BIOLOGIA PLANTARUM 45 (1): 77-84, 2002

Anatomical and histochemical changes of Norway spruce buds induced by simulated acid rain

J. SOUKUPOVÁ*, J. ALBRECHTOVÁ*[1], H. SVOBODOVÁ* and J. OPATRNÁ**

*Department of Plant Physiology, Faculty of Science, Charles University, Viničná 5, CZ-12844 Prague 2, Czech Republic**
*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojová 135, CZ-16502 Prague 6, Czech Republic***

Abstract

The study was focused on changes of anatomical and histochemical parameters of buds of 4-year-old Norway spruce (*Picea abies* L. Karst) trees subjected to simulated acid rain (SAR). Solutions of pH 2.9 and 3.9 were applied by spraying on shoot and/or by watering for two years. No macroscopic changes of buds or needles were observed in connection with SAR application and the only induced change was 2-week earlier onset of bud break in all treated variants compared to the control. Two-year treatment caused decrease in number of leaf primordia and increase in number of living bud scales in treated dormant buds while these parameters remained unchanged in the control buds. Treatments with solution of pH 2.9 caused decrease of flatness of bud apical meristem during the vegetative season. Increased activity of non-specific esterase in treated buds occurred during dormancy and bud break and the enhanced accumulation of phenolic compounds was detected at the beginning of shoot growth. Changes in histochemical parameters of bud tissues were induced mainly by spraying of shoots and can thus be qualified as primary damage.

Additional key words: histochemistry, image analysis, non-specific esterase, phenolic compounds, *Picea abies*.

Introduction

Atmospheric pollution during the last fifty years severely affected forest areas, and in some cases led to forest decline. Forest damage was first described via macroscopic symptoms, such as colour changes of foliage, premature leaf shedding, defoliation and altered crown architecture. Since that time changes of needle anatomy, biochemistry and metabolism induced by atmospheric pollution have been intensively studied (*e.g.* Huttunen *et al.* 1990, Moss *et al.* 1998). Buds are the primary element determining crown architecture. Anatomy of vegetative buds of Norway spruce have been already published (Gruber 1989, Hejnowicz and Obarska 1995) but little is known about bud structural and chemical changes under stress conditions (Matschke 1994, Bílková *et al.* 1999).

Non-specific esterase (NE) is a complex of enzymes hydrolysing ester bonds (EC 3.1.1.). They play important role in metabolism of fatty acids and lipids and they are involved in the establishment and reorganisation of cell walls (Bordenave *et al.* 1995, Moore *et al.* 1996). Thus, changes in activity and localisation of NE have been studied as a marker of elongation and differentiation of cells (Guglielmino *et al.* 1997) and altered activity and isoenzyme composition of NE was described as part of

Received 27 December 2000, *accepted* 24 May 2001.
Acknowledgements: The study was supported by the NASA grant NAG5-5192 and the grant J13/9811310003 of the Ministry of Education of the Czech Republic. Initially, the work was supported by the Central European University (Budapest, Hungary), grant No. ERSS 91-1. Special thanks are due to Dr. Jan Pokorný from the Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň for his encouragement and enabling us to conduct the SAR experiment in the field station Lužnice of the Institute. Dr. B.N. Rock (University of New Hampshire, U.S.A) is gratefully acknowledged for his inspiring discussions and strong support in our work and Dr. Dubová (Masaryk University, Brno) and Prof. Karel Beneš (South Bohemian University, České Budějovice) for their advice in histochemistry.
[1] To whom correspondence should be addressed; fax: +420 2 21953306, e-mail: albrecht@natur.cuni.cz.

plant host-pathogen interactions (Oven and Torelli 1994). Environmental factors such as temperature (Krasnuk *et al.* 1978) or water stress (Taskakorie *et al.* 1985) can affect the activity of NE related closely to the physiological and metabolic states of cells. It is also claimed to be a good indicator of the toxic effect of heavy metals (Maier 1978) and water pollution (Cachot *et al.* 1994).

Phenolic compounds are another group of compounds, often studied in coniferous needles (Polle *et al.* 1994), which is considered to be stress related. Induced depositions of soluble phenolics, tannins and lignin are documented as a response to changes in mineral nutrition (Kainulainen *et al.* 1996), invasion of pathogens (Mace and Howell 1974), wounding (Borg-Oliver and Monties 1993) and anthropogenic pollutants (Kainulainen *et al.* 1995). An increasing amount of catechin was observed in needles of Norway spruce of different damage classes as a consequence of atmospheric pollution (Richter and Wild 1992) as well as after exposure to ozone treatment (Booker *et al.* 1996).

