Long-term effects of CO_2 enrichment on bud phenology and shoot growth patterns of Norway spruce juvenile trees

R. Pokorný[1], I. Tomášková[1], I. Drápelová[2], J. Kulhavý[2], M. V. Marek[1,2]

[1]*Laboratory of Plant Ecological Physiology, Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Brno, Czech Republic*
[2]*Institute of Forest Ecology, Mendel University in Brno, Brno, Czech Republic*

ABSTRACT: Bud phenology and shoot elongation growth were monitored on Norway spruce (*Picea abies* [L.] Karst.) trees grown inside glass domes with adjustable windows for six years under ambient (355 µmol $CO_2 \cdot mol^{-1}$) and elevated (700 µmol $CO_2 \cdot mol^{-1}$) atmospheric CO_2 concentrations CO_2. Each treatment consisted of two stand densities – sparse (5,000 trees·ha^{-1}) and dense (10,000 trees·ha^{-1}). The age of spruce trees was 10 years at the beginning of the experiment. Elevated CO_2 slightly accelerated the consequential bud germinating phases and it significantly induced shoot elongation growth, especially of sun-exposed shoots in a stand with sparse density. This accelerated growth lasted one to three weeks after full bud development in E compared to A. At the end of the growing season the total shoot length did not show any differences between the treatments. We supposed that limiting nitrogen supply to needles slowed down subsequent shoot elongation growth in E treatment. Nevertheless, faster shoot growth in elevated CO_2 conditions can enhance the carbon sink in spruce due to prolongation of the growing season.

Keywords: bud; elevated CO_2; Norway spruce; phenology; shoot length

Phenology is principally concerned with the dates of the first occurrence and duration of natural events in the plant annual cycle. Temperature (as the factor accompanied with higher air CO_2) is regarded as an important environmental factor inducing plant growth, manifested by bud flushing and shoot development (Hannerz 1999). However, not only the temperature (Linkosalo et al. 2000) but also other environmental factors – global radiation or amount of precipitation (e.g. Bigras, D'Aoust 1993; Häkkinen 1999) and fertilization – may act as stimulators of plant growth (Roberntz 1999). For example, the increasing nutrient supply lengthened the growing season and plants flushed earlier in spring and set buds later in autumn (Murray et al. 1994; Roberntz 1999). Recently, earlier flowering and an extended period of active plant growth across much of the northern hemisphere have been interpreted as responses to global climate change (Cleland et al. 2006). Yet, Schwartz et al. (2006) showed the onset of spring starting earlier across the Northern Hemisphere. Under elevated CO_2 conditions, an acceleration of bud phenology (Repo et al. 1996; Jach, Ceulemans 1999) is reported, others showed a dilution response (i.e. the positive effect of elevated CO_2 on tree phenology is diminished over time, Linkosalo [2000]) or no effects (Olszyk et al. 1998; Roberntz 1999; Kilpelainen et al. 2006; Slaney et al. 2007). Even among various tree species clones there is a variability of phenological responses which indicated that there are many factors reshaping the seasonality of ecosystem processes (Murray

et al. 1994; Johnsen, Seiler 1996; Centritto et al. 1999; Bigras, Bertrand 2006). Nevertheless, thermal requirements for bud burst, or elevated air temperature, were found to be of greater importance compared to the impact of elevated CO_2 in many studies (e.g. Repo et al. 1996; Hanninen et al. 2007). From the aspect of frost injury, both the timing of bud break and the bud set are important for trees growing under elevated atmospheric CO_2 conditions (Karnosky 2003). Earlier bud burst and acceleration of bud phenology result in prolongation of the shoot growth period and in a subsequent enhancement of wood production (Beuker 1994). The effect of long-term (months and years) CO_2 enrichment on phenology of Norway spruce was investigated by few authors, and the ecosystem level approach was missing. According to results from branch investigation (Robertnz 1999) or short-term studies (Slaney 2007), Norway spruce was found unaffected by elevated CO_2 in bud break as well as in shoot elongation growth.

