Available online at www.sciencedirect.com

Forest Ecology and Management 225 (2006) 337–348

Forest Ecology and Management

www.elsevier.com/locate/foreco

Shoot growth processes, assessed by bud development types, reflect Norway spruce vitality and sink prioritization

Tomás Polák [a], Barrett N. Rock [b], Petya Entcheva Campbell [c], Jitka Soukupová [a], Blanka Solcová [d], Karel Zvára [e], Jana Albrechtová [a,f,*]

[a] *Department of Plant Physiology, Faculty of Science, Charles University, Vinicná 5, CZ-128 44, Prague 2, Czech Republic*
[b] *Complex Systems Research Center, University of New Hampshire, Morse Hall, Durham, NH 03824-3525, USA*
[c] *Joint Center for Earth Systems Technology, University of Maryland Baltimore County and GSFC/NASA, Biospheric Sciences Branch, Code 614.4, Greenbelt, MD 20771, USA*
[d] *Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojová 135, CZ-165 02, Prague 6, Czech Republic*
[e] *Department of Probability and Mathematical Statistics, Faculty of Mathematics and Physics, Charles University, Sokolovská 83, CZ-186 75, Prague 8, Czech Republic*
[f] *Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 43 Pruhonice, Czech Republic*

Received 31 March 2005; received in revised form 15 November 2005; accepted 2 January 2006

Abstract

Crown defoliation, is a product of the tree crown status from the past several years of growth, which can be misleading if used as a stress indicator when assessing current Norway spruce vitality. To evaluate recovery processes in previously damaged trees a newly developed macroscopic indicator based on proportions of bud development types was investigated. In 1998 and 1999, 474 mature Norway spruce trees were sampled for macroscopic measurements, 293 trees for tree-ring increments and 40 trees for study of metabolic markers (concentration of chlorophylls, phenolic compounds and lignin). Study sites were located in the Czech Republic, in the Sumava Mts, a relatively unpolluted and undisturbed area, and in the Krusne hory Mts, which exhibited a whole range of tree damage corresponding to a gradient of increasing air pollution load. Three categories of trees were identified which reflect current levels of intensity of shoot formation and capacity to replace potential needle loss by activation of buds with growth potential: accelerated, stabilized and decreased shoot growth. Relative to sink prioritization, we concluded that the highest vitality occurred in trees with stabilized shoot growth and the lowest vitality in trees with accelerated shoot growth. In conifers, the amount of allocated assimilates to low priority carbon sinks (such as stem growth, production of protective chemical compounds and reproductive organs) depends on what is remaining from the shoot growth processes which are of the highest priority. Significant inverse relationships were found between the intensity of shoot growth, tree-ring increments, production of reproductive organs and concentration of phenolic compounds in the needles. The highest allocation of assimilates to crown recovery occurred in trees with crown defoliation of 50–69% indicating forest recovery was observed in the most heavily damaged areas impacted by air pollution in the past. The present criterion of bud development types has potential for forestry management applications as it is easily applied in the field and, in contrast with standard forestry measurement of crown defoliation, it reflects accurately tree recovery and decline processes under multiple stress impacts.

Keywords: Tree vitality; Buds; Carbon allocation; Sink/source concept; Crown defoliation; Norway spruce (*Picea abies*)

1. Introduction

According to Dobbertin and Brang (2001) tree vitality can be defined as 'the ability of a tree to assimilate, survive stress, react to changing conditions, and reproduce'. Crown defoliation (needle loss) is a non-specific symptom of Norway spruce (*Picea abies*/L./Karst.) vitality widely used in forest practice. It has been questioned whether defoliation is a valid indicator of tree vitality, since it does not reflect early stages of crown recovery (Salemaa and Jukola-Sulonen, 1990; Cudlín et al., 2001; Dobbertin and Brang, 2001). The processes leading to crown regeneration in mature trees (i.e. to forest recovery) enable replacement of lost assimilative organs caused by the

---

* Corresponding author. Tel.: +420 2 2195 1694/3185; fax: +420 2 2195 1704/3306.
E-mail address: albrecht@natur.cuni.cz (J. Albrechtová).

0378-1127/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.foreco.2006.01.027

impact of multiple stresses and promotes tree resistance to defoliation (Chen et al., 2001). Therefore, the assessment and understanding of crown recovery and decline processes might be an important tool for forestry management practice for taking appropriate measures in time, or for identification of resistant or susceptible individuals to environmental factors.

The term 'tree growth' often refers to stem increments (e.g. Schulz and Härtling, 2003), however, tree growth comprises both shoot and stem increments. In the present study, the production of assimilative organs and wood formation is separated. Therefore, we use the term 'shoot growth' for production of shoots, while 'stem growth' is used for depiction of stem radial increments.

Crown regeneration of Norway spruce trees is accomplished by formation of two types of shoots, which differ in the duration of the dormant period between bud set and bud burst (Kozlowski, 1971). Regular shoots are differentiated following vegetative season after bud set from dormant buds. Secondary shoots, also called adventitious, epicormic or proventitious shoots (Gruber, 1994), are initiated at least two seasons after bud set. The buds giving origin to secondary shoots are called 'buds with growth potential' since they contain healthy meristems and they include dormant and latent buds (Albrechtová, 1997; Polák et al., 2003, 2004). Buds with growth potential resume development and form secondary shoots depending on their development program or in response to environmental cues (Shimizu-Sato and Mori, 2001). They normally do not resume their growth activity unless the trees are stressed as, for example, due to insect infestation (Powell, 1974), damage and defoliation (Halle et al., 1978) or pruning (Ishii et al., 2000).

One of the tools used to identify and quantify intensity of shoot growth and thus intensity of crown regeneration is based on the activity of apical meristems contained in the buds. Any disruption of bud development reduces the biomass and/or quality of foliage produced in the following year (Straw et al., 2000), while bud metabolic status reflects the photosynthetic activity of needles and transport capacity (Lipavská et al., 2000). Therefore, buds and photosynthetically active needles are mutually dependent. On the basis of a detailed anatomical study, Albrechtová (1997) developed a macroscopic criterion for classification of individual vegetative bud development types, allowing fast estimation of current Norway spruce vitality. The method is applicable to 1-year and older shoots and separates three types of vegetative buds according to their activity and regenerative potential: (1) active (regular) buds producing regular shoots; and two inactive bud types developed in two alternative ways depending on a combination of external and internal factors. Inactive bud types may have meristematic regions (2) that are dead or lost (an aborted bud) or (3) those regions that remain viable at least until the following season (a bud with growth potential).

Buds containing apical meristems are considered, among the plant tissues, to be a strong sink for carbon (e.g. Lipavská et al., 2000; Svobodová et al., 2000). The allocation of carbohydrates to individual physiological processes is in accordance with the principles of sink/source concept, proposed to explain how and why resource availability influences the allocation of plant resources to shoot growth and stem growth (Wareing and Patrick, 1975; Honkanen and Haukioja, 1994). It assumes that different physiological processes compete for resources and variations in the strengths of sources (e.g. leaves) and sinks (actively growing meristems) influence resource allocation and quantitative changes in growth. Running and Gower (1991) and Klap et al. (2000) suggested that conifers allocate carbon to different tissues or physiological processes according to the following prioritization: (1) maintenance respiration; (2) shoot growth and storage; (3) root growth and storage; (4) stem growth and storage; (5) synthesis of protective chemical compounds; (6) production of reproductive organs.

The majority of mature trees are not able to produce such an amount of carbohydrates to supply all tree sinks and physiological processes. Further reduction of resource availability is assumed in damaged trees with high crown defoliation, and/or poor physiological status of remaining needles. One of the assumptions of the sink/source concept is, if the amount of carbohydrates available for allocation is limited, that the sink with highest priority is preferentially supplied. In conifers, since shoot growth is the primary sink for assimilates (Schreuder and Brewer, 2001), a decrease in resource availability has the most negative influence on tissues responsible for processes that are in lower priorities for carbon allocation, such as stem increment growth (Honkanen et al., 1999; Nicolini et al., 2001), production of protective chemical compounds and reproductive organs (Kaitaniemi et al., 1999; Zvereva and Kozlov, 2001).