On the basis of our previous study (Bílková *et al.* 1996, 1999) and results of other authors (Bordenave 1995) we proposed to study changes of anatomical structure, localisation of non-specific esterase and amount of phenolic compounds in buds of Norway spruce induced by simulated acid rain (SAR) during the annual bud development. Design of SAR experiment was chosen in order to test following hypotheses: *1)* simulated acid rain will not affect quantitative anatomical parameters of spruce buds, *2)* simulated acid rain will not cause any changes in the activity of non-specific esterase and the accumulation of phenolic compounds in bud meristems, and *3)* primary damage caused by the direct application of SAR on the foliage has the same symptoms as the secondary damage caused by the uptake of SAR by root system.

Materials and methods

Plants and experimental design: The SAR experiment established in the field station of the Institute of Botany of the Academy of Science was located in Lužnice (South Bohemia). The station, being located in the Protected Landscape Area of Třeboňsko with negligible indigenous acidic deposition, ensured low interference with experimental treatment.

One hundred and fifty three-year-old nursery-grown trees of *Picea abies* (L.) Karst were potted and placed into plastic basins. Removable transparent plastic covers were used a couple of hours after the application of solutions to ensure the prolonged influence of the SAR. Five experimental treatments (30 seedlings per treatment) differed in pH of solution (3.9 or 2.9), and in the method of SAR application: watering of soil (R) or soil watering and spraying of a shoot (RS). Solutions were applied once a week to the soil (100 cm^3 per tree) and sprayed three times per week (20 cm^3 per tree) over the shoot. Trees from the control treatment (C) were watered and sprayed with distilled water. The solution with pH 3.9 simulating the precipitation quality in the Krkonoše Mts. (Vávra 1992) contained 22.0 mg dm^{-3} SO_4^{2-}, 6.2 mg dm^{-3} NO_3^-, 2.0 mg dm^{-3} NH_4^+ and the solution of pH 2.9 contained 3.3 times higher ion concentrations. The watering of soil containing root systems was stopped with the first frosts.

First-order terminal buds were sampled first in January 1995 (first dormancy - late stage of dormancy) after one year of the SAR treatment. Sixteen collections were made throughout the growing season 1995: once per week during bud break (28 March - 31 May 1995) and at the two-week intervals during the period of shoot growth and differentiation of a pre-formed bud (June - September 1995). The last two collections were performed in the following dormant season in November and December 1995 (second dormancy - early stage of dormancy).

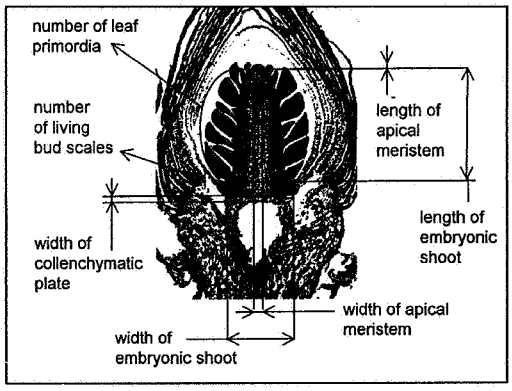

Fig. 1. Quantitative anatomical characteristics measured on dormant buds of Norway spruce.

Bud anatomy: Medial longitudinal sections were prepared by paraffin method (Johansen 1940). Chloroform was included into dehydration series to soften the tissue and vacuum oven was used to improve sample infiltration. Sections were stained with alcian blue and nuclear fast red (Beneš and Kamínek 1973). Approximately 12 buds per treatment were studied each period of dormancy and three buds were measured per treatment and sampling date during vegetative season. Quantitative characteristics (Fig. 1), *i.e.* the height and width of embryonic shoot, the height and width of apical meristem, thickness of collenchymatic plate, were measured with an ocular ruler. Number of cell layers of collenchymatic plate, number of leaf primordia and number of dead and living bud scales were counted.

Activity of non-specific esterase and determination of phenolic compounds: Sampled buds (8 per treatment) were immediately fixed in 4 % formaldehyde in phosphate buffer (pH 7.2) for 90 min, and infiltrated in 7.5 % sucrose for 10 min under low pressure. Sections (30 μm) were cut on a cryo-microtome *SME* (*Cryotome Shandon*, Pittsburgh, USA), and incubated for 3 h in a mixture of naphtol-AS-acetate as a substrate and Fast Blue B as a coupling reagent (Bílková *et al.* 1999). The specificity of the reaction was tested by incubation of parallel sections in mixture in which substrate was replaced by phosphate buffer. The Fast Blue B reacts specifically with phenolic compounds (O'Brien and McCully 1981) resulting in the reddish-brown reaction product. Thus, fit was possible to localise simultaneously the activity of NE (blue reaction product) and phenolics (red reaction product) on one section. Additionally, the vanillin-HCl test (Gardner 1975) was applied on free-band sections. Sections, mounted in glycerol-gelatine (Johansen 1940), were captured by video camera. The amount of blue reaction product was evaluated with the image analysis software *LUCIA M* (*Laboratory Imaging*, Prague, Czech Republic) as described Bílková *et al.* (1999). The activity (A) of non-specific esterase was computed using the formula: A = - (MR/MB - mr/mb) The areas of the tested tissue containing a blue reaction product as well as the reference tissue (without blue colouring) were marked. The "Mean Red" and "Mean Blue" were measured in tissues with positive reaction (MR, MB) and in the reference tissues without reaction (mr, mb). Because A is the ratio of optical parameters no units are given in Figs. 8 and 9.