In the present study phenological responses of juvenile Norway spruce trees which had been grown under elevated CO_2 conditions inside glass domes for six years were investigated. Then the following questions were solved:

(1) Are there any differences in bud phenology between ambient and elevated CO_2 treatments? Do these differences change with the time of cultivation?
(2) Does the dynamic change in shoot elongation growth?
(3) Does the total shoot length differ?

MATERIAL AND METHODS

The long-term impacts of elevated CO_2 on the spring bud phenology and subsequent shoot elongation growth of a Norway spruce (*Picea abies* [L.] Karst.) stand were investigated at the research site Bílý Kříž in the Beskids Mts. (northeastern part of the Czech Republic, 908 m a.s.l.). Since autumn 1996 spruce trees were grown under two treatments inside the domes with adjustable-windows (DAW) which differed in atmospheric CO_2: ambient (A, 355 μmol CO_2·mol^{-1}) and elevated (E, A + 355 μmol CO_2·mol^{-1}). The environmental conditions inside the DAWs were comparable in both treatments. Specifically, as Urban et al. (2001) described, the iron frames of DAW with dimensions 9 × 9 × 7 m and their windows reduced penetrating PAR (photosynthetically active radiation) by 26% on average. Air temperatures inside and outside the DAW differed insignificantly (0.2°C on average). Relative air humidity inside the DAW was significantly ($P < 0.05$) lower than outside (by –9.6% on average). The soil conditions did not differ between the treatments, except for slightly higher soil temperatures (by 0.5°C) in comparison with outside. The water supply was checked automatically in both treatments and compared to the soil moisture outside the DAWs (Virrib, Amet, CR). In this locality, natural soil contents of mineral nitrogen and available nitrogen forms are low throughout the whole soil profile (Formanek 2000). The geological bedrock is built of Mesozoic Godula sandstone (flysch type) and is overlaid by ferric Podzols. The mean annual air temperature was 5.4°C in the last 10 years (i.e. from 1995 to 2005). The annual precipitation amount was 1,400 mm (last 10-year average). N deposition in the open area reached ca 10 kg·ha^{-1} (NO_3 and NH_4 forms; Kulhavý et al. 2000).

In autumn 1996, the trees were planted within the control plot and DAWs as 10 years-old saplings (mean tree height 1.6 m, and stem diameter at one tenth above the ground 22.1 mm) at a triangular spacing per treatment: 1.25 × 1.25 m (s – sparse subtreatment with stand density of 5,000 trees·ha^{-1}) and 0.9 × 0.9 m (d – dense subtreatment with stand density of 10,000 trees·ha^{-1}). Totally, there were 56 trees per treatment. At the beginning of growing season 1998 all trees were slightly fertilized by Silvamix-forte (N+P_2O_5+K_2O+MgO, 17 g·m^{-2}) and Ureaform (urea-formaldehyde condensate, 21 g·m^{-2}) just to avoid yellowing.

The methodology of Murray et al. (1994) was used to identify five phenological phases of spring bud development (class: 0 – dormant bud, 1 – slight swelling, 2 – swollen bud, 3 – green needle/leaf clearly showing through the bud scales, and 4 – leaf per needle elongation). Shoot elongation growth was observed on exposed and shaded apical (ExA and ShA) and exposed and shaded lateral (ExL and ShL) buds/shoots. The sun-exposed shoots were supposed to be located up to the 4th whorl – counted downward from the tree top and shaded (Sh) shoots continuously below. Five trees per subtreatment were continuously monitored. On each tree, we observed identical terminal, lateral and apical buds/shoots. Monthly, needle samples of five shoots were scanned (Astra 1220 P, UMAX; Taiwan). The image analysis software ACC (Sofo Brno, Czech Republic) was used to estimate the projected needle area. Needles were dried (48 h, 80°C) and weighed (by 1405 B MP8-1 model, Sartorius, Germany) for nitrogen (N) content analysis. From Ex and Sh crown parts, five shoots per subtreatment were cut. LECO CNS-2000 automatic elemental analyzer (LECO Corporation, St. Joseph, MI, USA) was used for N

content analysis in needles. Mixed needle samples of 200 mg dry weight per subtreatment and crown part were analyzed. Commercial standards (Sulfamethazine and Alfalfa) delivered by LECO corporation were used for the calibration procedure. After full shoot development, specific leaf area (SLA) was estimated. Five shoots were sampled from both treatments and subtreatments. Obtained needles were scanned according to their age, dried and weighed using the same laboratory device and software as for nitrogen estimation. SLA was calculated as the projected needle area to dry needle mass ratio.

