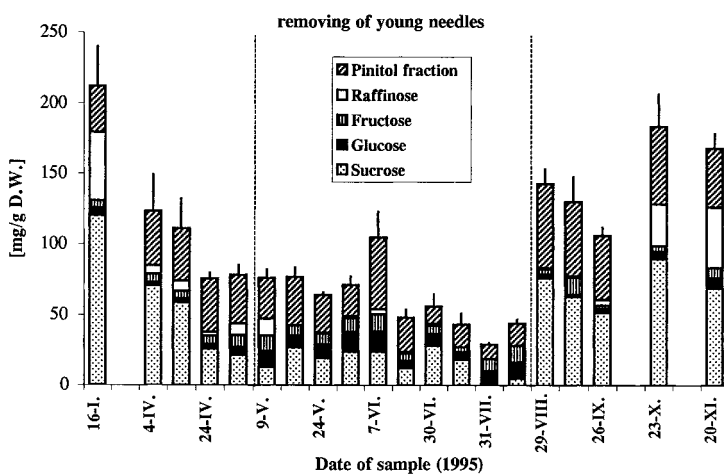


Figure 5. Annual dynamics of soluble NSS (sugar) content in buds of Norway spruce. Partitioning between individual components of the sugar spectrum. Young 4-year old trees. D.W.: dry weight. Bars: STD (n = 10) for total sugar content. Removing of young needles corresponds to the sampling system described in Fig. 2.

nitrol as a main compound, proved by two different HPLC detections. Thus, we decided to refer to this last named group as a pinitol fraction. Other compounds in pinitol fraction have in both HPLC systems very similar retention times to the pinitol one. We suppose them to be other cyclitols (inositol derivatives). For Norway spruce, the spectrum of sugars similar to

those we detected in buds was identified by other authors in needles (Lux et al. 1997), with the exception that only pinitol was identified instead of a larger group of probably related compounds. Cyclitols, including pinitol, are supposed to be involved in stress tolerance and may have roles as cryoprotectants, desiccation protectants, and hydroxyl radical scav-

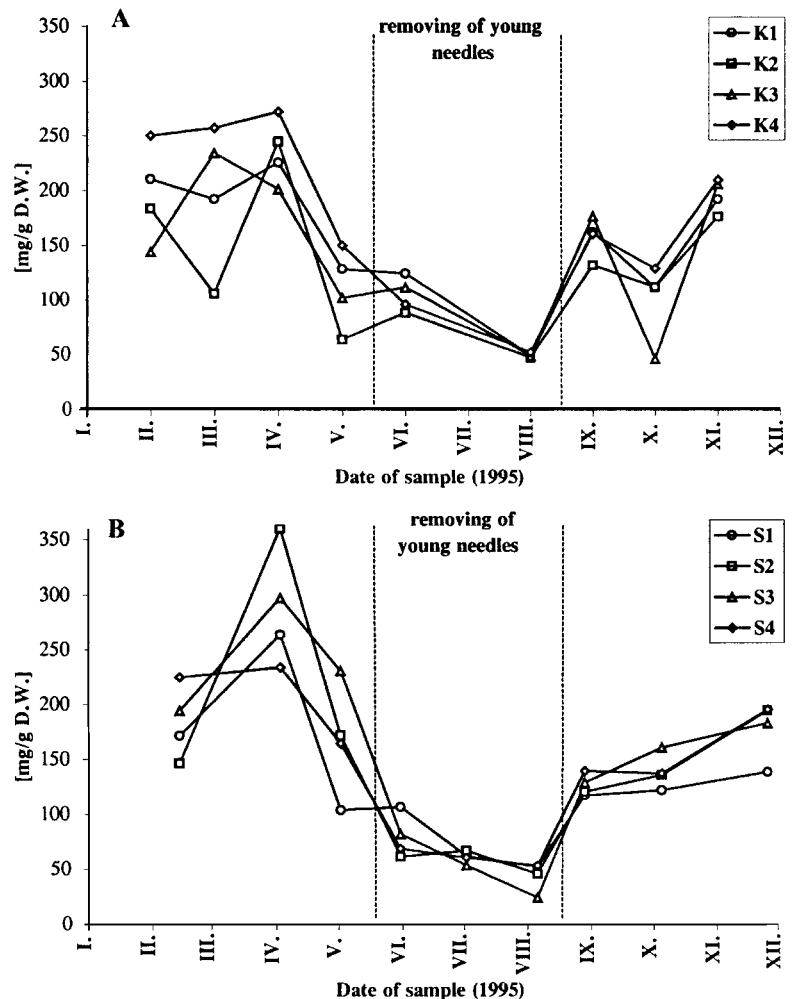


Figure 6. Annual dynamics of soluble NSS (sugar) content in buds of Norway spruce. Adult trees from mountain stands. A) K1–K4: trees from the sampling site in the Krkonoše Mts. B) S1–S4: trees from the sampling site in the Šumava Mts. D.W.: dry weight. Removing of young needles corresponds to the sampling system described in Fig. 2.

engers (e.g. Obendorf 1997). Their presence in buds of Norway spruce may have some importance in stress reactions as for young trees we detected the increased pinitol fraction content at June 7 (Fig. 5) after a dramatic change in mean daily temperature (instant 10 °C increase accompanied by no precipitation for 10 days). Nevertheless, in some plants pinitol can mainly play a metabolic role together with other metabolic sugars, such as glucose and sucrose (Ichimura et al. 1998). The content of the pinitol fraction in our material was remarkable during the whole year, and did not show so pronounced a dynamics. We cannot conclude precisely what role of the pinitol fraction in spruce bud physiology prevails.