The type of localisation of phenolics (granular, vacuolar or drop-like) in cells were recorded according to our previous study (Bílková *et al.* 1999) on each occasion. The percentage of area filled with dark stained phenolic compounds was measured on a bud section using the automatic area measurements of image analysis.

Statistical analysis: Statistical comparison was made by means of software *NCSS 6.0*. Normally distributed data were analysed by analysis of variance (*ANOVA*) and differences among treatments were detected by the Tukey-Kramer test. The Kruskal-Wallis and Kruskal-Wallis Z tests were used in case of other data distributions.

Results

After two-year exposure to simulated acid rain we did not observe any macroscopic or morphological damage, such as needle yellowing or bud abortion, which could be ascribed to the effect of experimental treatment. On the another way the only differences were found on anatomical and histochemical levels of observation. They could be characterised as early, macroscopically non-visible damage.

Anatomical changes: Development of Norway spruce buds was divided into five developmental stages according to Owens *et al.* (1977). The bud annual development (Fig. 2), started in January with the dormant stage (First dormancy, Fig. 1). During bud break (April - mid May), leaf primordia started to grow and apical meristem formed new bud scales (stage of early bud scale initiation, Fig. 3*A*). In late spring and early summer, characterised by shoot growth, the apical meristem kept forming bud scales gradually covering the bud meristems (stage of late bud scale initiation, till the end of June). Shoot growth stopped in the middle of summer and a pre-formed bud started to differentiate by the production of new leaf primordia. This phenological phase can be divided further, based on primordia formation, into developmental stages of early (Fig. 3*B*, July) and late leaf primordia initiations (Fig. 3*C*, till the end of September). Anatomical structure typical for the dormant state was achieved in October.

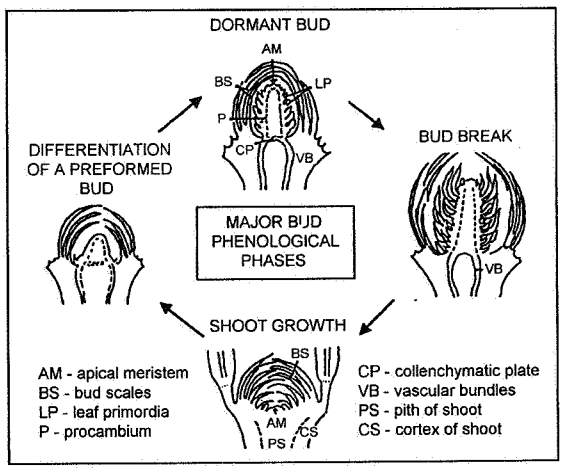

Fig. 2. Description of bud anatomy during the major phenological phases of vegetative season.

All quantitative parameters of control dormant buds did not differ significantly in both dormant periods, thus, we may consider them as general for our type of material: the average height of embryonic shoot was 1437 ± 107 μm (n = 24), ratio of length to width of embryonic shoot was 1.62 ± 0.12, ratio of length to width of apical meristem was 0.34 ± 0.02 and thickness of collenchymatic plate was 90.35 ± 8.29 μm. Control buds had in average 5.3 ± 0.5 cell layers of collenchymatic plate, 8.0 ± 0.9 leaf primordia and 1.7 ± 0.4 living bud scales. Changes of