Mann-Whitney *U*-test within STATISTICA software (StatSoft Inc., Tulsa, USA) was used for statistical analysis of data. χ^2-test was used to test the significances of differences between the treatments for date-marked measurements. Study design can be characterized as pseudo-replication due to one dome per treatment (Hurlbert 1984).

RESULTS AND DISCUSSION

At the end of growing season 2002, the mean tree height and stem diameter (at one tenth of tree height above the ground) were 3.5 m and 5.7 cm and 3.3 m

and 5.6 cm in A and E treatments, respectively. These parameters differed insignificantly.

Bud phenology was observed on apical and lateral buds during the growing seasons 1997–2002. The beginning of the growing season was considered as that date in spring when the mean daily temperature was higher than 5°C for five consecutive days (for comparison: May 2 in 1997 and 2002, April 21 in 2001). At the beginning of the experiment, both the lateral and apical buds in E treatment started their development earlier than those in A treatment (insignificantly, 3–5 days). Moreover, the buds of trees in E treatment were fully developed about one week sooner. After six years of cultivation, the bud break still started earlier, mainly in exposed crown parts, in E compared to A treatment (insignificantly, 5 to 7 days). Statistically significant differences ($P << 0.01$) were found in late bud development phases (the 3rd and the 4th phase) between A and E treatments for sparse subtreatment (Fig. 1a, c). There E buds developed faster. These differences were found on both the exposed (Ex) and shaded (Sh) crown parts in apical (ExA, ShA) as well as lateral (ExL, ShL) buds (results from shaded crown parts are not
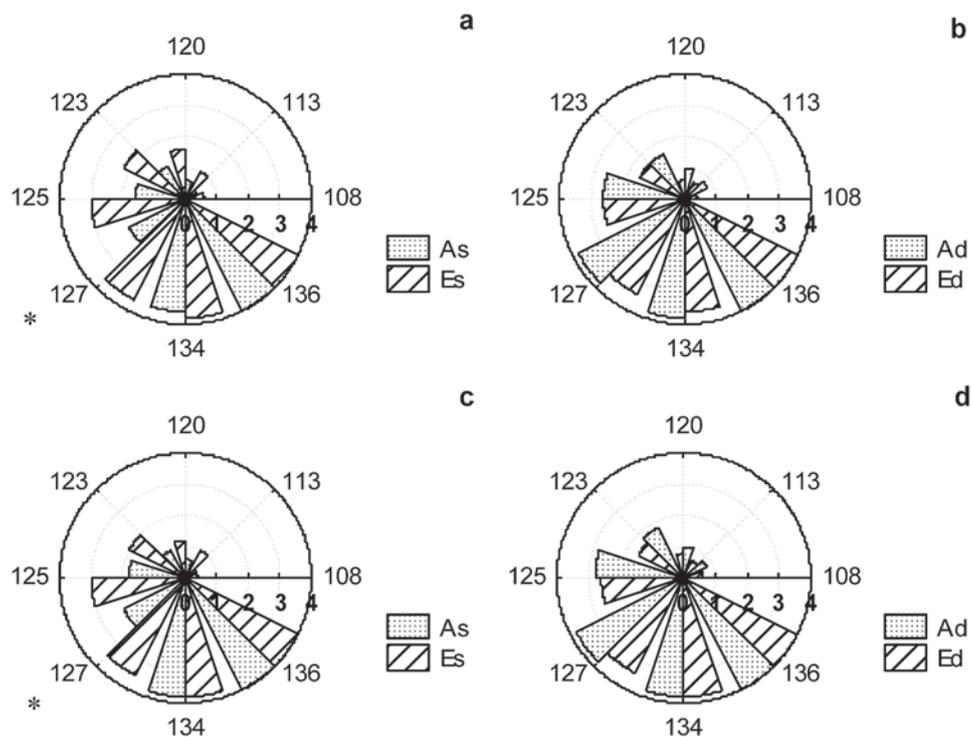

