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Physiological responses to air pollutants

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When investigating the phenomenon of large scale 'forest decline', particularly its appearance in so-called clean air regions, plant physiology has gained considerable importance. Especially tree physiology, which deals with the life processes of trees, has again become interesting not only for scientists, but also for the ecologically conscious public (Eschrich, 1987).

Trees are long-lived organisms which over many decades pass through various stages of development (seedling, sapling, young growth and old growth), each with its own distinctive physiological characteristics. In addition to these variations, sensitivity varies during the daily and annual rhythm. Since trees tower over all other forms of vegetation, they have a definite advantage in the struggle for light. Furthermore, they have evolved a system of compartmentalization which allows them the loss of larger plant parts without substantially affecting their chances for survival. Because of these attributes they possess a dominant position in a forest. Nevertheless, the term forest not only includes all closely interacting trees located in a defined area, but it also includes the complex structure of interactions between trees, bushes, herbs, animals, the soil including the organisms that live in and on it and the special climatic conditions. In the forest ecosystem with its diversity in vegetation and animal life, a near equilibrium is reached between decomposition and synthesizing processes. Even though this equilibrium is rather labile because of permanent natural changes, it still works very efficiently to maintain nutrients in the system. An additional attribute is the ability of trees, whose tops are strongly coupled with the atmosphere, to filter out dust and trace elements, which are then integrated into the nutrient cycle. It is precisely this large filtering capacity that appears increasingly to be a disadvantage for the forest in light of the present atmosphere load of pollutants.

Because of some of the attributes already discussed, it is understandable that trees have not often been studied by plant physiologists. Some of the difficulties in investigating trees range from the carrying out of experiments on tall trees and forest stands to the interpretation of the gathered data. Small trees, which are easy to handle as test objects, are usually only a few years old and, therefore, are not comparable in their physiological reactions to mature trees in forest stands. Large trees, however, are practically impossible to place in an experimental situation. This is especially true from an aboveground microclimatic perspective. James Bonner once said, "everything that can be done, can be done better with peas", but, unfortunately, this does not apply to the study of woody, long-lived plants.

The central problem of experimental forest research lies in the decision whether one carries out the experiments in labs or chambers with artificial but controlled conditions or undertakes field studies with realistic conditions, but with the influence of many uncontrollable environmental factors. Whenever the clarification of special questions or specific mechanisms concerning tree damage is desired, the first type of experiments would be chosen. The results of fumigation experiments on young plants could be utilized for interpreting some effects when air pollution is the dominant stress factor. This experimental approach is not adequate for more precise analysis of the interaction between air pollution and the forest ecosystem, where not only emission stress is at hand, but a complex system involving many stress factors (Lefohn and Krupa, 1988). Also, fumigation experiments using open-topped chambers may not correctly model the coupling between forest trees and the atmosphere (as reported by Dr. Jarvis in these proceedings).

This is surely a reason why today not enough tree-specific physiological information is available which is needed to explain the intricate phenomenon of 'forest decline' in its varied manifestations.

The fact that knowledge about physiological behavior of trees has become of great importance today, leads to two consequences for tree physiologists — a pleasant and an unpleasant one. The pleasant consequence is not only the increased appreciation of tree physiology, but also the increase in funds for research. The latter aspect has even allured some physiologists away from peas — though perhaps only for a short period of time. The unpleasant consequence manifests itself in the growing impatience of politicians and the general public. They expect tree physiologists to bring forth prompt and clear statements about the causes of the present forest damage. From what has already been said about research problems with trees, it is evident that, in tree physiology, it seems to be almost impossible to get quickly, universally applicable research results. 'Forest decline' is a complex phenomenon which has only surfaced as a major research focus in the past few years.

Without delving into a discussion about the causes of 'forest decline', most scientists agree that diverse air pollutants of the acidic or oxidative type play a significant role in this problem.