To test possible sink prioritization of such physiological processes, trees with different levels of physiological status corresponding to a gradient of tree vitality were selected from four areas in the Czech Republic, where the main stress factor was air pollution load. Symptoms of tree damage caused by the impact of stress factors can be identified not only at macroscopic level, but before that also at pre-visual microscopic and metabolic levels (e.g. Soukupová et al., 2000). One of the biomarkers of crown status at the metabolic level is the concentration of photosynthetic pigments in needles. The amount of photosynthetic pigments in conifers is dependent on various environmental factors, including nutrient imbalance (Solberg et al., 1998) and atmospheric pollutants (Rock et al., 1988; Soukupová et al., 2000). Phenolic compounds including lignin are one of the groups of carbon-based protective chemical compounds, which contribute to resistance against herbivores (Honkanen et al., 1999). Changes in deposition of soluble phenolics and lignin are documented as a response to various stresses, including herbivores (Tuomi et al., 1989) and atmospheric pollution (Kainulainen et al., 1995).

The effect of atmospheric pollution on the Norway spruce crown status was widely studied. Acid deposition is known to reduce foliage mass (Klap et al., 2000; Schreuder and Brewer, 2001), tree-ring increments (Solberg and Moshaug, 1999), chlorophyll and lignin content in the needles (Soukupová et al., 2000), to reduce carbohydrate allocation to low priority carbon sinks including production of protective chemical compounds and thus, to weaken strategies of plants for defence against diseases (Honkanen et al., 1999), etc. Acidic pollution was also found to affect many physiological processes of Norway spruce buds, e.g. changes in contents of non-structural saccharides (Lipavská et al., 2000; Svobodová et al., 2000) and increased activity of non-specific esterase (Soukupová et al., 2002).

The primary aim of the present study was to separate shoot growth categories of mature Norway spruce trees using bud developmental types that reflect the current intensity of primary shoot formation and the potential of tree recovery in the future. Our second aim was to test whether shoot growth categories correlate with the assumptions of the sink/source concept for conifers regarding stem growth, synthesis of protective chemical compounds in the needles, and production of reproductive structures. The third aim was to apply the newly developed shoot growth categories toward determination of tree vitality in the four different mountainous areas in Czech Republic that exhibit different levels of air pollution loading.

2. Material and methods

2.1. Study sites

A total of 116 30 m × 30 m plots were selected for study in 1998 and 1999 in at least 60-year-old homogenous Norway spruce forests in two mountain regions of the Czech Republic (Table 1 and Fig. 1). Area 1 (Modrava) was selected in the relatively healthy, unpolluted Sumava Mts, in the less-industrialized southwestern part of the country, representing the least polluted region in our study. The growth of forests in the Sumava Mts was previously limited primarily by the insufficient nutrition of mountain soils (Podrázský, 1999). Three remaining study areas were located in the Krusne hory Mts, along a strong gradient in tree damage, which was caused by acidic airborne pollution in northwestern Bohemia. Area 2 (Prebuz) was located in the western, relatively healthy and least polluted part of the Krusne hory, and areas 3 (Boží Dar) and 4 (Kovárská) were located in the central Krusne hory Mts, which was heavily polluted and damaged. Data of monthly average SO_2 deposition for all four areas between 1996 and 1999 are given in Table 1. The elevation of the sites varied from 800 to 1100 m, but the slope, soil and climatic conditions did not vary for selected sites.

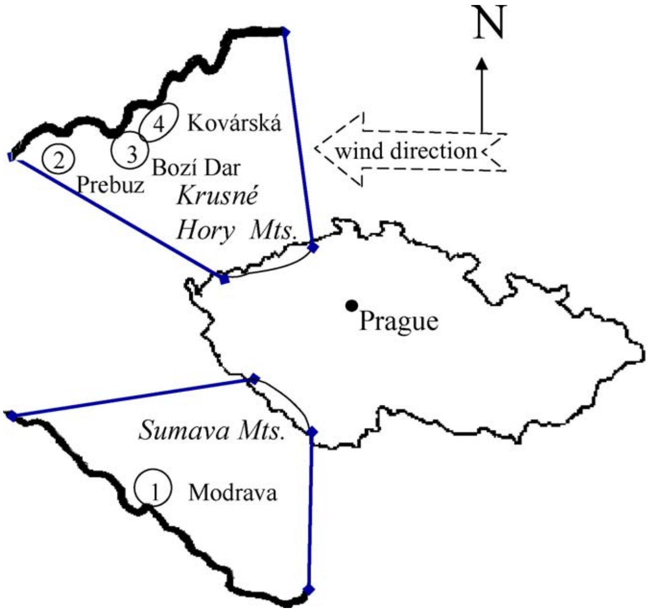

Fig. 1. Location of the research areas in Sumava (area 1 near Modrava) and Krusne hory Mts (from the western to the central parts: Prebuz, area 2; Bozí Dar, area 3; Kovárská, area 4) Czech Republic. The arrow indicates the direction of prevailing winds bringing air pollution from adjacent sources, what caused a strong gradient in air pollution in Krusne hory Mts.

2.2. Tree sampling

The study was conducted during August 1998 and 1999, when shoot growth was complete and current-year needles were fully developed and physiologically mature. Five representative trees were selected to cover the variability of

Table 1
The altitude and air pollution data from 1996 to 1999 for the four study areas in the Sumava and Krusne hory Mts, Czech Republic

	Sumava Mts		Krusne hory Mts	
	Area 1 (Modrava)	Area 2 (Prebuz)	Area 3 (Bozí Dar)	Area 4 (Kovárská)
Number of sites (1998, 1999)	12, 13	22, 11	11, 14	18, 15
The average altitude of the sites (m a.s.l.) ± S.E.	1100 ± 21.9	880 ± 18.6	990 ± 23.5	839 ± 19.4
The closest monitoring station (distance to study areas)	Churánov (10 km)	Prebuz (<5 km)	Medenec (5 km)	Prísecnice (<5 km)
Monthly average deposition of SO_2 in 1996–1999 (mg m^{-3})[a]				
1996	–[b]	26	66	57
1997	5	14	36	25
1998	2	6	17	13
1999	2	3	7	4
Monthly average deposition of NO_x in 1996–1999 (mg m^{-3})[a]				
1996	–	17	27	24
1997	6	15	23	19
1998	6	12	15	14
1999	6	11	17	11

[a] Data from Czech Hydrometeorological Institute (http://www.chmi.cz).
[b] Not measured.

crown defoliation inside all study sites in 1998 (a total of 315 trees from 63 sites); in 1999 the number of representative trees was reduced to three per site (a total of 159 trees from 53 sites). The indicators discussed in this study includes standard macroscopic measurements of (1) foliage biomass (crown defoliation, needle retention); (2) physiological status of assimilative organs (needle discoloration, total chlorophylls *a* and *b* concentration); (3) macroscopic classification of individual bud development types (regular to aborted bud ratio, proportion of buds with growth potential); (4) dendrochronology parameter (tree-ring increments); (5) reproduction parameter (cone production); (6) selected metabolic parameters (phenolic and lignin concentration).

2.3. Standard macroscopic measurements of tree vitality

Standard forestry macroscopic measurements were based on crown defoliation, using a classification table developed for individual spruce phenotypes, needle discoloration, needle retention and cone production. Tree damage class (TDC) was set according to the crown defoliation and needle discoloration: TDC 0 = defoliation 0–9%, TDC 1 = 10–29%, TDC 2 = 30–49%, TDC 3 = 50–69%, and TDC 4 = 70–99%. In the case of needle discoloration, the TDC was set one level higher. We did not find any Norway spruce with TDC 4 within our study sites. Needle discoloration, including yellowing and browning, was assessed as a binary variable (1, present; 0, no discoloration) for representative trees from all sites and proportional values were determined for the four areas under study and shoot growth categories. Needle retention corresponded to foliage longevity and was defined in years. Cone production was classified in 2002, the closest subsequent seed year after sampling at all study sites. The same trees used in 1998 were evaluated semi-quantitatively from the ground and values ranged from 0 to 3 (0, no cones; 1, mild; 2, medium; 3, intensive cone production).