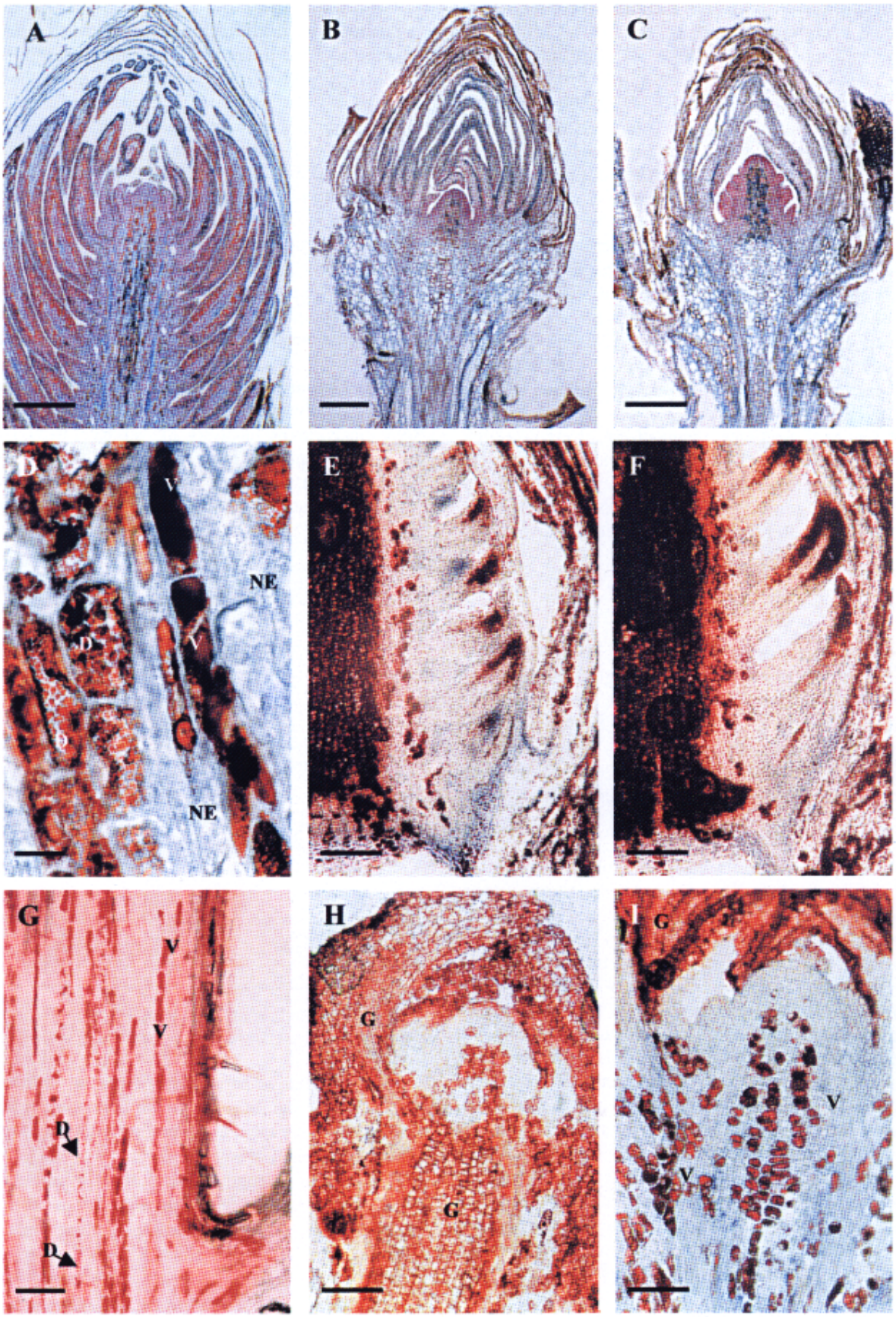
A
B
C
D
V
NE
V
D
NE
E
F
G
V
V
D
D
H
G
G
I
G
V
V

Fig. 3. Anatomical structure and localisation of non-specific esterase and phenolic compounds in buds of Norway spruce. Figs. *A* - *C* are stained with alcian blue and nuclear fast red to visualise meristematic parts (red colour). Non-specific esterase and phenolic compounds on Fig. *D* - *F* and *H* - *I* are visualised by the reaction with Fast Blue B; blue colour - non-specific esterase, brownish-red colour - phenolic compounds; D - drop-like phenolics, G - granular phenolics, NE - non-specific esterase, V - vacuolar phenolics (light microscopy - bright field).
A - Median longitudinal section of bud during bud break; early bud scale initiation; outgrowth and differentiation of leaf primordia formed in previous year (*bar* = 300 μm). *B* - Median longitudinal section of bud during differentiation of a pre-formed bud; early leaf initiation; bud apical meristem is pointed and starts to form new leaf primordia; cells of future bud cavity start to vacuolise (*bar* = 200 μm. *C* - Median longitudinal section of bud during differentiation of a pre-formed bud; late leaf initiation; most leaf primordia were already initiated; future bud cavity and collenchymatic plate were more pronounced (*bar* = 200 μm). *D* - Subcellular localisation of non-specific esterase and phenolic compounds within cells (*bar* = 20 μm). *E*, *F* - Comparison of the NE activity in the zone of differentiation of vascular bundle, collenchymatic plate and the youngest bud scale below the apical meristem in treatment *D*, the most treated variant (*E*) and control treatment (*F*) during bud break (4 April) (*bar* = 100 μm). *G* - Histochemical proof of condensed tannins (dark red) in growing shoot under bud meristem (31 May); stained with vanillin-HCl (*bar* = 100 μm). *H*, *I* - Comparison of accumulation and type of phenolic compounds in buds of treatment D (*G*) and K (*H*) during early shoot growth (31 May) (*bar* = 100 μm).

most of quantitative parameters observed among treatments had prevalently inconsistent character. However, when comparing the first and second dormancies, all treated variants showed the significant decrease in number of leaf primordia (Fig. 4*A*) and increase in number of living bud scales (Fig. 4*B*) while these parameters remained unchanged in the control buds.

