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Oxidative stress symptoms in Norway spruce needles (*Picea abies* L. Karst.)

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Abstract

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We observed the deformation level in mesophyll cells in Norway spruce (*Picea abies* L. Karst.) needles displaying characteristic features of ozone damage. There were examined mesophyll cells under discoloured spots (zones) in epidermis and hypodermis. We evaluated modifications in their water potential as well as relative occurrence of healthy and damaged cells, and determined relation between healthy and diseased mesophyll cells expressed by the cell shape index indicating the degree of the damage.

Key words

cell shape index, Norway spruce (*Picea abies* L. Karst*)*, mesophyll, reactive forms of oxygen

Introduction

The spruce stands in middle altitudes are often exposed to harmful influence of both biotic and abiotic factors. These factors cause stress, activated mainly by the reactive forms of oxygen. Molecular oxygen and its radical derivatives, which are initiated mainly by photochemical reactions in presence of excessive amount of UV radiation, may lead to photooxidation of photosynthetical pigments, particularly chlorophyll *a* (BERGENDI, 1988). Hydrogen dioxide, when present in chloroplasts even in small concentrations, inhibits the CO_2 fixation. This results in disruption of oxidation-reduction balance of cells and oxidation damage to cell components (MANGEL and ZIEGLER, 1986; OSWALD and ELSTNER, 1986; HIPPELI and ELSTNER, 1997). The cell water potential reduction and its deformation is very common. Plants have developed a defensive anti-oxidation system against the above mentioned oxidative damage. This system consists of an enzyme complex including for instance ascorbatperoxidaze, superoxiddismutaze, catalaze, and a non-enzymatic part containing a zeaxantine, α-toco-ferol, β-carotene, ascorbat. Anti-oxidative defence activation influences the plant's vulnerability and tolerance to stress in plants (PITERKOVA et al., 2005; PURVIS et al., 1995; DOTZLER et al., 1990). The defence of enzymes occurs in several isoforms. The amount of cytokinins in general decreases under unfavourable conditions, and certain phytohormones become important for defensive response to stress. In the damaged one-year-old needles of the studied Norway spruce growing in the 7th and 8th forest vegetation zones were found different levels of cell deformations, changes in their water potential, and in relative frequency of healthy and damaged cells.

Our research objectives were:

- To determine the extent of deformation of mesophyll cells of Norway spruce (*Picea abies* L. Karst.) below epidermal and hypodermal decolorized zones in the damaged Norway spruce needles
- To measure the ratio of damaged to undamaged cells, and to determine a shape index for damage to the studied needle segments
- To determine level of deformation for damaged cells and differences in their water potential.

Material and methods

Plant material

One-year-old needles of damaged spruce trees (*Picea abies* L. Karst.). Their characteristic feature was discolouration in form of visible rounded yellow spots 1–2 mm in diameter (Fig. 1).

Locality

Lysá hora, the Moravskoslezské Beskydy Mts, peak part, 8th vegetation zone, forest type 8Z2 – peak bilberry rowan pine grove (Sorbeto Piceetum), altitude 1,310 m above the sea level. A severely damaged adult pine stand, main soil types: ranker, podzol and cryptopodzol with raw humus, very acidic, clay-sand soil, skeletal to boulder, fresh moisture.

Fig. 1. One-year-old needles of Norway spruce with characteristic symptoms of ozone-caused damage. There are hyaline or yellow chlorosis spots visible on the needles. Locality: Lysá hora, 1,310 m above the sea level

Histology

Sampling of one-year-old needles, year-class 2007, was carried out on 14. 5. 2008 at 14.30. The samples were fixed with a FAA (formalin aceto-alcohol) fixation solution. They were blocked, particular damaged segments were cut, separated, and processed by application of histopathological and histochemical methods. They were embedded Bio-Plastic (at the temperature of 58 °C) and paraffin blocks were cut off with a rotary microtome HM 325, MICROM GmbH (Germany). The thickness of the slices was – if possible – from 4 to 7 µm. We used with advantage silanised glass and a special Superfrost electrostatic underlying glass. The material was dyed with malachite green with acid fuchsine and toluidine blue, Van Gieson, Groccot, were selected as the most suitable dyestuffs. The picture was processed with a microscope ZEISS Axiostar Plus connected with a camera OLYMPUS C5060 WZ and a multimedia PC. The software used was Quick Photo Camera 2.1, GIMP 2.2.