Fig. 1. Temporal development (day of the year on the circumference) of apical (a, b) and lateral (c, d) buds is shown by the column size among concentric circles for five phases of flushing (centre – dormancy and circles – phenological phases: 1 – slight swelling, 2 – swollen bud, 3 – green needle/leaf clearly showing through the bud scales, and 4 – leaf/needle elongation) during the growing season 2002. Ambient (A; 355 μmol $CO_2 \cdot mol^{-1}$) and elevated atmospheric CO_2 treatments (E; A + 355 μmol $CO_2 \cdot mol^{-1}$) and subtreatments (the 2nd letter in note): s – sparse (5,000 trees·ha^{-1}) and d – dense (10,000 trees·ha^{-1}). Asterisks denote statistical significant differences
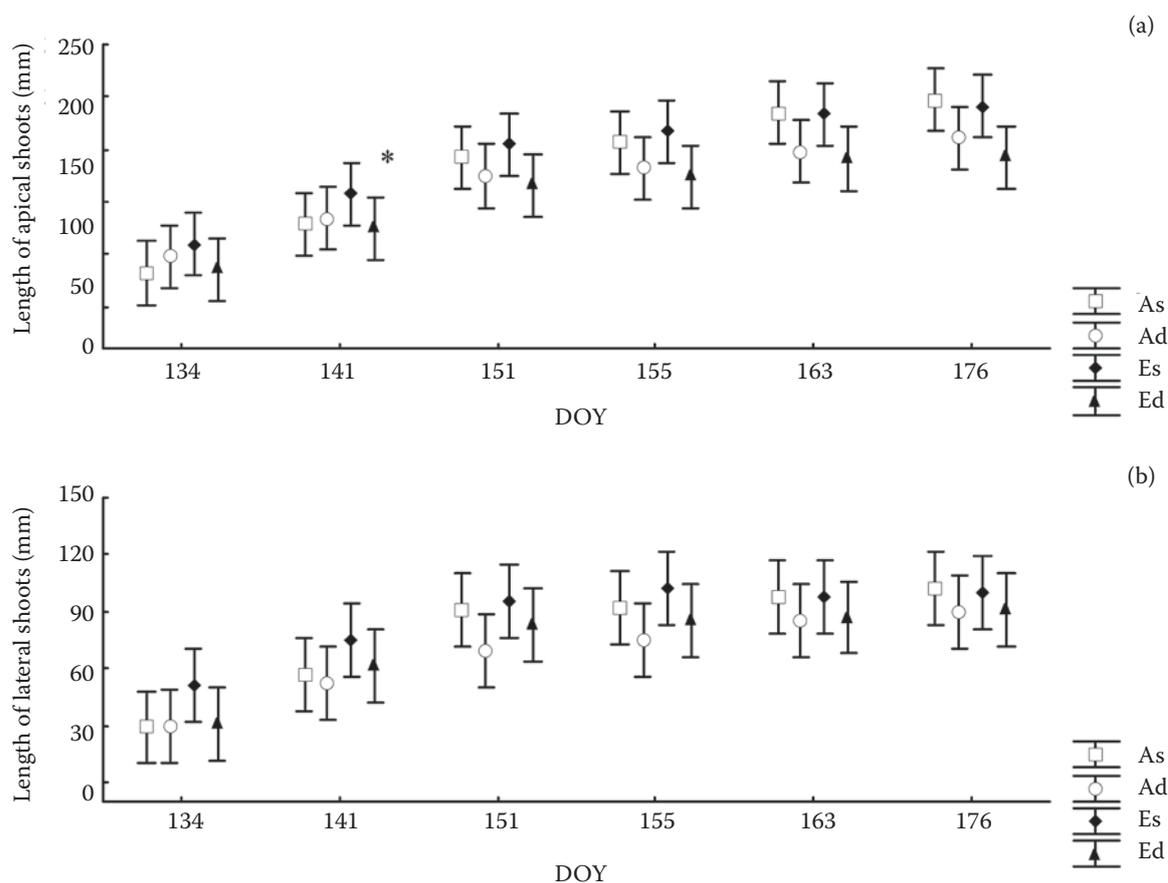
Fig. 2. Dynamics of the mean length increment of apical (a) and lateral (b) shoot at ambient (A; 355 μmol $CO_2 \cdot mol^{-1}$) and elevated atmospheric CO_2 treatments (E; A + 355 μmol $CO_2 \cdot mol^{-1}$) and subtreatments (the 2nd letter in note): s – sparse (5,000 trees·ha^{-1}) and d – dense (10,000 trees·ha^{-1}) after six years of fumigation in 2002. DOY designates day of the year, error bars indicate standard deviation