Comparison of quantitative anatomical parameters during vegetative season was difficult mainly due to variation connected with shifts in timing of developmental stages of buds of different treatments. The onset of bud break in buds of control (C) was two weeks delayed. Some differences were found in the seasonal changes of length to width ratio of apical meristem. Independently on a treatment the curve describing this characteristic had major maximum at the transition from early to late leaf initiation (June). Meristems of the treatments 3.9R and

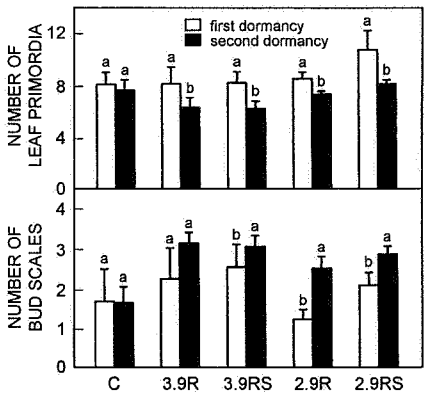

Fig. 4. Number of leaf primordia (*A*) and number of living bud scales (*B*) in buds of all treatments 3.9R, 3.9RS, 2.9R, 2.9RS and control C. *Bars* indicate SD, $n = 5 - 8$. Data with common letters ate not significantly different ($P < 0.05$).

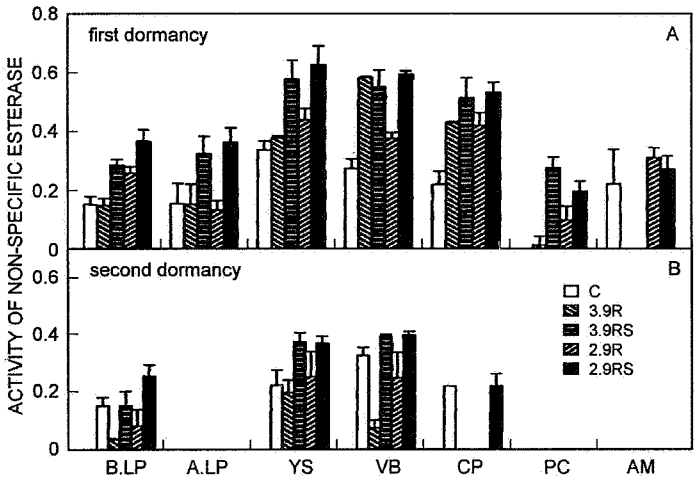

Fig. 5. Activity of NE in buds of all treatments 3.9R, 3.9RS, 2.9R, 2.9RS and control C. *A* - First dormancy - January 1995; activity of NE in 3.9RS and 2.9RS treatments was significantly higher at $P < 0.01$, $n = 8$. *B* - Second dormancy - December 1995; activity of NE in 3.9RS and 2.9RS treatments was significantly higher at $P = 0.07$, $n = 6$. The activity of NE is given as a ratio of absorbances. *Bars* indicate SD. Missing columns means that activity of NE was not detected at that tissue and treatment. B.LP - base of leaf primordia, A.LP - apex of leaf primordia, YS - the youngest bud scales, VB - vascular bundles, CP - collenchymatic plate, P - procambium, AM - apical meristem.

3.9RS were more pointed than control ones at their maximums of length to width ratio of apical meristem than those of control treatment, while the meristems of the treatments 2.9R and 2.9RS were flatter.

Non-specific esterase: Activity of non-specific esterase was localised in small cytoplasmic compartments (Fig. 3*D*). During the first dormancy the NE activity was detected in a majority of bud tissues: collenchymatic plate, the youngest bud scales, zone of differentiation of vascular tissues, in leaf primordia and the apical meristem (Fig. 5*A*). The highest activity of NE was found in buds from the 3.9RS and 2.9RS treatments. The esterase activity gradually decreased during bud break (Fig. 6) but trend of enhanced activity in treatments 2.9RS (Fig. 3*E*) and 3.9RS (Fig. 3*F*) prevailed.