Cell deformation modelling

For estimation of the water potential we used the Höfler's diagram (Fig. 2). It is difficult to determine the exact volume of the cell, i.e. the ratio of its volume with one hundred percent content of water (later on as "healthy cell"). We estimated it through assuming that oxidative stress during a high insolation event results in water loss. The cell is distorted only in one dimension – the width (w) – whereas both length (l) and height (h) remain most unchanged. We could assume relative volume of the cell in simplified point of view, which is adequate to the width of both diseased and healthy cell ratios. This relation was named the cell deformation level (Fig. 3). The original width undistorted of cell (full turgor) is usually unknown. From the measurements carried out on undeformed mesophyll cell across the radial cut, we could conclude that their very variable width was roughly linear dependent on the cell's length. This fact was revealed by linear regression. Since we regard the cell length in the process of enhanced water loss in autonomous areas as a constant, we can use the above discussed linear function for estimation of the original width in deformed cells of particular length, and calculate the cell deformation level considered (in our simplified model) as identical with relative the volume of the cell (Salisbury and Roos, 1992).

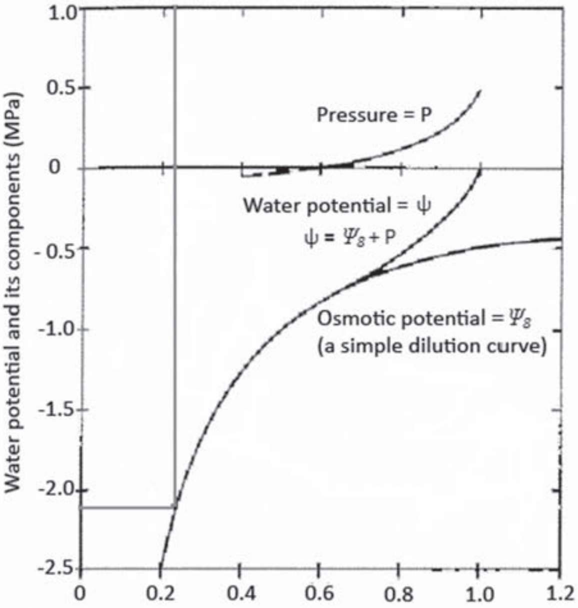

Fig. 2. Hőflers diagram (according to Salisbury and Ross, 1992). Graphic expression of relation between osmotic potential Ψs, pressure potential P and water potential Ψw

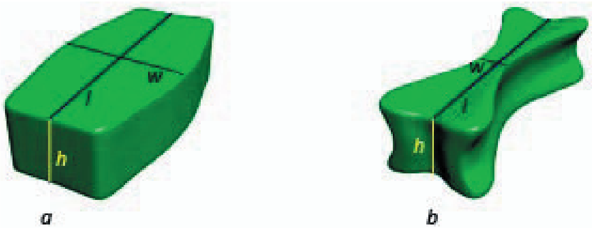

Fig. 3. Model of mesophyll cell deformation affected by reactive forms of oxygen (ozone) a – intact cell, b – deformed cell, l – length of cell, w – width of cell