For cryoprotection, the ratio between particular sugars might prove to be crucial (Liu et al. 1997, Obendorf 1997). Sucrose is known to be able to retain the liquid-crystalline state of membranes under osmotic stress caused by cold, draught, or salinity. Raffinose is supposed to improve the sucrose protective quality and also to inhibit the tendency of sucrose to crystalline and hence to lose its protective effect under stress conditions (Caffrey et al. 1988). We recorded dramatic

changes in raffinose content during annual cycle (Fig. 5). Only in late autumn and winter did raffinose make a substantial contribution to the total sugar content. Conifers are known for the more remarkable accumulation of raffinose family oligosaccharides (RFO) instead of sucrose, during autumn and winter, and in needles, RFO are known to play a role in frost hardening and frost tolerance (Kandler and Hopf 1982, Hampp 1992, Hinesley et al. 1992, Wiemken and Ineichen 1993, Lux et al. 1997). Our observation of accumulation of raffinose in buds before dormancy, and its presence during dormant state, supports this conclusion. The start of raffinose accumulation in September corresponded to the decrease in average daily temperatures under 10 °C, which was recorded at the end of August for young trees (Fig. 1). It is known that at least in some plants, two distinct pools of raffinose exist: 1) a large storage pool which is probably involved in stress tolerance as well; and 2) a transport pool (Bachman et al. 1995). We suppose that in our material, all raffinose belongs to the first type of pool, as during the period with the greatest photosynthetic activity the raffinose content is very low.

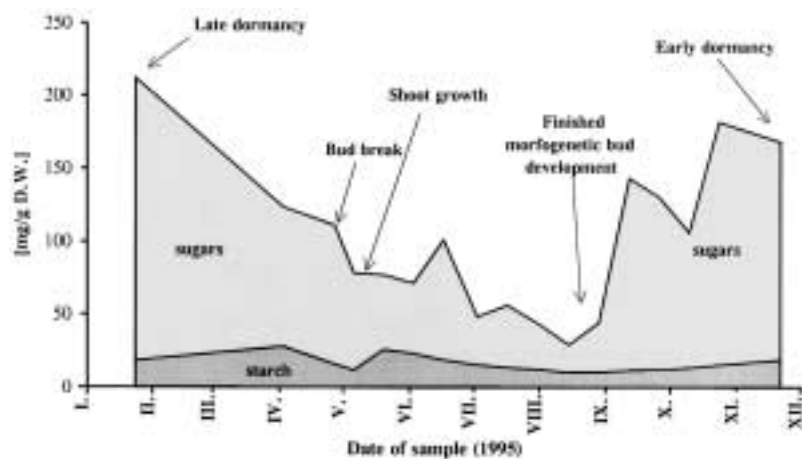


Figure 7. Annual dynamics of the total NSS content (sugars and starch) in the buds of Norway spruce in the context of bud morphogenetic development. Young 4-year old trees.

Total non-structural saccharide (NSS) content

No clear-cut causal relationship of starch-to-sugar conversion was observed, i.e. between the intensity of starch deposition/mobilisation and the height of organ sugar level. It is possible to conclude that the dynamics of the NSS content reflected the major morphogenetic and developmental changes occurring during the annual cycle of vegetative bud development (Fig. 7). The late dormant state was characterised by the highest content of sugars, and about ten times lower the amount of starch. At the time of bud break, the changes in carbon partitioning favouring starch accumulation were detected. Simultaneous rapid lowering of the sugar content during this time was accompanied by simultaneous formation of small starch grains localised in needle primordia. As the current year needle primordia were still covered by bud scales, changes were the results of re-organisation of existing reserves or imported sugars rather than deposition of new products of photosynthesis. Transformation from a strong sink to an almost self-sufficient organ since the assumed beginning of the photosynthetic activity of young needles was apparent during shoot elongation. During the processes of both bud morphogenetic differentiation in late summer and bud cold hardening in autumn, a massive sugar accumulation, accompanied by relative starch accumulation, took place as buds act as a strong storage sink again. During dormant state, this sink attractiveness was supposedly minimal.

In conclusion, total non-structural saccharides show considerable dynamics, both in starch and sugars. Our study proved that either dynamics of structural changes or changes in carbohydrate status followed for young or adult trees from two geographical locations had the same patterns allowing generalisation. The observed changes in timing between adult and young trees could be explained by altitude and considered to be climate-driven. Also, the trees from higher altitudes possess several potentially adaptive features, expressed as changes in physiological and anatomical charac-

teristics (Oleksyn et al. 1998). This also explains why it was not possible to statistically compare the annual dynamics observed for buds of young and adult trees.

Our study also points at the presence of carbohydrate-mediated frost protection of buds of Norway spruce. Harmonious development of bud meristems is the indispensable prerequisite for the proper development of crown architecture in subsequent vegetation periods, supporting the ability of trees to survive under all types of ambient environmental conditions. Buds and photosynthetically active needles are mutually dependent. On one hand, bud metabolic status reflects photosynthetic activity of needles and transport capacity. On the other hand, morphogenetic capability of apical meristem reflecting bud metabolic status is an indispensable basis for the next year's photosynthetic organ formation. Thanks to the key role of bud meristem in this relationship, changes in the metabolic status of buds can reveal the disturbances in this sink-source transition cycle, storage metabolic transition, or stress tolerance. Information about the development of the bud carbohydrate status during the whole annual cycle increases common knowledge of tree physiology, and can serve as a background for further understanding stress reactions of tree species.

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