2.4. Macroscopic criterion for classification of bud development types

One sunlit branch was systematically collected for analysis, using pruning poles, from the middle third of the production portion of the crown from all representative trees (in total 474

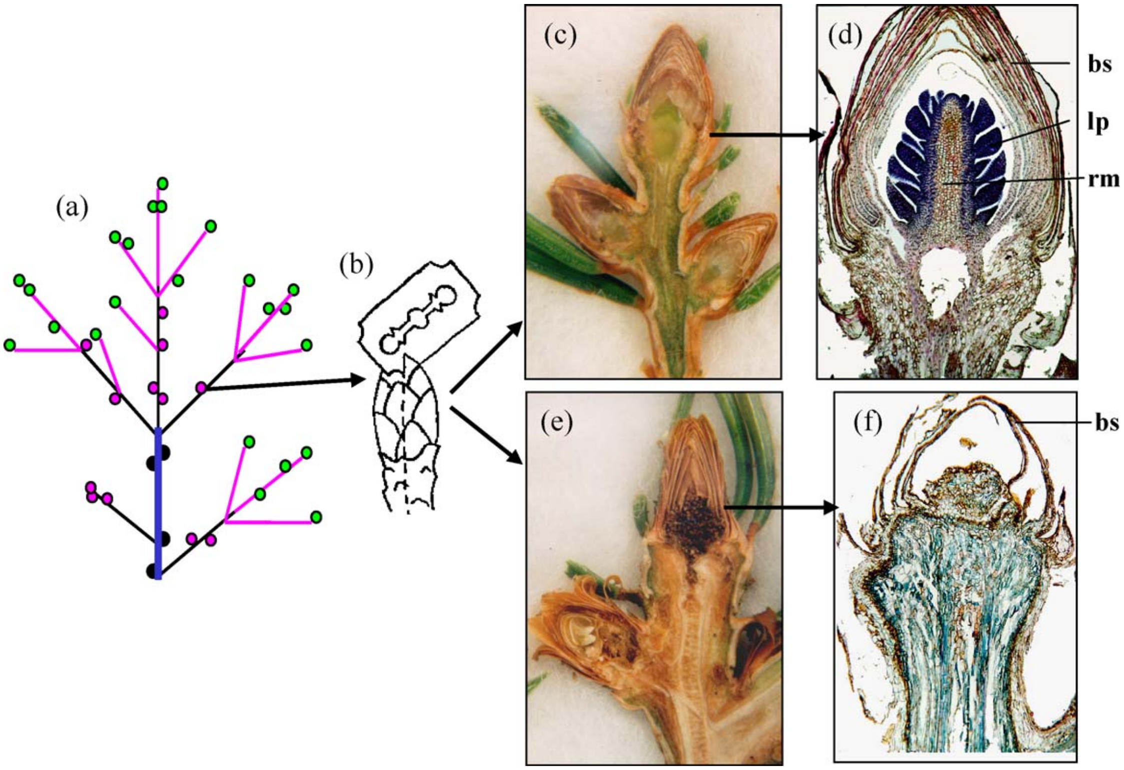

Fig. 2. Macroscopic criterion for classification of bud development types. (a) Schematic picture of branch architecture of Norway spruce trees. The blue line represents a 2-year-old shoot. Black-colored symbols represent structures differentiated on a 2-year-old shoot: either inactive buds, which were not studied, or regular 1-year-old shoots (black color). Macroscopic criterion for classification of bud development types was applied to these 1-year-old shoots. Regular buds corresponding to current-year primary shoots are marked by red lines and inactive buds—either buds with growth potential or aborted by red dots. Newly differentiated buds on current-year shoots are green-colored and these buds are not part of this study. The criterion for classification of bud development types cannot be applied to these buds since they have not differentiated into active or inactive buds yet. (b) Inactive buds on 1-year-old shoots (either terminal or lateral) were classified after cutting a longitudinal bud section with a razor blade into two halves as (c) buds with growth potential with green and vital meristematic tissue in the centre of a bud and (e) aborted buds with brown and dead meristematic region. Macroscopic criterion was applied during August, when regular buds (current-year shoots) are fully developed. (d, f) Prepared median longitudinal sections of Norway spruce buds. (d) Dormant bud with growth potential has vital bud meristems: (lp) meristematic leaf primordia and (rm) rib meristem covered by (bs) bud scales. (f) Aborted bud lacks any meristematic tissues even though it still can be covered by (bs) bud scales. Sections were prepared by paraffin method (Johansen, 1940) with small modifications (details are given in Soukupová et al., 2002), stained with alcian blue and nuclear fast red (Benes and Kamínek, 1973).

trees: 315 in 1998 and 159 in 1999). Development of the vegetative bud of Norway spruce is described in detail by Soukupová et al. (2002). In the beginning of the vegetative season, resting (dormant) buds formed in the previous vegetative season break dormancy and a new primary shoot is formed by regular shoot growth, therefore, these buds are called regular buds. Some buds do not pass throughout the annual growth cycle and stay inactive during the next vegetative season (Kozlowski, 1971). By cutting a longitudinal section of an inactive bud with a razor blade (Fig. 2b), a bud with growth potential and an aborted bud can be distinguished. The bud with growth potential includes green meristematic tissues in its center (Fig. 2c and d), while an aborted bud is characterized by dead, brown meristematic tissues (Fig. 2e and f) (Albrechtová, 1997). The green color of viable buds with growth potential is due to chlorophyll contained in the needle primordia (e.g. Lepedus et al., 2001) and these buds may form secondary shoots during subsequent seasons. The brown color of an aborted bud is due to chlorophyll loss and high lignin and phenolics content of the dead meristematic tissue.

To determine current shoot growth processes of Norway spruce, only the buds (either terminal or lateral) formed on 1-year-old shoots were analyzed (Fig. 2a). These are the youngest shoots where regular buds can be identified as those giving origin to regular shoots. Thus, bud samples collected in August 1998 were formed on 1997 shoots (Fig. 2a). At least 70 buds per branch (approximately 30–50 cm long distal segment of a branch) formed on 1-year-old shoots were classified into one of these three categories and transformed into a percentage scale. Total number of evaluated buds in this study was more than 33,000 (more than 70 buds per tree).

2.5. Shoot growth categories

Two variables were used to interpret the results of macroscopic classification of bud development types: the ratio of regular to aborted buds and the proportion of buds with growth potential. The higher the ratio of regular to aborted buds, the more intensive primary shoot formation. Low values of the ratio correspond to high bud mortality rate and/or to a low proportion of regular buds and, thus, they indicate a low intensity of the primary shoot formation. The higher the proportion of buds with growth potential, the higher the tree's capacity to replace the loss of assimilative organs in future by formation of new secondary shoots under stress conditions.

We wanted to address the following question: can the 474 trees studied be grouped into clusters in which the shoot growth of Norway spruce is described by a ratio of regular to aborted buds and the proportion of buds with growth potential? Norway spruce trees were grouped by cluster analysis into three categories which reflect shoot growth accomplished via bud meristems and they, in principle, correspond to accelerated shoot growth, stabilized shoot growth and decreased shoot growth. The definition and brief description of shoot growth categories is given in Table 2.

2.6. Dendrochronological data

Tree increment cores were extracted at breast-height from five representative trees at each site, during the summer of 1998. The total number of usable increment cores was 293. Ring widths series were cross-dated and checked for consistency and outliers using Cofecha software. Details regarding the dendrochronological collection are given in Entcheva (2000), Entcheva et al. (2004). To study relationship between current shoot growth processes and stem growth, and to avoid climatic and random fluctuations of radial growth, the means of last 3 years were used instead of the last increment, i.e. in 1998 and 1999 samples the means from 1996 to 1998 and from 1997 to 1999 were used, respectively.

2.7. Metabolic parameters

The branches for metabolic parameters were sampled from 40 selected trees located across study areas, which were a subset of trees selected for macroscopic classification of bud development types (20 trees in total were sampled for metabolic parameters in 1998, another 20 trees in 1999). First-, second-, and third-year needles were collected directly at the site and taken in a portable freezing box (−4 °C) to the laboratory, where they were weighed and placed in a freezer (−70 °C) within 8 h of collection to await analysis.

2.7.1. Determination of total phenolic material

Needles were frozen in liquid nitrogen, homogenized with a Heidolph RZR 2020 homogenizer (Heidolph, Kelheim, Germany) and extracted in boiling methanol. The total amount of phenolics was determined with Folin-Ciocalteau reagent (Singleton and Rossi, 1965) using gallic acid as a standard. Details are given in Soukupová et al. (2000).