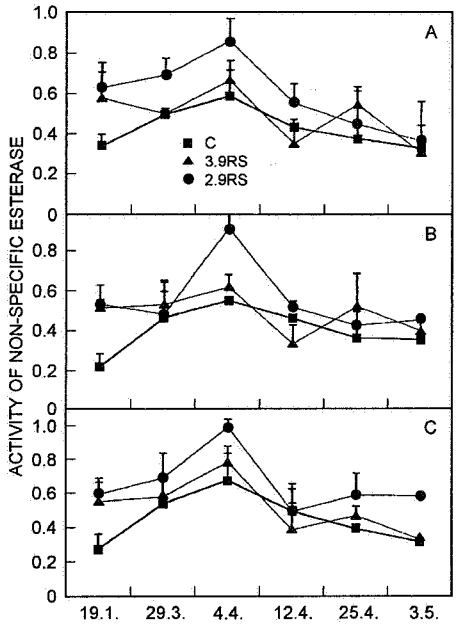

Fig. 6. Activity of NE in vascular bundles (*A*), the youngest bud scales (*B*), and collenchymatic plate (*C*) in buds of treatments 3.9RS and 2.9RS, and control C during the first dormancy (19 January 1995) and bud break (29 March - 3 May 1995). The activity of NE is given as a ratio of absorbances. *Bars* indicate standard deviation, $n = 4$.

It was most pronounced in the vascular bundles below the bud meristems (Fig. 6*A*), the youngest bud scales (Fig. 6*B*) and collenchymatic plate (Fig. 6*C*). During the growth of a new shoot, the NE activity was localised only in the vascular tissues below the bud meristems. The increased enzyme activity observed in treatments 3.9RS and 2.9RS disappeared. The NE activity reoccurred in the late summer during differentiation of pre-formed buds but no relation between the experimental treatment and NE activity was observed. During the second dormancy (Fig. 5*B*) no NE activity was detected in leaf primordia and apical meristem. Despite the differences in activity and localisation of NE during both periods of dormancy it was clear that the highest activity of NE was detected in treatments 3.9RS and 2.9RS). This difference was statistically significant ($P < 0.01$) in the first dormancy period and in the second dormancy period the difference was less pronounced ($P = 0.07$).

Phenolic compounds: All phenolic compounds detected in buds with Fast Blue B reacted positively with vanillin-HCl, which identified them as condensed tannins. As described in our previous study (Bílková 1999) phenolic compounds of buds of Norway spruce were divided according to their subcellular localisation into three groups called granular, vacuolar, and drop-like phenolics (Figs. 3*D,H,I*). Their localisation changed in connection with anatomical changes during the vegetative cycle.

Granular phenolics were the most typical for meristematic and non-differentiated tissues, while the vacuolar phenolics commonly occurred in a tissue at a progressed stage of differentiation. All types of phenolics were detected in all treatments of SAR. They occurred in all treatments at the same time but their total amount was dependent on a treatment. At the beginning of growth of a young shoot (31 May - 26 June), buds from treatments 3.9RS and 2.9RS accumulated higher amount of phenolics (measured as the percentage of area of a median bud section containing phenolics, Figs. 3*H,I* and 7). Most of those accumulated phenolics were of granular type. Thus, we regarded the accumulation of granular phenolics to be a consequence of the direct application of experimental solutions on a developing shoot. It was also observed that injured or dead (brownish) needles, which were infrequently observed on young growing shoots sprayed with SAR, contained a great amount of granular phenolics. Some of injuries were later healed with small round cells containing exclusively vacuolar phenolics.

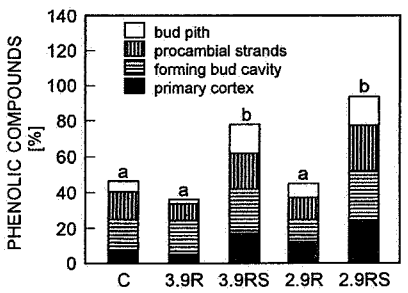

Fig. 7. Amount of phenolic compounds expressed as the proportional area of a median bud section. SAR treatments: 3.9R, 3.9RS, 2.9R, 2.9RS and control C. CS - cortex of young shoot, RM - rib meristem, VB - vascular bundles, PS - pith of young shoot. Data with common letters are not significantly different ($P < 0.02$) (analysis of variance).

Discussion

Only limited information is available concerning bud structural changes caused by stress factors. Matschke (1994) described flattening of apical meristem of Norway spruce buds as a consequence of the herbicide application. We did not observe significant differences in anatomical parameters among treatments in both dormant periods. However, during growing season we observed stimulating effect of application of solution pH 3.9 (treatments 3.9R and 3.9RS) on the ratio of length to width of apical meristem while the application of solution pH 2.9 caused pronounced flatness of apical meristem. Flatness of apical meristem may be regarded as an indicator of decreased capacity for establishment of morphogenetic gradients or fields (Carr 1984), and thus a lower capacity for formation of new primordial structures in the following vegetative season (n+1). This could be expressed by a reduced shoot growth in vegetative season n+2 (Owens *et al.* 1977).

Observed significant decrease of amount of leaf primordia for all treated variants in the second dormancy when compared with the first dormancy could indicate a lower growth capacity of embryonic structures in the following season. Temperatures during both growing seasons did not differ remarkably and therefore the decrease in the number of primordia may be considered as one of the consequences of the SAR.