shown). In dense subtreatments no difference in bud development phases between ambient and elevated CO_2 was found. Contrariwise, the development of apical and lateral buds in ambient dense subtreatment was often finished sooner (insignificantly) as compared to elevated dense subtreatment (Fig. 1b, d). Several authors concluded that enhanced air temperature accelerated both the bud development and the initiation and termination of shoot growth of Norway spruce more than did elevated CO_2 (Repo et al. 1996; Hanninen et al. 2007; Slaney et al. 2007). Analogously to higher temperature, early flushing relates to high N concentration and delayed bud break expected at low N availability (Murray et al. 1994; Bigras et al. 2001).

Shoot elongation growth was monitored in detail during the growing seasons 2001 and 2002 (i.e. after five and six years of CO_2 fumigation). The length of ExA, ExL, ShA and ShL shoots was significantly higher ($P < 0.05$) in E treatment compared to A treatment on May 22 and 31, June 7 and 26 in 2001 (data not shown), and on May 14 and 21 in 2002

(Fig. 2). Thus, differences in shoot length between the treatments were obvious during the first 35 days in 2001 and the first 7 days in 2002 after full bud development. In 2001, both types of sunny adapted shoots exposed to elevated CO_2 concentration (i.e. ExA and ExL) exceeded by even about 45–60% the shoot length of ambient shoots in sparse subtreatment. These differences disappeared after three to four weeks from the beginning of shoot elongation. In 2002, both apical and lateral shoots from sunexposed crown parts in E treatment were longer (by 19 and 37%, respectively) compared to A treatment. E shoots from shaded crown parts were also longer (by 16–17%) than A ones. In shaded crown parts of both treatments the difference in shoot length increased by up to 30% one week after full development of buds, but then these differences rapidly decreased, especially in dense subtreatments. Even when the large average percentage differences were shown, they were not mostly statistically significant due to high data variability. In early spring, longer shoots by about 16–60% for one to three weeks in
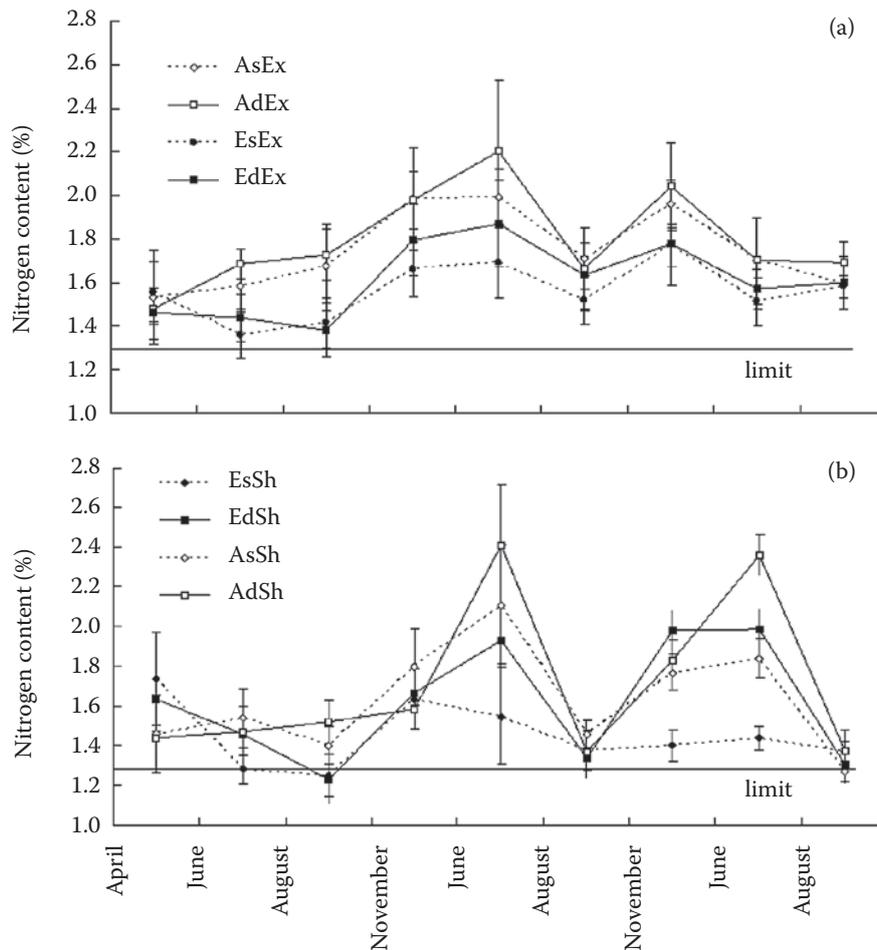
Fig. 3. Variation of nitrogen content within the sun (whorl II, add- Ex) (a) and shade (whorls < IV, add- Sh) (b) adapted current needles of young Norway spruce trees grown at ambient (A; 355 μmol $CO_2 \cdot mol^{-1}$) and elevated atmospheric CO_2 treatments (E; A + 355 μmol $CO_2 \cdot mol^{-1}$) and subtreatments (the 2nd letter in note), s – sparse (5,000 trees·ha^{-1}) and d – dense (10,000 trees·ha^{-1}) during the years 2000–2002 (month–year). Whiskers passed the mean values denote standard deviation. There are statistical significant differences in nitrogen content between treatments during the investigated period except April 2000 and August 2001 and August 2002 (asterisks were not applied for better lucidity of the figure)