2.7.2. Determination of lignin content

In order to isolate cell walls, a pellet of previously homogenized needles was suspended in methanol and washed

Table 2
Definition and brief description of Norway spruce shoot growth categories

Shoot growth category	Ratio of regular/aborted buds	Buds with growth potential	Brief description of the shoot growth category
Accelerated shoot growth	>7	≤20%	Intensive primary shoot formation and low potential for secondary shoot formation
Stabilized shoot growth	Not relevant	>20%	High potential for secondary shoot formation
Decreased shoot growth	≤7	≤20%	Low primary shoot formation and low potential for secondary shoot formation

with a series of solvents: methanol 1 M NaCl, 1% sodium dodecylsulphate, water, methanol, 1:1 chloroform/methanol (Lange et al., 1995). Total lignin content was determined using thioglycolysis (Lange et al., 1995). The absorbance of the extracted lignin was measured at the maximum absorption of 225 nm using a Unicam Helios α spectrophotometer. The amount of lignin is given as an absorbance of extracted lignin per unit of fresh weight. Details are given in Soukupová et al. (2000).

2.7.3. Determination of chlorophyll content

The concentrations of chlorophylls *a* and *b* were determined spectrophotometrically. On average, 0.5 g of frozen needles was placed in 10 ml of dimethylformamide (DMF, Porra et al., 1989) and left in the dark at 8 °C for 5 days until the needles were bleached. The absorbance of extracts was then measured at 647, 664 and 750 nm using a Unicam Helios α spectrophotometer. The amounts of chlorophyll were then calculated according to Wellburn (1994). Details are given in Albrechtová et al. (2001) and Soukupová et al. (2000).

2.8. Statistical analysis

The shoot growth categories were set according to cluster analysis using the quadratic Euclidean distance measure and the Ward criterion (Brosius, 1989). When data were normally distributed, differences of continuous variables among tree shoot growth categories were analyzed by ANOVA and Tukey–Kramer test. Non-normal data were log-transformed. Ordinal variables were analyzed by Armitage test. Significance level for all tests was $\alpha = 0.05$. Statistical analyses were performed using the NCSS 2001 software (Hintze, 2001).

3. Results and discussion

3.1. Shoot growth categories and the assumptions of sink/source concept

The average TDC assessed on the basis of crown defoliation was twice as high for trees with accelerated shoot growth as for trees with stabilized and decreased shoot growth (Fig. 3a). Needle retention for trees with accelerated shoot growth reached only 7 years, whereas, it was approximately 9 years for trees in other two shoot growth categories (Fig. 3b) indicating that trees with accelerated shoot growth had the lowest biomass of needles. The physiological status of the needles of trees with accelerated shoot growth was not very good, since the average needle discoloration was significantly higher (Fig. 3b) and concentration of total chlorophylls *a* and *b* in 1 and 2-year-old needles was lower than for trees in stabilized shoot growth category (Fig. 4a). The low needle biomass and relatively poor physiological status of remaining needles suggests that the total amount of carbohydrates available for allocation to sinks in trees with accelerated shoot growth was limited.

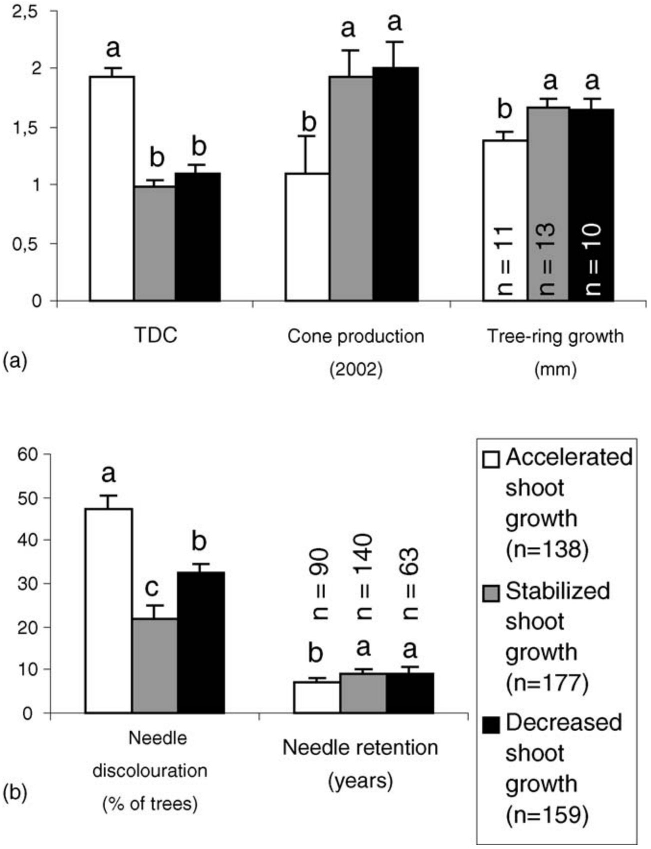

Fig. 3. (a) Tree damage class (TDC), cone production, tree-ring increments, (b) needle discoloration and needle retention for Norway spruce trees grouped according to the newly defined shoot growth categories. All indicators were measured in 1998 and 1999 except of the cone production, which was measured in the closest reproductive year 2002. Means; bars above columns correspond to S.E.; columns with common letters are not significantly different (ANOVA); $\alpha = 0.05$; *n* for each group (except of tree-ring increments and cone production) is given in the legend; total $n = 293$ for tree-ring increments, $n = 34$ for the cone production and $n = 474$ for other parameters.

One of the assumptions in the sink/source concept is that carbohydrates tend to be allocated preferentially to the most limiting physiological processes (Salemaa and Jukola-Sulonen, 1990; Mooney and Winner, 1991), which in the case of heavily defoliated trees will be to the production of new shoots (Schreuder and Brewer, 2001). To keep degradative and regenerative processes in the crown in balance, heavily defoliated trees must maximize the use of available buds. In conifers, the amount of allocated assimilates to low priority carbon sinks (such as stem growth, production of protective chemical compounds and reproductive organs) would depend on what is remaining from the shoot growth processes (Herms and Mattson, 1992). For trees with accelerated shoot growth, low carbon allocation to reproduction processes would be expected in comparison with other categories. This was documented by data from the closest subsequent cone production year in 2002 (Fig. 3a). We recorded that the proportion of generative buds for trees with increased shoot growth was only 1%, whereas, it was 43% for trees with stabilized shoot growth (Polák et al., 2004). The negative trade-off between the intensity of shoot growth and production of reproductive structures in low pollution sites was described also by Zvereva and Kozlov (2001) for *Salix* sp. Reduced cone mass and seed viability were also reported by Kaitaniemi et al.