The first dormancy (January 1995), during which the higher amount of living bud scales was observed in all treated variants, represented late stages of dormancy, while collections during second dormancy (December 1995), when the number of living bud scales was higher, were made in earlier stage of dormancy. Since the number of living bud scales of control buds remained unchanged in both dormancies, their increased number in all treatments cannot be attributed to the differences in timing of collections, but to the effect of treatment. In the autumn during differentiation of a pre-formed bud, outer bud scales gradually wither and become a part of mechanical bud protection against harsh environmental conditions. It is possible to assume that delayed necrotisation of living bud scales could, on structural level, indicate a prolonged vegetative state. Documented two-week delay of bud break onset for control trees also indicates prolongation of vegetative period of treated buds. Regardless that these differences were observed for short periods of the year only, they can be very important, because earlier bud break and delayed frost hardening in autumn could make trees more susceptible to frosts occurring during those both marginal periods of vegetative season.

The highest activity of NE as well as the greatest difference among treatments was observed in tissues located in the basal part of a bud. These tissues have mostly protective or transport functions (Matschke 1994). Based on findings of other authors the phenomenon of the highest activity of NE at the basal part of a bud could be partly attributed to progressed differentiation of those tissues (Beneš 1971). High activity of NE during differentiation was reported many times in vascular tissues (Godbold *et al.* 1993, Bílková *et al.* 1999). Moreover, vascular tissues being the only tissues where the activity of NE was detected during the whole year were the best responding tissues to SAR treatment. High NE activity during the differentiation of the vascular tissues was recorded by other authors (Guglielmino *et al.* 1997). NE is known to play a role in the differentiation of the cell wall (Smith and O'Brien 1979) and therefore, a potential reason for high enzymatic activity could be the progressive differentiation in vascular tissues.

The observed increase in the NE activity of treatments 3.9RS and 2.9RS could be a result of higher energetic demand connected with higher requirement for repair processes taking place in meristems. The increased activity of NE in treatments 3.9RS and 2.9RS implies, that NE activity was not only dependent on pH of experimental solution, but mostly on the direct application of experimental solution on buds and foliage causing the primary damage. The increased activity of NE as a consequence of stress or damage was documented also in other studies (Taskakorie *et al.* 1985, Cachot *et al.* 1994).

Our observations confirmed an earlier finding that the occurrence of granular and vacuolar phenolics had a developmental connection (Bílková *et al.* 1996). As in case of activity of NE, the greatest influence of SAR on phenolic accumulation was observed in treatments sprayed with SAR on aboveground parts (treatments 3.9RS and 2.9RS) at the beginning of growth of a new shoot. During that time bud meristems had only limited protection against SAR treatment. Accumulation of phenolics could help to isolate the most important and vulnerable tissue (apical meristem) from the negative influence of treatment. A similar pattern was documented during the wound healing or pathogen attack, when the accumulation of phenolics tended to isolate and preserve surrounding healthy tissue (Oven and Torelli 1994).

Based on the results discussed above it seems that the main effects of simulated acidic pollution is not to mediated through root system, *i.e.* having a character of secondary damage, but a major role in tree damage should be attributed to direct application of pollutants on foliage. This primary damage of trees by acidic pollutants was more severe than secondary damage.

References

Beneš, K.: Histogenesis and localisation of non-specific esterase in root tip. - Biol. Plant. **13**: 110-121, 1971.

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

Bílková, J., Opatrná J., Albrechtová, J.: The effect of stress on physiological state of buds of Norway spruce (*Picea abies* L. Karst.): III. Activity of non-specific esterase. - Biologia (Bratislava) **51**: 470, 1996.

Bílková, J., Opatrná, J., Albrechtová, J.: Histochemical detection and image analysis of non-specific esterase activity and the amount of polyphenols during annual bud development in Norway spruce. - J. exp. Bot. **50**: 1129-1138, 1999.

Booker, F.L., Anttonen, S., Heagle, A.S.: Catechin, proanthocyanidin and lignin contents of loblolly pine (*Pinus taeda*) needles after chronic exposure to ozone. - New Phytol. **132**: 483-492, 1996.

Bordenave, M., Goldberg, R., Huet, J.C., Pernollet, J.C.: A novel protein from mung bean hypocotyl cell walls with acetyl esterase activity. - Phytochemistry **38**: 315-319, 1995.

Borg-Oliver, O., Monties, B.: Lignin, suberin, phenolic acids and tyramine in the suberized wound-induced potato periderm. - Phytochemistry **32**: 601-606, 1993.

Cachot, J., Romana, L.A., Galgani, F.: *In vivo* esterase activity in protoplasts as a bioassay of environmental quality. - Aquat. Bot. **48**: 297-312, 1994.