Results and discussion

We observed significant cell deformations across radial cross-section of damaged needle segments. The cells were deformed by characteristic contraction accompanied with water potential drop. Deformed cells penetrate irregularly into the zone of cells which are still healthy. The intact cells are predominantly mitotic active, and their undisturbed protective mechanisms meet their function, and withstand the stress (Fig. 4). It was found that the average length of undeformed and deformed mesophyll cells was the sum. The deformation level of damaged cells was defined based on the ratio between widths of deformed and original cell of the same length. The median is of 0.289 (Fig. 5). Relative volume of damaged cells is proportional to the level of their deformation. Median of the water potential has the value of – 2.12 MPa, standard deviation is 1.12 MPa (Fig. 6). Cell width to height ratio is expressed through the cell shape index. Median of the shape index was established in monitored segments as follows: for healthy cells in the amount of 0.591, and for diseased ones 0.187. Standard deviation achieved 0.220 for intact cells, while 0.081 for damaged ones (Fig. 7). Dependence of the healthy cell width to its length is approximation linear (Fig. 8). Cell shape index indicates certain disease level showing at the final stage irreversible modification take place in mesophyll tissues. Its scope has adverse impact to the energy management and weakens metabolic functions of the cells. It was found that even single year needles often starts to form segregation layer in abscise zones and phelogen activity is increasing there, this results in premature aging, featured with subsequent defoliation of needles from the stem (Fig. 9). Scaling the leaf area down takes place for needles damaged by ozone (and by other reactive forms of oxygen), resulting also in vitality weakening of the whole tree. Premature elder needle shedding is a consequence of it. In the highest stations of middle-mountain the age of said needles reaches three, rarely even four years.

Conclusions

Structural analysis of the needles damaged by oxidative stress showed permanent changes in both covering and mesophyll tissues. Scope of damage achieved up to conductive tissues. Histological image showed qualitative deformations within locations of damaged cells. Frequent autonomous zones of atrophied cells featured with osmotic activity were observed within mesophyll. Local occurrence of the hypertrophy of fully turgescent cells has been found. Oxidative stress accelerates needle ageing as well as their premature fall.

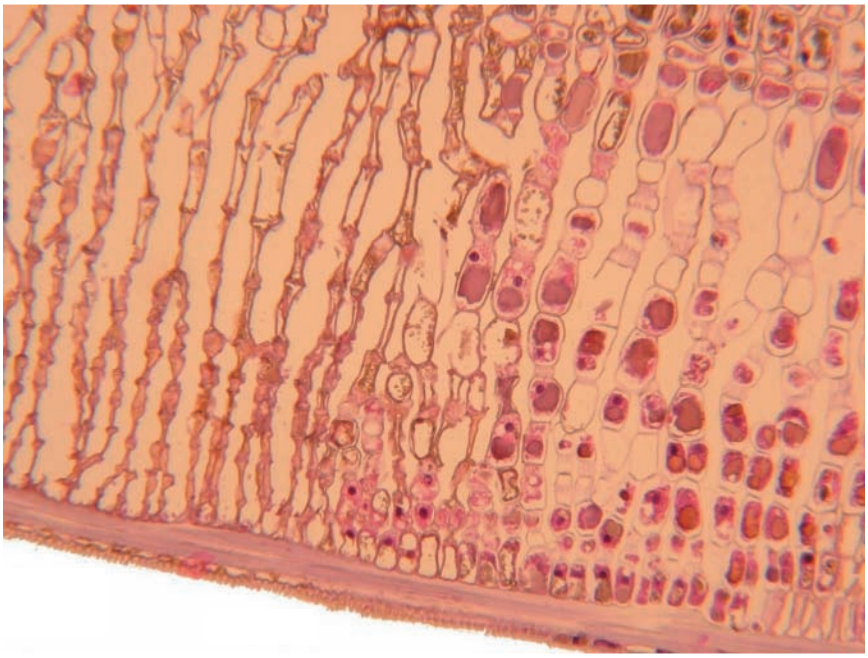

Fig. 4. Radial cross-section of damaged cells, zone of mitotic activity and healthy cells. Coloring: Hematoxylin-eosin