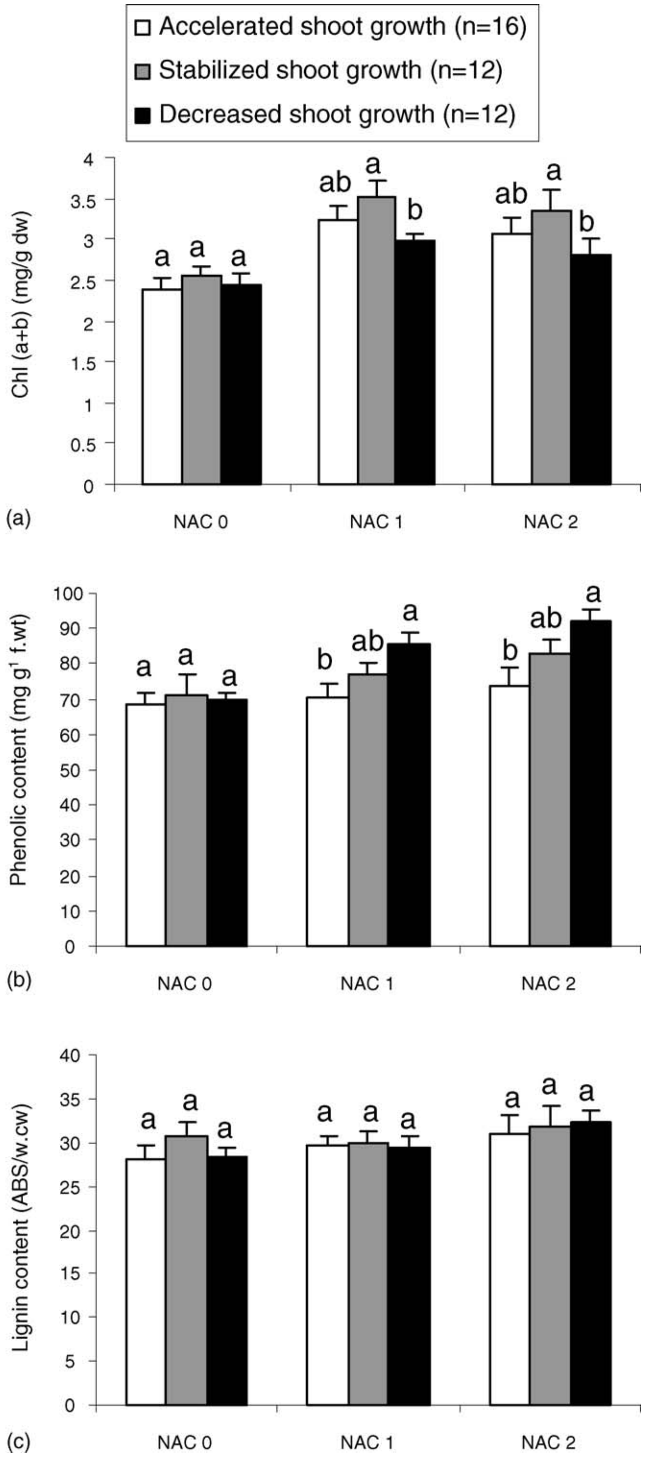

Fig. 4. Metabolic markers of current-, 1-, and 2-year-old needles of Norway spruce trees grouped according to the newly defined shoot growth categories. (a) Total amounts of chlorophylls *a* and *b* ($\text{chl}_a + \text{chl}_b$). (b) Phenolic content in mg per gram of fresh weight. (c) Lignin content. ABS, absorbance at 225 nm; NAC, needle age class; wcw, weight of cell walls. Means; bars above columns correspond to S.E.; columns with common letters inside each NAC are not significantly different (ANOVA); $\alpha = 0.05$; total $n = 40$.

(1999) for trees with high crown regeneration. The average tree-ring increment was only 1.37 mm for trees with accelerated shoot growth and nearly 1.7 mm for trees in the other two shoot growth categories (Fig. 3a). Nicolini et al. (2001) revealed a similar pattern of intensive crown regeneration combined with a very sharp reduction in stem cambial activity and tree-ring dimensions. Low tree-ring increments of the trees with accelerated shoot growth from polluted site during several past years are also documented from representative core in comparison with increments of the trees with decreased shoot growth from polluted site and stabilized shoot growth from unpolluted site (Fig. 5).

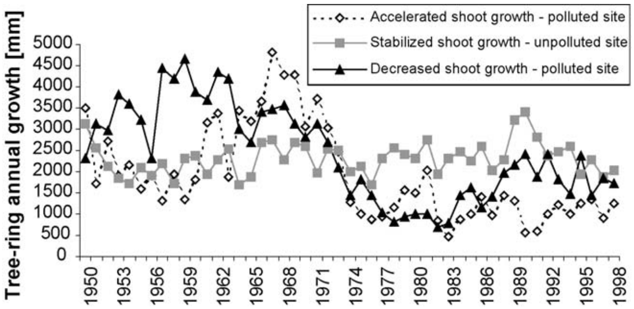

Fig. 5. Tree-ring annual growth of a representative tree for each growth category during the years 1950–1998: tree cores of categories accelerated shoot growth and decreased shoot growth from polluted areas (area 4 in both cases) and stabilized shoot growth from unpolluted area (area 1).

Induced deposition of protective chemical compounds such as phenolics has been documented for various stress factors including atmospheric pollutants (Vogelmann and Rock, 1988; Moss et al., 1998; Soukupová et al., 2000). In current year needles we did not find any difference in relation to shoot growth intensity. However, for 1 and 2-year-old needles the concentration of phenolic compounds was significantly lower for trees with accelerated shoot growth in comparison with the other two shoot growth categories (Fig. 4b). The trade-off between shoot growth and production of phenolics was found by others, e.g. by Honkanen et al. (1999) for pines. Phenolic compounds are known to affect tree resistance to herbivore attack (e.g. Waring and Cobb, 1992). It is apparent, that due to the low concentration of protective chemical compounds, Norway spruce trees with accelerated shoot growth have high risk of being attacked by herbivores and pathogens.

It is generally accepted that increased cell-wall lignification is a non-specific reaction of plants to stresses. Thus, it could be expected that trees with higher TDC would have needle mesophyll cells with higher cell wall lignification. However, various contrary findings were found as a response of needles to different stresses (Entcheva, 2000; Soukupová et al., 2000). Based on the concept of sink/source prioritization (Running and Gower, 1991; Klap et al., 2000), the lowest lignin content could be expected for trees with accelerated shoot growth. We did not find any significant relationship between lignin concentrations in needles and shoot growth category (Fig. 4c). One possible explanation could be that heavily defoliated trees with accelerated shoot growth were located particularly in the more polluted areas 3 and 4 (Fig. 6), which have been heavily affected by SO_2 pollution prior to our study (Table 1). The mechanism of lowered lignification of needles affected by SO_2 could be that sulphite (the product of detoxification of SO_2) competitively inhibits peroxidases involved in lignification,

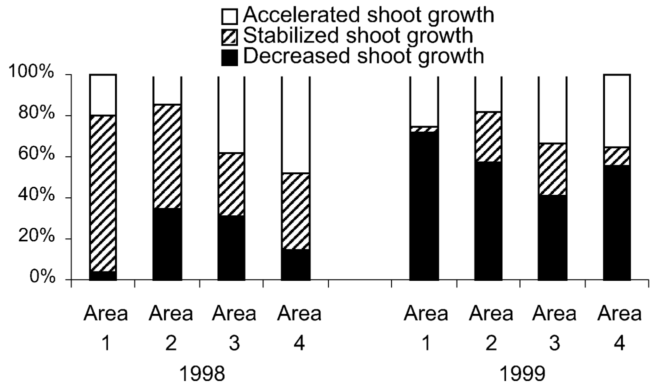

Fig. 6. Proportion of Norway spruce trees classified according to the shoot growth category for all study areas in 1998 and 1999; total $n = 474$.

causing a decrease in lignification in affected tissues (Pfanz and Oppmann, 1991).

In general, intensive production of shoots might result from weakened apical dominance, as it was suggested by Zvereva and Kozlov (2001) for trees from more polluted sites. Physiological mechanisms underlying the weakened apical dominance in trees from more polluted sites may result from changes in the activity of soluble peroxidases, which might be involved in the oxidation of indoleacetic acid (IAA) (Whitmore, 1976), a main hormone (auxin) involved in phenomenon of apical dominance. In our case, the activity of soluble peroxidases was found to be significantly greater in the needles from highly defoliated trees located in the heavily polluted sites from the areas 3 and 4 (Soukupová et al., 2000), the majority of which revealed accelerated shoot growth (Fig. 6). Greater activity of soluble peroxidases might have reduced auxin concentrations in plant tissues and helped to release the buds from apical dominance.

The trees from stabilized and decreased shoot growth categories had equal values of TDC, needle retention, tree-ring increments and cone production intensity (Fig. 3a and b). Both categories significantly differed in the physiological status of assimilative organs, which was significantly better for trees with stabilized shoot growth. Total concentrations of chlorophylls *a* and *b*, which is considered to be a sensitive indicator of physiological status of the needles, was the highest for trees with stabilized shoot growth for 1 and 2-year-old needles (Fig. 4a) This corresponded with the lowest occurrence of needle discoloration (Fig. 3b). The chlorophyll concentrations in needles of all trees under study were still above minimal physiological value, which is reported to be 2.2 mg/g dry weight for Norway spruce needles (Siefermann-Harms, 1994).