E compared to A treatment enable for E trees to be a higher positive carbon sink through the larger leaf area. At the end of shoot elongation growth, E shoots showed similar lengths like A ones (± 7%). Therefore, the total shoot length was unaffected by elevated CO_2. Slaney et al. (2007) and Hanninen et al. (2007) pointed out that the elevated air temperature as an accompanying effect of elevated CO_2 accelerated bud development as well as the initiation and termination of shoot growth but did not elevate CO_2 itself.

Nitrogen content was found higher in E needles compared to A ones only before budding in early spring in 1998. The long-term effect of elevated CO_2 was responsible for a decrease in needle N content. The gradient of needle N content per subsamples was as follows: Ambient-sun needles > Ambient-shade needles > Elevated-sun needles > Elevated-shade adapted needles. The critical needle N content was established as 1.3% for Norway spruce (Innes 1993). In the consecutive shoot growth nitrogen is reallocated to current needles, but the concurrently ingoing dilution effect contributed to a decrease in needle N content. Therefore, the highest variability of N content within the current needles occurs during the months of May and June (Fig. 3). In August, when the shoot growth was completed, the lowest needle N content and its variability among the samples were found. Hrstka et al. (2005) showed that the elevated CO_2 treatment leads to a decrease in N concentration in leaf tissues and amount of Rubisco enzyme. Marek et al. (2002) and Urban (2003) demonstrated a suppression of E shoot growth following the significant decrease in carbon assimilation efficiency reported as photosynthetic down-regulation. Therefore, changes in the shoot extension rate under

elevated CO_2 may be explained by varying N-content in needles (HRSTKA et al. 2005) or by different production of growth phytohormones or by another regulative process (reviewed by URBAN 2003). We supposed that the primarily decreasing amount of nitrogen availability slowed down the subsequent shoot development growth in E treatment compared to A treatment. Additionally, SLA values of E current needles were lower (64 ± 12 $cm^2 \cdot g^{-1}$, mean ± standard deviation) compared to the A ones (72 ± 12 $cm^2 \cdot g^{-1}$). Especially, newly formed needles in E treatment became more dense (i.e. with lower SLA) than in A treatment (about 3–5%).