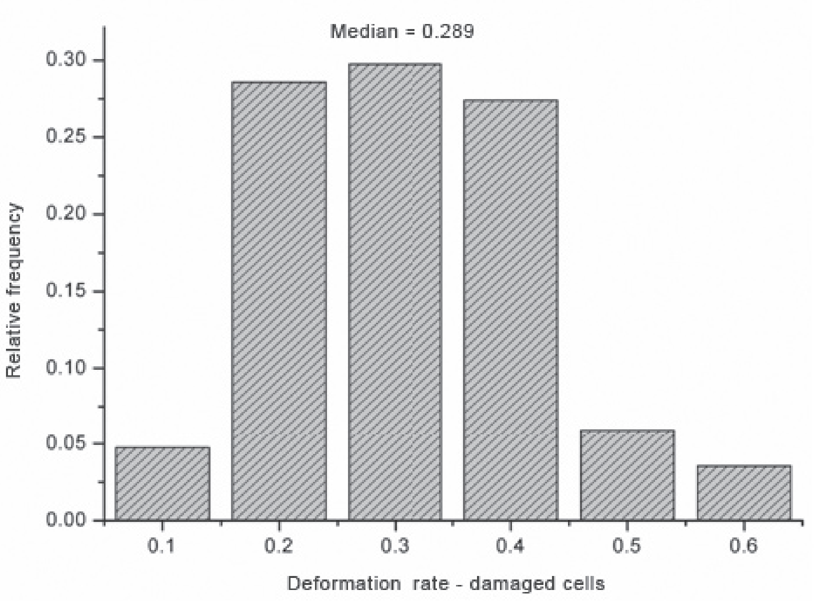

Fig. 5. Deformation level in damaged cells

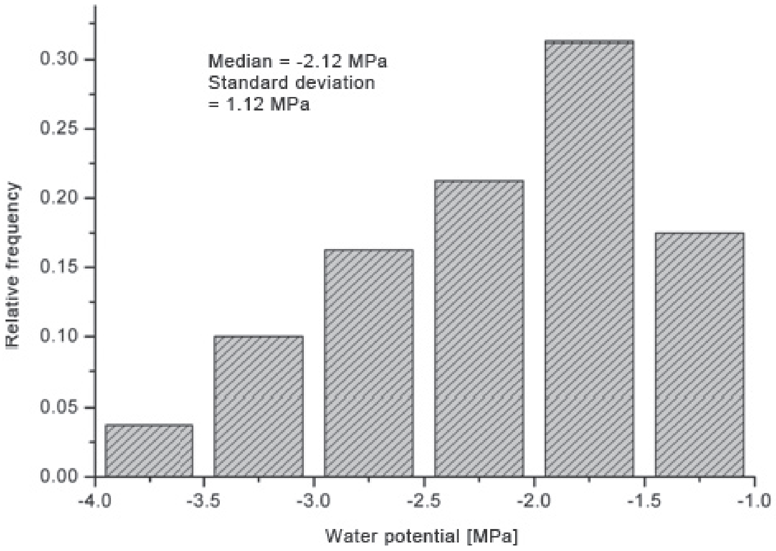

Fig. 6. Water potential of damaged cells

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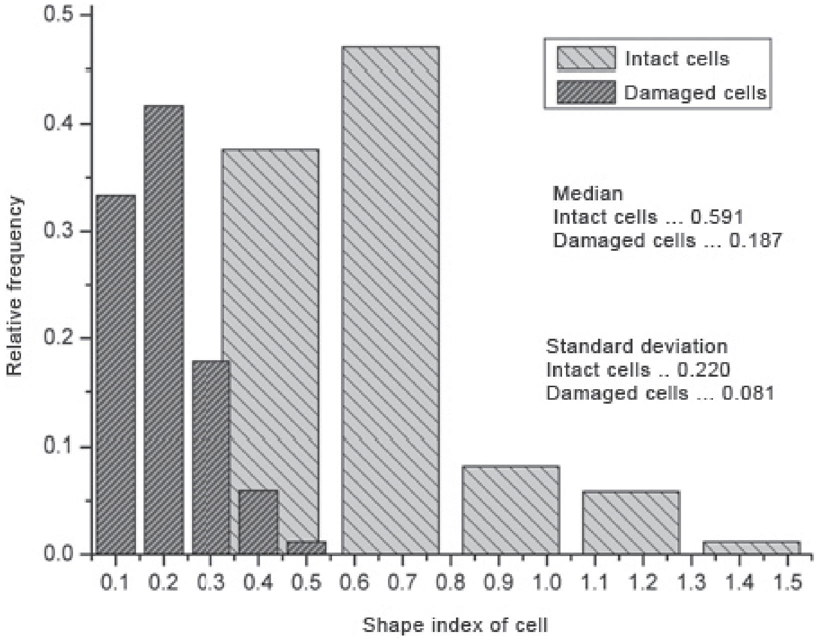