Tree vitality can be defined as the ‘ability of a tree to assimilate, to survive stress, to react to changing conditions, and to reproduce’ (Dobbertin and Brang, 2001). According to this definition, trees with accelerated shoot growth had the lowest tree vitality, because the indicators of foliage biomass, physiological status of assimilative organs, stem growth, reproduction, and production of protective chemical compounds revealed the lowest values (Figs. 3a,b and 4a,b). The highest vitality seems to be exhibited by trees with stabilized shoot growth. According to metabolic markers, these trees were in the best physiological status (Fig. 4a–c). It is apparent that stabilized shoot growth may optimize resource-use efficiency or other attributes associated with long-term survival, wood production and reproductive success. A high proportion of buds with growth potential enable trees to rapidly replace potential needle loss in the future by formation of new secondary shoots, if needed.

3.2. Shoot growth categories and tree vitality gradient

3.2.1. Area 1: Sumava Mts

In 1998, the indicators of foliage biomass and physiological status of assimilative organs revealed the best crown status of trees from area 1 located in Sumava Mts and area 2 from the western part of Krusne hory Mts (Fig. 7c–e), corresponding well with the lowest air pollution load in the four areas (Table 1). In the area 1, 77% of trees were characterized by stabilized shoot growth (Fig. 6). Surprisingly in 1999, the situation dramatically changed for the worse in the area 1. The average TDC significantly increased, reflecting an increased defoliation (Fig. 7e). The proportion of buds with growth potential decreased from 30% to 10%, the ratio of regular to aborted buds from 9 to 4, and proportion of aborted buds increased from 6% to 24% (Fig. 7a and b). In 1999, 72% of all trees exhibited a decreased shoot growth. We suggest that an intensive bark-beetle attack (mostly *Ips typographus* and other species of xylophagus insects) was the primary stress factor leading to decline of tree vitality between two consecutive seasons in this region, since after 1998, the bark beetle outbreak has reached the scale of a calamity. While in 1992 the proportion of highly damaged and declining spruce trees in Sumava Mts was determined to be 9% in 1999 it was 48% (Zemek and Herman, 2001). The recorded increased rate of bud abortion could have been another contributing factor, in addition to the biotic cause. The negative role of environmental stress factors on the physiological state of bud meristems may result in damage-induced changes in the ability of meristems to compete for resources (Honkanen and Haukioja, 1994). Buds containing apical meristems belong to the most important plant sinks (e.g. Lipavská et al., 2000; Svobodová et al., 2000). Damage may modify sink strength (Honkanen and Haukioja, 1994; Honkanen et al., 1999) leading eventually to bud abortion. Weakened even-aged plantations of spruce suggest an inability to resist to air pollution, snowbreaks and windfall, resulting in deteriorating of forest health (Moravec et al., 2002; Wild et al., 2004). Based on our study, the prognosis for the area 1 would have been that continual impact of stress factors would accelerate crown defoliation. When a loss of needles exceeds a certain threshold value, the tree usually responds by accelerated shoot growth to replace the needle loss. Based on statistical analysis we have assessed the threshold value of crown defoliation to be 30%, the value for transition from mildly (TDC = 1) to moderately damaged trees (TDC = 2) and corresponds to an increase in primary shoot formation as indicated by the regular to aborted bud ratio and decrease in the proportion of buds with growth potential.

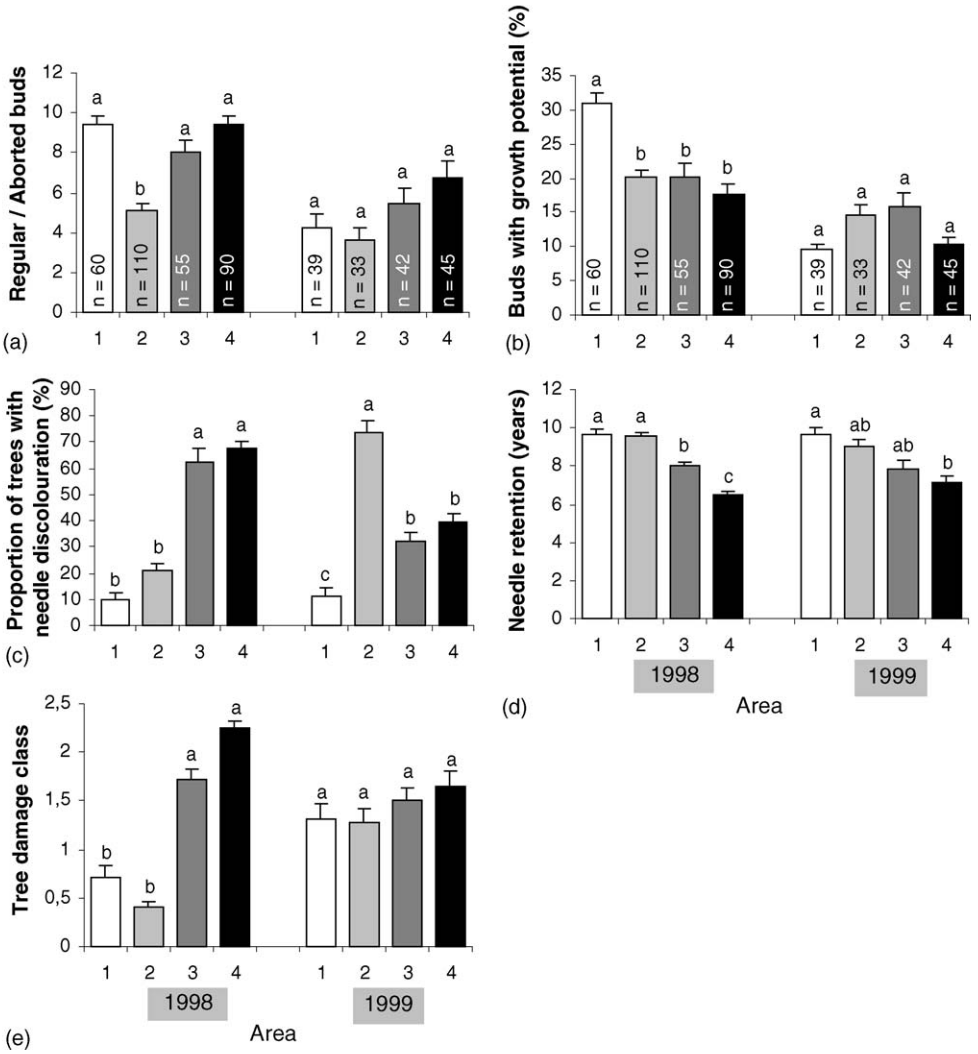

Fig. 7. Norway spruce tree characteristics of (a, b) shoot growth sink (regular to aborted bud ratio and proportion of buds with growth potential); (c) physiological status of assimilative organs (needle discoloration); and (d, e) foliage biomass (needle retention, tree damage class) for all study areas in the Krusne hory and Sumava Mts in 1998 and 1999. Means; bars above columns correspond to S.E.; columns with common letters inside each year are not significantly different (ANOVA); $\alpha = 0.05$; total $n = 474$.

3.2.2. Area 2: western part of Krusne hory Mts

The average crown defoliation as well as proportion of chlorotic trees of this relatively healthy (in 1998) area 2 from the western Krusne hory significantly increased in 1999 (Fig. 7c and e). The proportion of trees with decreased shoot growth increased from 1998 to 1999 from 40% to 58%, respectively. These negative changes were probably caused by overall worsening of the physiological state in this region during winter 1998/1999, which exhibited a massive needle yellowing in the spring of 1999. The causes of this winter damage has been assumed to be long-term accumulation of pollutants, the exhaustion of self-regulation capacity of older trees and the destabilization of sensitive spruce ecosystems (Modrzyński, 2003; Srámek et al., 2003). Acidification of the upper layers of the soil profile may be responsible for the critical extraction of nutrients from sorption complex, especially basic cations (such as Mg and Ca elements) in both soil and needles (e.g. Srámek et al., 2003). The prognosis for area 2 would have been the same as area 1, i.e. a major transition of trees to the accelerated shoot growth category. The winter damage has altered this situation, just as the insect damage has altered the conditions in area 1.