CONCLUSION

The long-term cultivation of spruce trees under elevated CO_2 led to insignificantly slight acceleration of bud breaks (3–5 days) and subsequent significant stimulation of initial shoot growth. Shoot growth especially of sun-exposed shoots of trees grown in sparse stand density was accelerated from one to three weeks. In these first weeks of shoot elongation, E shoots were significantly longer compared to A ones. Such extension in leaf area led to a highly positive carbon sink. This CO_2 stimulation effect disappeared at maximum within three to four weeks after full bud development and no significant differences between the treatments in the shoot length were observed at the end of growing seasons. The influence of elevated CO_2 on Norway spruce phenology was recorded during the first spring as well as during the sixth spring of experiment duration. High variability of responses can be caused by no uniform stand density and variable nitrogen availabi-lity. Global climate change is presumed to increase the air temperature. As the bud break is controlled mainly by the temperature, more expansive shoot and foliage extension should be expected in the future spring periods, especially in sparse Norway spruce stands with sufficient nutrient availability.

References

BEUKER E. (1994): Adaptation to climatic changes of the timing burst in populations of *Pinus sylvestris* (L.) and *Picea abies* (L.) Karst. Tree Physiology, *14*: 961–970.

BIGRAS F.J., D'AOUST A. (1993): Influence of photoperiod on shoot and root frost tolerance and bud phenology of white spruce seedlings (*Picea glauca*). Canadian Journal of Forest Research, *23*: 219–228.

BIGRAS F.J., RYYPPÖ A., LINDSTRÖM A., STATTIN E. (2001): Cold acclimation and deacclimation of shoots and roots of conifer seedlings. In: BIGRAS F.J., KOLOMBO S.J. (eds):

Conifer Cold Hardines. Dordrecht, Kluwer Academic Publisher: 57–88.

BIGRAS F.J., BERTRAND A. (2006): Response of *Picea mariana* to elevated CO_2 concentration during growth, cold hardening and dehardening: phenology, cold tolerance, photosynthesis and growth. Tree Physiology, *26*: 875–888.

CENTRITTO M., LEE H.S.J., JARVIS P.G. (1999): Long-term effects of elevated carbon dioxide concentration and provenance on four clones of Sitka spruce (*Picea sitchensis*). I. Plant growth, allocation and ontogeny. Tree Physiology, *19*: 799–806.

CLELAND E.E., CHIARIELLO N.R., LOARIE S.R., MOONEY H.A., FIELD C.B. (2006): Diverse responses of phenology to global changes in a grassland ecosystem. In: Proceedings of the National Academy of Sciences of the United States of America, *103*: 13740–13744.

FORMÁNEK P. (2000): Nitrogen transformation in anthropogenic influenced spruce ecosystems of Moravian-Silesian Beskids. [Ph.D. Thesis.] Brno, Mendelova zemědělská a lesnická univerzita v Brně: 246. (in Czech)

HÄKKINEN R. (1999): Statistical evaluation of bud development theories: application to bud burst of *Betula pendula* leaves. Tree Physiology, *19*: 613–618.

HANNERZ M. (1999): Evaluation of temperature models for predicting bud burst in Norway spruce. Canadian Journal of Forest Research, *29*: 9–19.

HANNINEN H., SLANEY M., LINDER S. (2007): Dormancy release of Norway spruce under climatic warming: testing ecophysiological models of bud burst with a whole-tree chamber experiment. Tree Physiology, *27*: 291–300.