Fig. 7. Cell shape index – indicator of a damage rate

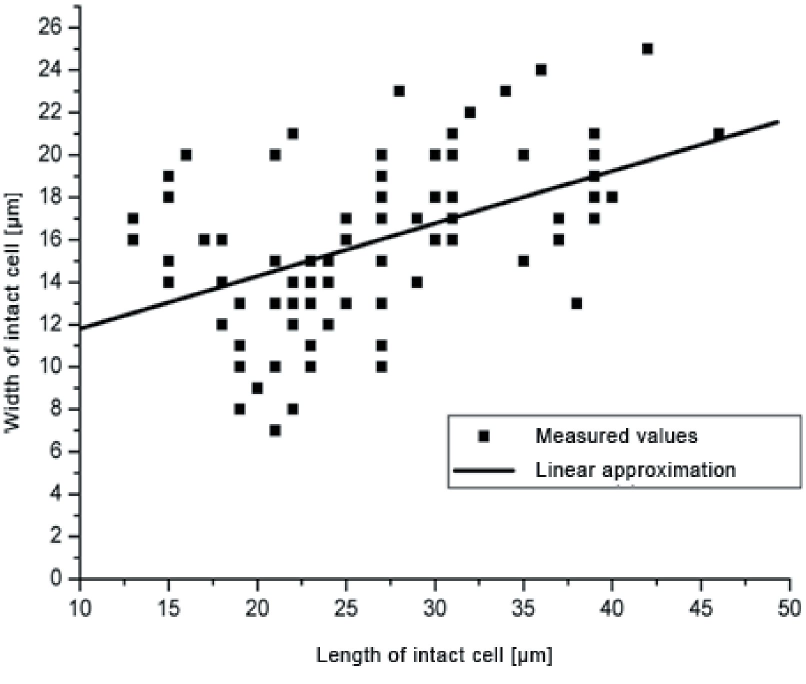

Fig. 8. Dependence of healthy cell width on its length

Fig. 9. Initiation of the defoliation with generating the segregation layer (A – one year old, B, C – two years old)

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Symptomy oxidativního stresu v jehlicích smrku ztepilého (*Picea abies* L. Karst.)

Souhrn

Ve vyšších polohách středohoří bývají smrkové porosty poškozeny oxidativním stresem způsobeným především vysokými koncentracemi ozonu. Molekulární kyslík a jeho radikálové deriváty vyvolávají fotooxidaci fotosyntetických pigmentů, což vede k snížení výkonu fotosyntézy a inhibici růstu smrků.

Dochází k narušení redoxní rovnováhy buňky, a tím k oxidačnímu poškození buněčných komponent. Nastává snížení vodního potenciálu buňky až na hodnotu – 2.12 MPa a její deformace.

Práce sleduje, ve zkoumaném souboru poškozených buněk jehlic smrku ztepilého, stupeň deformace mezofylových buněk v autonomních oblastech pod diskolorovanou zónou epidermis a hypodermis, poměr četnosti výskytu nemocných buněk ke zdravým buňkám, stanoví stupeň deformace nemocných buněk a změny jejich vodního potenciálu.

Při oxidativním stresu během vysoké insolace dojde k ztrátám vody, buňka se zužuje pouze v jednom rozměru, šířce, přičemž délka a výška buňky zůstává prakticky stejná.

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