3.2.3. Areas 3 and 4: central part of Krusne hory Mts

Spruce forests in area 3 and especially in area 4 (the central part of Krusne hory Mts) were heavily damaged by acidic air pollution in the past and were found to be seriously defoliated in 1998 (Fig. 7d and e). Proportion of chlorotic trees was higher than 40% and low values of needle retention indicated serious damage and high intensity of degradative processes in the crown. The proportion of trees characterized by accelerated shoot growth was high in comparison with the areas 1 and 2 (Fig. 6), indicating massive forest recovery in this area. The situation even improved in 1999 (Fig. 7c–e). Documented forest recovery there is likely a result of continuing long-term reduction in sulphur emissions during the 1990s (e.g. Ardo et al., 1997). The average TDC improved from 1998 to 1999 and decreased below value 2 corresponding to crown defoliation 10–30%, indicating a transition of trees from moderately damaged to mildly damaged trees. Similarly, the R.M.A.C.R. (2002) describes forest recovery in the area in the form of improved crown defoliation (decreased by 10%) in the central and eastern Krusne hory Mts from 1998 to 1999. The ratio of regular to aborted buds (the intensity of primary shoot formation) markedly decreased for healthy trees with TDC = 0 and 1, and to a lesser degree for TDC = 2 trees and remained high for TDC = 3, supporting our conclusions regarding the sink/source concept. The altered assimilate allocation to high priority sinks such as shoot growth results in intensive crown recovery of more heavily damaged trees. We suppose that reduction of the intensity of shoot formation for less defoliated trees in 1999 was caused by the fact that the loss of needles had been already compensated and resource availability seemed to be high enough to supply both high and low priority sinks by carbohydrates. The prognosis for areas 3 and 4, under the condition that the stress load will not significantly change in the future, would have been a continuing decrease in trees with accelerated shoot growth and an improvement of tree vitality. Heavily defoliated trees are supposed to continue in accelerated shoot growth, leading either to total recovery (lowering defoliation) or to energetic exhaustion or tree death (with defoliation remaining high).

4. Conclusions

Crown defoliation allows the classification of past foliar loss in a crown for conifers, but does not reflect current recovery or declining processes. Using the proportion individual bud development types as indicator (assessed on 1-year-old shoots) we separated the following shoot growth categories of Norway spruce associated with multiple impacts of stress factors: accelerated shoot growth, stabilized shoot growth and decreased shoot growth. These categories reflect the current trend of tree recovery or decline processes. This finding has direct implications in forestry practices for forecasting of forest stand dynamics and development. It will facilitate the selection of resistant trees for propagation and stressed susceptible trees for tree logging. Because of the simplicity of this method, it can be easily applied under field conditions and seem to have a practical potential for large-scale monitoring of Norway spruce decline and recovery events.

Our study supports the prioritization of sink/source physiological processes for conifers. The result of the present study points to the importance of the sink/source relationship of Norway spruce trees in recovery from heavy damage. We found that heavily defoliated trees (defoliation 50–69%) exhibited the most intensive crown regeneration, i.e. highest allocation of assimilates to the primary shoot growth processes. In addition to high defoliation, these trees are also weakened by insufficient carbohydrate allocation to low priority carbon sinks (e.g. reproduction, stem increment growth and synthesis of protective chemical compounds), which decreases their chance for sustainable survival.

From the long-term perspective, our data supported the conclusion that the vitality of Norway spruce forests in the Czech Republic previously heavily impacted by air pollution is in agreement with the conclusion of the R.M.A.C.R. (2002). This means that: (1) forests, which have been relatively healthy since the beginning of 1990s, have started to decline as a result of long-term impact of multiple stress factors, and (2) forests, which have been heavily damaged and survived the extreme stress conditions, have started to recover in the central and eastern Krusne hory, likely in response to improved air pollution conditions during the last decade. However, due to changes in soil conditions induced by long-term heavy acidic loads it can be expected, that even after improvement of air quality, the stress conditions are going to persist much longer. The local surviving spruce forests are still on the edge of ecological stability. Large-scale ameliorative measures of forestry management such as liming with crushed dolomite have been applied there since the 1970s and their effects and consequences on the long-term stand dynamics have yet to be evaluated.

Acknowledgements

The present research was supported by Grant Agency of the Academy of Science of the Czech Republic (KJB6111307). Participation of the last two authors during paper finishing was covered by NASA grant (NAG5-5192) and by a joint U.S.–Czech project (NSF-DEB 108385); and Ministry of Education (ME 658). We also thank Ryan Huntley, Shannon Spencer, Anja Whittington, and others from the University of New Hampshire, USA and other students from Charles University, CZ, who participated in the field research in 1998 and 1999.

References

Albrechtová, J., 1997. Development and growth of vegetative buds of Norway spruce (Picea abies/L./Karst.): The effect of acid rain. Summary of PhD Thesis. Charles University in Prague, Czech Republic (http://www.natur.cuni.cz/~albrecht/).

Albrechtová, J., Rock, B.N., Soukupová, J., Entcheva, P., Šolcová, B., Polák, T., 2001. Biochemical, histochemical, structural and reflectance markers of damage in Norway spruce from the Krušné hory used for interpretation of remote sensing data. J. For. Sci. 47 (Special issue), 26–33.

Ardo, J., Lambert, N., Henzlík, V., Rock, B., 1997. Satellite-based estimations of coniferous forest cover changes: Krusne hory, Czech Republic 1972–1989. Ambio 26, 158–166.

Benes, K., Kamínek, M., 1973. The use of aluminium lake of nuclear fast red in plant material successively with alcian blue. Biol. Plant. 15, 294–297.

Brosius, G., 1989. SPSS/PC+ advanced Statistics und Tables-Einführung und praktische Beispiele. McGraw-Hill, London.

Chen, Z., Kolb, T.E., Clancy, K.M., 2001. Mechanisms of Douglas-fir resistance to western spruce budworm defoliation: bud burst phenology, photosynthetic compensation and growth rate. Tree Physiol. 21, 1159–1169.

Cudlín, P., Novotný, R., Moravec, I., Chmelíková, E., 2001. Retrospective evaluation of the response of montane forest ecosystems to multiple stress. Ekol. Bratislava 20, 108–124.

Dobbertin, M., Brang, P., 2001. Crown defoliation improves tree mortality models. For. Ecol. Manage. 141, 271–284.

Entcheva, P., 2000. Remote Sensing of Forest Damage in the Czech Republic Using Hyperspectral Methods. PhD Dissertation, University of New Hampshire, NH, USA, pp. 277.

Entcheva, P., Rock, B.N., Martin, M.E., Neefus, C.D., Irons, J.R., Middleton, E.M., Albrechtová, J., 2004. Detection of initial damage in Norway spruce canopies using hyperspectral airborne data. Int. J. Remote Sens. 24, 5557–5583.

Gruber, F., 1994. Morphology of coniferous trees: possible effects of soil acidification on the morphology of Norway spruce and Silver fir. In: Godbold, D.L., Huttermann, A. (Eds.), Effects of Acid Rain on Forest Processes. J. Wiley-Liss, New York, pp. 265–324.

Halle, F., Oldeman, R.A.A., Tomlinson, P.B., 1978. Tropical Trees and Forests. An Architectural Analysis. Springer–Verlag, Berlin.

Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. Quart. Rev. Biol. 67, 283–335.

Hintze, J., 2001. NCSS and PASS. Number cruncher statistical systems. Kaysville, Utah (http://www.ncss.com).

Honkanen, T., Haukioja, E., 1994. Why does a branch suffer more after branch-wide than after tree-wide defoliation? Oikos 71, 441–450.

Honkanen, T., Haukioja, E., Kitunen, V., 1999. Responses of *Pinus sylvestris* branches to simulated herbivory are modified by tree sink/source dynamics and by external resources. Funct. Ecol. 13, 126–140.

Ishii, H., Clement, J.P., Shaw, D.C., 2000. Branch growth and crown form in old coastal Douglas-fir. For. Ecol. Manage. 131, 81–91.

Johansen, D.A., 1940. Plant Microtechnique. McGraw-Hill Book, New York.

Kainulainen, P., Holopainen, J.K., Oksanen, J., 1995. Effects of SO_2 on the concentrations of carbohydrates and secondary compounds in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) seedlings. New Phytol. 130, 231–238.

Kaitaniemi, P., Neuvonen, S., Nyyssonen, T., 1999. Effects of cumulative defoliations on growth, reproduction, and insect resistance in mountain birch. Ecology 80, 524–532.