HRSTKA M., URBAN O., MAREK M.V. (2005): Long-term effect of elevated CO_2 on spatial differentiation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity in Norway spruce canopy. Photosynthetica, *43*: 211–216.

HURLBERT S.H. (1984): Pseudoreplication and the design of ecological field experiments. Ecological Monographs, *54*: 187–211.

INNES J.L. (1993): Forest health, its assessement and status. Wallingford, CAB International.

JACH E.M., CEULEMANS R. (1999): Effects of elevated atmospheric CO_2 on phenology, growth and crown structure of Scots pine (*Pinus sylvestris*) seedlings after two years of exposure in the field. Tree Physiology, *19*: 289–300.

JOHNSEN K.H., SEILER J.R. (1996): Growth, shoot phenology and physiology of diverse seed sources of black spruce: I. Seedling response to varied atmospheric CO_2 concentrations and photoperiods. Tree Physiology, *16*: 364–373.

KARNOSKY D.F. (2003): Impacts of elevated atmospheric CO_2 on forest trees and forest ecosystems: knowledge gaps. Environment International, *29*: 161–169.

KILPELAINEN A., PELTOLA H., ROUVINEN I., KELLOMAKI S. (2006): Dynamics of daily height growth in Scots pine trees at elevated temperature and CO_2. Trees, *20*: 16–27.

KULHAVÝ J., FORMÁNEK P. (2001): Nitrogen transformation

in soil and nutrition conditions of young spruce stands in the Moravian-Silesian Beskids. Journal of Forest Science, *47*: 383–391

Linkosalo T., Carter T.R., Häkkinen R., Hari P. (2000): Predicting spring phenology and frost damage risk of *Betula* spp. under climatic warming: a comparison of two models. Tree Physiology, *20*: 1175–1182.

Marek M.V., Urban O., Šprtová M., Pokorný R., Rosová Z., Kulhavý J. (2002): Photosynthetic assimilation of sun versus shade needles under long-term impact of elevated CO_2. Photosynthetica, *40*: 259–267.

Murray M.B., Smith R.I., Leith I.D., Fowler D., Lee H.S.J., Friend A.D., Jarvis P.G. (1994): Effects of elevated CO_2, nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage. Tree Physiology, *14*: 691–706.

Olszyk D., Wise C., VanEss E., Apple M., Tingey D. (1998): Phenology and growth of shoots, needles, and buds of Douglas-fir seedlings with elevated CO_2 and (or) temperature. Canadian Journal of Botany, *76*: 1991–2001.

Repo T., Hanninen H., Kellömaki S. (1996): The effects of long-term elevation of air temperature and CO_2 on the frost hardiness of Scots pine. Plant, Cell and Environment,

19: 209–216.

Roberntz P. (1999): Effects of long-term CO_2 enrichment and nutrient availability in Norway spruce I. Phenology and morphology of branches. Trees, *13*: 188–198.

Schwartz M.D., Ahas R., Aasa A. (2006): Onset of spring starting earlier across the Northern Hemisphere. Global Change Biology, *12*: 343–351.

Slaney M., Wallin G., Medhurst J., Linder S. (2007): Impact of elevated carbon dioxide concentration and temperature on bud burst and shoot growth of boreal Norway spruce. Tree Physiology, *27*: 301–312.

Urban O., Janouš D., Pokorný R., Marková I., Pavelka M., Fojtík Z., Šprtová M., Kalina J., Marek M.V. (2001): Glass domes with adjustable windows: A novel technique for exposing juvenile forest stands to elevated CO_2 concentration. Photosyntetica, *39*: 395–401.

Urban O. (2003): Physiological impacts of elevated CO_2 concentration ranging from molecular to whole plant responses. Photosynthetica, *41*: 9–20.

Corresponding author:

Ing. Radek Pokorný, Ph.D., Ústav systémové biologie a ekologie AV ČR, v.v.i., Laboratoř ekologické fyziologie rostlin, Poříčí 3b, 603 00 Brno, Česká republika
tel./fax: + 420 543 211 560, e-mail: eradek@usbe.cas.cz