Carr, D.J.: Positional information and plant morphology. - In: Barlow, P.W., Carr, D.J. (ed.): Positional Controls in Plant Development. Pp. 441-460. Cambridge University Press, Cambridge - London 1984.

Gardner, R.O.: Vanillin-hydrochloric acid as a histochemical test for tannin. - Stain Technol. **50**: 315-317, 1975.

Godbold, D.L., Herms-Cremer, A., Hüttermann, A.: Stress bioindicators in Norway spruce. - In: Teller, A., Mathy, P., Jeffers, J.N.R. (ed.): Responses of Forest Ecosystems to Environmental Changes. Pp. 806-807. Elsevier Appl. Sci., London - New York 1993.

Gruber, F.: Die proleptische Triebbildung bei *Picea abies* (L.) Karst. - Beitr. Biol. Pflanz. **64**: 75-113, 1989.

Guglielmino, N., Liberman, M., Catesson, A.M., Mareck, A., Prat, R., Mutaftschiev, S., Goldberg, R.: Pectin methylesterases from poplar cambium and inner bark: localisation, properties and seasonal changes. - Planta **202**: 70-75, 1997.

Hejnowicz, A., Obarska, E.: Structure and development of vegetative buds from the lower crown of *Picea abies*. - Ann. Sci. forest. **52**: 433-447, 1995.

Huttunen, S., Reinikainen, J., Turunen, N.: Wintering response of conifers to acid rain treatment under northern conditions. - In: Kauppi, P., Antila, P., Kanttamies, K. (ed.): Acidification in Finland. Pp. 607-634. Springer-Verlag, Berlin - Heidelberg - New York 1990.

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

Kainulainen, P., Holopainen, J.K., Oksanen, J.: 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, 1995.

Kainulainen, P., Holopainen, J., Palomaki, V., Holopainen, T.: Effects of nitrogen fertilization on secondary chemistry and ectomycorrhizal state of Scots pine seedlings and on growth of grey pine aphid. - J. chem. Ecol. **22**: 617-636, 1996.

Kozlowski, T.T., Growth characteristics of forest trees. - J. Forest. **61**: 655-662, 1964.

Krasnuk, M., Witham, F.H., Jung, G.A.: Hydrolytic enzyme differences in cold-tolerant and cold-sensitive alfalfa. - Agron. J. **70**: 597-605, 1978.

Maier, R.: Blei und seine Auswirkung auf Aktivität und multiple Formen der Alpha-Naphtyl-Esterase in bleichgeten und verdunkelten Pflanzen. - Ber. deut. bot. Ges. **91**: 339-350,1978.

Mace, M.E., Howell, C.R., Histochemistry and identification of condensed tannin precursors in root of cotton seedlings. - Can. J. Bot. **52**: 2422-2426, 1974.

Matschke, J.: Das Alte stirbt damit das Junge lebt (Beschleunigte Alterung und Absterben der Triebspitzen - Schlüssel zur Erklärung des Baumsterbens?). - Landwirtschaftskammer Westfalen-Lippe, Münster, Beitrage zur Sache 43/94, 1994.

Moore, J., Bamforkh, C.W., Kroon, P.A., Bartolome, B.: Ferulic acid esterase catalyses the solubilization of beta-glucans and pentosans from starchy endosperm cell walls of barley. - Biotechnol. Lett. **18**: 1423-1426, 1996.

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

O'Brien, T.P., McCully, M.E.: The Study of Plant Structure: Principles and Selected Methods. - Termacarphi Pty, Melbourne 1981.

Oven, P., Torelli, N.: Wound response of the bark in healthy and declining silver firs (*Abies alba*). - IAWA J. **15**: 407-415, 1994.

Owens, J.N., Molder, M., Langer, H.: Bud development in *Picea glauca*. I. Annual growth cycle of vegetative buds and shoot elongation as they relate to date and temperature sum. - Can. J. Bot. **55**: 2728-2745, 1977.

Polle, A., Tilman, O., Seifert, F.: Apoplastic peroxidase and lignification in needles of Norway spruce (*Picea abies*). - Plant Physiol. **106**: 53-60, 1994.

Richter, C.M., Wild, A.: Phenolic compounds in needles of Norway spruce trees in relation to novel forest decline I. Studies of trees from a site in the northern Black Forest. - Biochem. Physiol. Pflanz. **188**: 305-320, 1992.

Smith, M.M., O'Brien, T.P.: Distribution of autofluorescence and esterase and peroxidase activities in the epidermis of wheat roots. - Aust. J. Plant Physiol. **6**: 201-219, 1979.

Taskakorie, A., Clerc, M., Thi, A.T.P., da Silva, J.V.: Mise en évidence de l'activité estérase dans les feuilles de cotonnier: action de la sécheresse sur cette activité enzymatique. - Compt. rend. Acad. Sci. Paris **301**: 343-349, 1985.