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Working party 'mechanisms of action' and indicator development 7.04.02

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Studying environmental problems in forest ecosystems, particularly in trees, the key question is: which changes or injury do we observe and what is their origin? An anthropogeneous stress factor is often hypothesized as being to blame and, in the case of air and soil pollution, experiments are carried out to prove the dose-response relationship with focus on the pollutant uptake and plant growth. In the past 20 years the experimental conditions to test air pollution have constantly improved using increasingly sophisticated technical installations from greenhouses, climate chambers, open-top chambers, to branch and single tree chambers, and recently different free air release fumigations systems in tree plantations or forest canopies - all with the purpose of measuring the effects in a near-natural system. Parallelly, research has deepened into the 'mechanisms of action' of different stress factors, by analysis of leaf/branch or entire small tree gas exchange, anatomical and biochemical responses. Introductory work has been done using molecular biology methods. Applications using bioindication in the field (initially with exposed sensitive plants and recently with the natural vegetation) have been developed. Results from ozone injury assessments have been reported in different sessions of this meeting during which much new work is dedicated to understanding the effects of increased ozone, CO_2, N & S, and UV-B, whereas concurrent climatic, biotic and other anthropogeneous stress factors are considered less.

As shown in different presentations during the workshop, the principal role of microscopy in stress physiology is to make the link between stress factors and visible symptoms on the one hand, and cellular and biochemical effects on the other. The workshop attracted 63 persons, the majority of whom are not directly involved as speakers or in presenting posters but acknowledge the widespread need of microscopy techniques for validating the effects of different stress factors.

Two presentations focused on technical aspects. A paper about PAM imaging presented a novel technique to map and quantify changes in chlorophyll fluorescence. In conjunction with traditional microscopy techniques, it should allow new advances in functional anatomy of chloroplasts. The other presentation demonstrated that routine aldehyde fixation deactivates the proanthocyanidins. As a consequence, the crucial role of the tannins in stress reactions has been overlooked because of the widespread and unquestioned use of aldehydes in fixation pretreatment.

Microscopy, in particular, has proved its value in diagnosing several stress factors and therefore ascertaining the observed visible symptoms to render them suitable for a bioindication (monitoring, in particular for ozone) purpose, and that, irrespective of species and site. Physiological and biochemical processes could be localised by cytochemistry and their mechanisms of action better understood. Increasing experience in electron microscopy is used to detect stress associated structural changes as occurring in conifer chloroplasts on a subcellular level potentially before visible symptoms appeared. The number of peroxisomes, mitochondria and chloroplast plastoglobuli in conifer species is associated with light and/or ozone. It was stressed that exposure of the organ and tissue to light, as an important factor enhancing ozone injury, has to be considered in assessments, sampling and microscopical analysis. The latest results scale the 'mechanisms of action' through ozone down from the structural to the biochemical and molecular level, e.g. ozone-induced increase of H_2O_2 (detected with a specific staining) and transcript levels of catalase appeared to be restricted to the apoplast without ultrastructural injuries in tolerant aspen clones, whereas in sensitive plants they continued to the plasma membrane, cytosol and chloroplasts. The latter effect was absent in the treatment with elevated CO_2, showing the influence of the carbon household. At a cellular and ultrastructural level, the compartmentation pays a vital role; this was also evident in the investigation of products from the secondary metabolism. Their presence, quantity and site within tissue cells and cell compartments give important indications for detecting different stress factors. An innovation was the detection of heavy metals in situ by cytochemical methods.

The influence of leaf anatomy on the relative sensitivity of native vegetation was shown, however, woody plants are more easily used for ozone monitoring than herbaceous species, which have very variable life cycles, tend to senescence after flowering and are more often infected by fungi. In contrast to ozone, increased UV-B did not have significant effects so far in the Finnish peatland experiments, but increased the flavonoids in birch leaves, whereas in Scots pine needles it was argued that xeromorphic features increase the resistance to UV-B light. Microscopy was used to explain the anatomical features induced by nutrient deficiency (as shown for boron). Traditional methods used for long term monitoring could now show structural ameliorations in the vegetation around industries after changing production techniques in eastern countries.

Several interesting directions for future research can be outlined in synthesis from the presentations and the resulting animated discussion:

- Microtechnical level:
- Importance of fixation, sampling, selection of the staining and observing method as a decisive step. A critical approach is required according to the interactions between the preparation chemicals and the observed plant structures. New advances can be expected by research labs, having different technical specialities, joining in common research investigations.
- Extension of the list of bioindications usable for differential diagnosis of more stress factors and in a widened list of plant species as a long term goal. Investigations in this domain are urgently required with an increasingly stressed plant environment in a changing world. Joining microscopical and biochemical techniques is an efficient way of overcoming the limitations imposed by both approaches (respectively the significance and the specificity of the observed changes)
- Actuality of certain stress factors as outlined by recent advances in research and changes in the global situation:
- Effects of drought and heat in mesophilous and climacical species
- Quantitative contribution of light with and without ozone which leads to photobleeching and other visible symptoms
- Biotic and abiotic stress factor interactions:
- Role of biotic stress (air pollution by fungal spores), which may be underestimated
- Predisposition to biotic infection by anthorpogeneous pollution or environmental change
- Cellular reactions against different biotic attack,- also as founding criteria of differential diagnosis
- Understanding the changes in cell physiology underlying microscopical stress symptoms, noteably concerning:

- Vesiculations in the cytoplasm
- Changes in chloroplast structure
- Changes in vein structure caused by nutrient deficiencies

 Linking microscopical symptoms to higher observation levels:

- Estimation of the significance of leaf and needle symptoms for whole tree physiology
- Differentiation of species- and climate-specific (North vs. South) natural aging and senescence (the use of these terms is not clearly defined)
- Investigation of plant reactions to anthopogeneous stress in Africa, South America and Asia

The workshop and the focus on microscopy with an open discussion in a friendly atmosphere were highly appreciated.