These air pollutants along with other abiotic and biotic stresses account for those conditions which could inhibit physiological processes. Since these physiological processes determine the quantity and quality of tree growth limited by genetic potential and directed by environmental conditions, physiology as a science should be strongly anchored in forestry. Unfortunately, the role of physiology in this branch of science — as Kramer (1986) regrettably determined — was often not correctly understood. This has turned out to be a disadvantage because it is difficult to discuss changes when one does not possess sufficient information about the original conditions. For example, the first signs of injury from 'forest decline' have been found at the macroscopic level, even though the causes of these disturbances are found on the cellular and subcellular levels. An important task for the plant physiologist is to determine the mechanisms responsible for such damage and, if possible, also the primary cause of it. Physiological criteria, however, should also help to quantify and differentiate the damage to trees. Above that, they should be capable of following the course of destruction and its effects from the primary injury, which should be detected as early as possible, until the death of the tree. Reports have only recently been released concerning physiological and biochemical reactions of trees and shrubs to different air pollutants (Kozlowski and Constantinidou, 1986) as well as physiological and biochemical changes within damaged trees (Lange and Zellner, 1986; Ziegler, 1986) and about the effects of gaseous air pollutants on forest trees from a plant physiological point of view (Weigel *et al.*, 1989). The topics discussed mainly in these papers are listed in Table I.

The various test parameters listed in the table changed in the presence of air pollutants, however, the mechanism of change has not been specified. Many of these parameters also behave in a similar way when exposed to other abiotic or biotic stresses, such as frost, heat, light, drought as well as fungus infection and insect attack. The isolated observation of these parameters is not useful when trying to place the reaction on a whole tree basis. For example, it is unrealistic to determine the vitality of a whole tree or canopy from changes in chlorophyll fluorescence in a few needles. In order to be able to apply plant physiological criteria as an effective determinant, the suggestions from Weigel and Jäger (1985) to compile and combine

Table I. Parameters of metabolism in plants affected by pollutants (from Weigel *et al.*, 1989).

Process/size/property	*Tested parameter*
Photosynthesis *(in vitro/in vivo)*	chlorophyll fluorescence CO_2-fixation gas exchange/stomatal function various photosynthetic functions (ATP-production, electron transport, enzymes and metabolites involved in Calvin-cycle)
Water relations	transpiration stomatal function water potential permeability of cuticle
Respiration *(in vitro/in vivo)*	gas exchange mitochondrial functions enzymes involved in respiration
Nutrient relations	N-, P-, K-, S-, Mg-, Ca-, Fe-contents
Enzyme activities	peroxidase, superoxide dismutase, catalase, glutamate dehydrogenase, nitrite/nitrate reductase, ribulose-1,5-bisphosphate, carboxylase, malate dehydrogenase, *etc.* phytohormone levels
Metabolite pools	amino acids, polyamine, carbohydrates, protein, phenolics, organic acids, nucleic acids, lipids, ascorbic acid, *etc.* photosynthate allocation
Pigment turnover	chlorophyll, carotenes, anthocyanins
Membrane integrity	ethane-, malondialdehyde production, lipid peroxidation permeability of membranes
Buffering capacity	
Surface/ultrastructure	
Biorhythms of plants	electrical conductance in leaves

various physiological, biochemical and chemical parameters to build a chain of evidence should be tried. In this manner, at least an indication of overlying toxicity principles can be achieved, such as the general acid effect, the formation of radicals and the role they play as well as the destruction of membrane systems.

Unalterable assumptions for the investigation of pollution effects using physiological and biochemical parameters must include the local emission situation and the consideration of the climatic conditions.

The knowledge of reactions which take place on the plant's surface and inside it allows inferences about the various resistance mechanisms of trees in contact with air pollutants. According to Levitt (1980), two strategies can be distinguished: avoidance and tolerance. While avoidance strategies include the cuticle and the stomata, tolerance plays a part whenever gaseous air pollutants penetrate into the leaves or needles. A few examples will demonstrate this.

The cuticular wax layers of the leaves from trees present themselves as the primary target for air-borne pollutants (Huttunen and Soikkeli, 1984). These layers function as a protection against wind, non-stomatal transpiration, frost, pathogenic and insect attack as well as against the penetration of air pollutants. Their erosion and destruction introduce, on the one hand, a loss of the barrier which prohibits the intake of pollutants and, on the other hand, facilitates the leaching of essential nutrients, leading to an increase in the effects of the damage already done by pollutants. Destruction of the cuticle has been observed after the impact of various acidic air pollutants (Ulrich, 1980; Huttunen and Laine, 1983; Godzik and Halbwachs, 1986), even though this has often been discussed in connection with ozone penetration. According to Baig and Tranquillini's (1976) observations in the Alps, the thickness of the cuticle from spruce and stone pine needles decreases as the elevation and wind-exposure increase, which is at the same time connected with a higher transpiration rate. These factors determine not only the timber line in temperate zones, but could also be used to explain the often observed exceptional sensitivity of trees in the ridge areas of mountains. The ozone concentrations which generally increase with the elevation (Smidt, 1983; Bucher *et al.*, 1986) are correlated with a reduced quantity and poorer quality of cuticle. Therefore, these ozone concentrations can lead to relatively severe damage to trees, especially under unfavorable weather conditions and shortening of the vegetation period.