Klap, J.M., Voshaar, J.H.O., De Vries, W., Erisman, J.W., 2000. Effects of environmental stress on forest crown condition in Europe. Part IV. Statistical analysis of relationships. Water Air Soil Poll. 119, 387–420.

Kozlowski, T.T., 1971. Growth and Development of Trees. Academic Press, New York.

Lange, B.M., Lapierre, C., Sanderman, H., 1995. Elicitor-induced Spruce stress lignin—structural similarity to early developmental lignins. Plant Physiol. 108, 1277–1287.

Lepedus, H., Cesar, V., Ljubesic, N., 2001. Chloroplast ultrastructure and chlorophyll levels in vegetative buds and needles of Norway spruce (*Picea abies* L. Karst.) Period. Biol. 103, 61–65.

Lipavská, H., Svobodová, H., Albrechtová, J., 2000. Annual dynamics of the content of non-structural saccharides in the context of structural development of vegetative buds of Norway spruce. J. Plant Physiol. 157, 365–373.

Modrzyński, J., 2003. Defoliation of older Norway spruce (*Picea abies* /L./ Karst.) stands in the Polish Sudety and Carpathian mountains. For. Ecol. Manage. 181, 289–299.

Mooney, H.A., Winner, W.E., 1991. Partitioning response of plants to stress. In: Mooney, H.A., Winner, W.E., Pell, E.J. (Eds.), Response of Plants to Multiple Stresses. Academic Press, San Diego, pp. 129–141.

Moravec, I., Cudlín, P., Polák, T., Havlícek, F., 2002. Spruce bark beetle (*Ips typographus* L.) infestation and Norway spruce status: is there a causal relationship? Silva Gabreta 8, 255–264.

Moss, D.M., Rock, B.N., Bogle, A.L., Bílková, J., 1998. Anatomical evidence of the development of damage symptoms across a growing season in needles of red spruce from central New Hampshire. Environ. Exp. Bot. 39, 247–262.

Nicolini, E., Chanson, B., Bonne, F., 2001. Stem growth and epicormic branch formation in understorey beech trees (*Fagus sylvatica* L.) Ann. Bot. Lond. 87, 737–750.

Pfanz, H., Oppmann, B., 1991. The possible role of apoplasctic peroxidases in detoxifying the air pollutant sulphur dioxide. In: Lobarzewski, J., Greppin, H., Penel, C., Gaspar, T.G. (Eds.), Biochemical, Molecular and Physiological Aspects of Plant Peroxidases. University of Geneva, Geneva, Switzerland, pp. 401–417.

Podrázský, V., 1999. Neglected aspects of calamity in Sumava Mts. Lesnická práce 8, 353–354 (in Czech).

Polák, T., Albrechtová, J., Rock, B.N., 2003. Development of macroscopic markers of Norway spruce damage in the Krusne Hory between 1998 and 2000. Ekol. Bratislava 22 (Supp. 1), 284–287.

Polák, T., Albrechtová, J., Cudlín, P., Moravec, I., 2004. Proportion of bud development types—new indicator of current tree health status? Ekol. Bratislava 23 (Supp. 2), 113–123.

Porra, R.J., Thompson, W.A., Kriedemann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim. Biophys. Acta 975, 384–394.

Powell, G.R., 1974. Initiation and development of lateral buds in *Abies balsamea*. Can. J. For. Res. 4, 458–469.

Report of the Min. of Agric. of Czech Rep., 2002. Report on Forestry of the Czech Republic by December 31, 2002, Forest Management Institute, Brandýs nad Labem (http://www.uhul.cz/en/zz/2002/content.php).

Rock, B.N., Hoshiyeki, T., Miller, J.R., 1988. Comparison of in situ and airborne spectral measurements of the blue shift associated with forest decline. Remote Sens. Environ. 24, 109–127.

Running, S.W., Gower, S.T., 1991. Forest-BGC. A general-model of forest ecosystem processes for regional applications. 2. Dynamic carbon allocation and nitrogen budgets. Tree Physiol. 9, 147–160.

Salemaa, M., Jukola-Sulonen, E.L., 1990. Vitality rating of *Picea abies* by defoliation class and other vigour indicators. Scan. J. For. Res. 5, 413–426.

Schreuder, M.D.J., Brewer, C.A., 2001. Persistent effects of short-term, high exposure to chlorine gas on physiology and growth of *Pinus ponderosa* and *Pseudotsuga menziesii*. Ann. Bot. Lond. 88, 197–206.

Schulz, H., Härtling, S., 2003. Vitality analysis of Scots pines using a multivariate approach. For. Ecol. Manage. 186, 73–84.

Shimizu-Sato, S., Mori, H., 2001. Control of outgrowth and dormancy in axillary buds. Plant Physiol. 127, 1405–1413.

Siefermann-Harms, D., 1994. Light and temperature control of season-dependent changes in the α- and β-carotene content of Spruce needles. J. Plant. Phys. 143, 488–494.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics and phosphomolybdic–phosphotungstic acid reagents. Am. J. Enol. Viticul. 16, 144–158.

Solberg, S., Rindal, T.K., Ogner, G., 1998. Pigment composition of Norway spruce needles suffering from different types of nutrient deficiency. Trees 12, 289–292.

Solberg, S., Moshaug, E., 1999. Crown condition of Norway spruce: within-stand relationships and competition. Forestry 72, 329–336.

Soukupová, J., Albrechtová, J., Svobodová, H., Opatrná, J., 2002. Anatomical and histochemical changes of Norway spruce buds induced by simulated acid rain. Biol. Plant. 45, 77–84.

Soukupová, J., Cvikrová, M., Albrechtová, J., Rock, B.N., Eder, J., 2000. Histochemical and biochemical approaches to the study of phenolic compounds and peroxidases in needles of Norway spruce (*Picea abies*). New Phytol. 146, 403–414.

Straw, N.A., Fielding, N.J., Green, G., Price, J., 2000. The impact of green spruce aphid, *Elatobium abietinum* (Walker), and root aphids on the growth of young Sitka spruce in Hafren Forest, Wales: effects on height, diameter and volume. For. Ecol. Manage. 134, 97–109.

Svobodová, H., Lipavská, H., Albrechtová, J., 2000. Non-structural carbohydrate status in Norway spruce buds in the context of annual bud structural development as affected by acidic pollution. Environ. Exp. Bot. 43, 253–265.

Srámek, V., Fadrhonsová, V., Lomský, B., 2003. Liming and fertilizing of forest ecosystem in Czech Republic during 2000–2004. Lesnická práce 4, 184–186 (in Czech).

Tuomi, J., Niemela, P., Jussila, I., Vuorislao, T., Jormalainen, V., 1989. Delayed budbreak: a defensive response of mountain birch to early-season defoliation? Oikos 54, 87–91.

Vogelmann, A.F., Rock, B.N., 1988. Anatomy of red spruce needles from forest decline sites in Vermont. Environ. Exp. Bot. 28, 19–26.

Wareing, P.F., Patrick, J., 1975. Source-sink relations and the partition of assimilates in the plant. In: Cooper, J.P. (Ed.), Photosynthesis and Productivity in Different Environments. Cambridge University Press, Cambridge, pp. 481–499.

Waring, G.L., Cobb, N.S., 1992. The impact of plant stress on herbivore population dynamics. In: Bernays, E. (Ed.), Insect–Plant Interactions. CRC Press, Ann Arbor, pp. 167–225.

Wellburn, A.R., 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–314.

Whitmore, F.W., 1976. Binding of ferulic acid to cell walls by peroxidases of *Pinus elliottii*. Phytochemistry 15, 375–378.

Wild, J., Neuhäuslová, Z., Sofron, J., 2004. Changes of plant species composition in the Sumava spruce forests, SW Bohemia, since the 1970s. For. Ecol. Manage. 187, 117–132.

Zemek, F., Herman, M., 2001. Bark beetle—a stress factor of spruce forests in the Bohemian Forest. Ekol. Bratislava 20, 95–107.

Zvereva, E.L., Kozlov, M.V., 2001. Effects of pollution-induced habitat disturbance on the response of willows to simulated herbivory. J. Ecol. 89, 21–30.