The stomata can play a role similar to the cuticle with respect to the avoidance of absorption of gaseous substances, when the absorbed substance causes the stomata to close. Indeed, from the studies of Black (1982) and Mansfield and Freer-Smith (1984), it has been shown that stomata can open or close as a result of the penetration of pollutants. Considering the complexity of stomatal function, it is hard to make general statements about the behavior of stomata in a certain emission situation, particularly for field studies. The results of changes in stomatal aperture or regulation – for instance, reduction of pollution intake coupled with a reduced CO_2 absorption or an increase in transpiration which leads to an excessive water loss – both could greatly affect the plant's metabolism.

Only after the penetration of wet or dry deposited pollutants through the cuticle or the stomata are metabolic processes affected both physically and biochemically. These processes considered together form the internal resistance (Hällgren, 1984; Unsworth, 1981). The magnitude of the internal resistance is responsible for the tolerance of a plant species with respect to air pollutants. This internal resistance is determined among other things by the solubility of each pollutant in the water of the cell wall, which, when considered singly, could be used to rank the internal toxicity of the various pollutants. Also vital for the plant's tolerance strategy is its ability either to degrade the penetrated pollutant or to inactivate it through chemical binding or to metabolize it into non-toxic reaction products. The latter case is likely with those pollutants containing essential elements, such as SO_2 or NO_x.

Ozone is an example of a pollutant which degrades inside the plant. Even though, when compared to SO_2, NO_2 or HF, ozone demonstrates less solubility, its high chemical reactivity with unsaturated fatty acids, aromatic compounds and sulfhydril groups necessitates the maintenance of a steep concentration gradient between the outside air and the inside of the plant. Various radicals also take part in the phytotoxic effect of ozone (Tingey and Taylor, 1982; Elstner, 1984). They are not only a result of the reaction of ozone molecules with sulfhydril groups or aromatic and olefinic compounds, but also stem from reactions of ozone with the water in the cell wall. Equally possible is the formation of hydroxyl-, hydroperoxy- and superoxide anion radicals. The reactions of ozone and radical oxygen compounds with the unsaturated fatty acids of the biomembrane lead to the formation of lipid radicals and, in the presence of oxygen, lipid peroxides and lipid hydroperoxides (Bus and Gibson, 1979; Halliwell and Gutteridge, 1985). Elstner (1984) takes the process of lipid peroxidation as the initial reaction for destruction of the membrane system, which is responsible for the life preserving compartmentalization of the cell. The process of the destruction of the membrane promotes both the damaging of cuticular wax and the leaching of nutrients.

Since radicals are also found in normal metabolism, cells have developed a mechanism to eliminate them. Enzymes, such as superoxide dismutase (SOD), catalase and peroxidase, or molecules produced by the cell itself, such as ascorbate, which acts as an anti-oxidant, play a decisive role in the plant's detoxification system and, therefore, also in its tolerance. The increase in SOD found in poplar leaves as well as pine and spruce needles after fumigation and also in 'forest decline' areas points to a participation of the oxygen radical in the damaging of trees.

Fluoride-containing air pollutants serve as an example of how penetrated toxic ions in the cell are taken out of the plant's metabolism by chemical binding, for example, with Ca and Mg cations.

The tolerance of forest trees with respect to sulfur- and nitrogen-containing air pollutants is dependent upon their ability to transform these compounds, so that they can be utilized in their own metabolism. The oxidation of sulfur to sulfate occurs either enzymatically or in a radical chain reaction. The increases in nitrite- and nitrate-reductase activities after NO_2 impact also indicate a change in metabolism, as in the increase of sulfur-containing glutathione after SO_2 impact (Wellburn, 1982; Grill *et al.,* 1982).

The synergistic effects observed with many pollutant combinations are more understandable when considering that the detoxification mechanism for one of the pollutants may be blocked in its function by the other one.

The demand on scientists from the applied areas of forestry to contribute more to the solution of real problems concerning forests cannot be quickly fulfilled by tree physiologists because of the difficulties in experimentation, as demonstrated at the beginning of this paper. A step in the right direction has been the realization that 'forest decline' is not a monocausal problem. Each new bit of information acquired in tree physiology is of scientific importance, when we keep in mind that it represents only a small part of a complex phenomenon.

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