Ascorbic Acid as Indicator of Damage to Forest. A Correlation with Air Quality

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EPR investigations of spruce needles under *in vivo* conditions are capable of the detection of several radicals attributed to the plant photosystem. Furthermore, at room temperature an absorption due to ascorbic acid radical and other paramagnetic oxidation products can be detected additionally in the dark-adapted needles as well as under illumination. A systematic investigation of the ascorbic acid signal intensity in spruce needles grown in the vicinity of air quality control units indicates two different oxidative mechanisms, depending on the state of illumination. Ozone plays a key role but is not the hazardous agent monitored by the ascorbic radical immediately. However, its signal reflects the present state of the antioxidative system in the needles and provides an early and simple diagnosis of the oxidative interactions of air pollutants with the photosynthetic apparatus of big trees.

The first EPR signal of photosynthetic material was observed by Commoner [1] in 1954. Since, this spectroscopy and the multiresonance methods ENDOR and TRIPLE were developed to the most powerful tools for investigation of the primary processes of photosynthesis. The main compounds of the electron transport chain were characterized, and the knowledge of the complex mechanism could be enhanced remarkably [2–4] by studying chloroplasts and chloroplast fragments. The same methods can be applied to whole needles of conifers or to leaf fragments of 2 cm^2 size [5]. Basically, by investigation of these entire photosynthetic organs all known paramagnetic compounds involved in photosynthesis can be detected under appropriate conditions. Furthermore, at room temperature a 6-line-signal which can be assigned to Mn^{2+}, a doublet absorption due to ascorbic acid radical in aqueous solution and a signal with $g \approx 2.02$ can be detected, additionally [6]. The intensities of these absorptions show significant variations depending on the time at which the material was collected. The Mn^{2+} signal observed under the conditions used has no direct connection to manganese bound in the oxygen evolving complex [7]. The meaning of this absorption will be discussed elsewhere.

As the interaction of chemicals with the electron transport chain is known for many years [8], we presume a correlation of these signals with anthropogenic air pollutants. For this reason we started at first a systematic investigation of the ascorbic acid radical concentration in spruce needles grown in the vicinity of air quality control units.

Materials and Methods

Small Norway spruce branches (*Picea abies* L.) were collected from several trees in Vienna and the Rottenburg/Tübingen area over a period of one year. This material was transmitted carefully, as fast as possible, to the laboratory in Tübingen and submitted to EPR investigations at room temperature as described elsewhere [5]. The assignments of the different signals observed to individual radicals were done with the help of the *g*-factors. Their

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limit of error is estimated to be less than 2×10^{-4}. For evaluation of the intensities of the ascorbic acid radicals, the background signals caused by PS I or PS II, respectively, were suppressed by double Fourier transformation. From those spectra, the average of the amplitudes of both HFS compounds was deduced and corrected for differences of the individual samples using the intensities of the photosignals. The values obtained in this way are accurate to about 10%. All trees investigated are growing close to air quality control units measuring the concentrations of O_3, NO_2 and SO_2, continuously. These data were provided by the community of Vienna and the Umweltbundesamt Berlin.

Results and Discussion

Photosignal II of spruce needles normally reveals a pattern shown in Fig. 1, upper trace. This signal is well known from investigations of chloroplasts or their fragments. Significant contributions of the ascorbic acid hyperfine structure to this absorption were firstly detected in the spectra of trees grown in the area Jägerwiese (north of downtown Vienna) shown in Fig. 1, lower trace, whereas the superposition with Photosignal I was already described [5, 6].

Comparison of the two spectra clearly shows the sharp additional signals in the center part of the lower spectrum which is not present in the signal representing the normal Photosignal II_{slow} in spruce needles. Furthermore, a new absorption at 3443 G, corresponding to $g = 2.02$, is observed. This signal may be tentatively attributed to a sulfur centered radical which may arise under oxidative conditions, likewise.

A systematic investigation of the ascorbic acid radical intensity versus the time for a tree located in the Lobau (near Vienna) in proximity of an oil refinery results in the dependence given in Fig. 2. At this location, the concentration of the trace gases O_3, NO_2 and SO_2 is low all over the year, and the intensity of the ascorbic acid radical in the dark-adapted needles (Fig. 2, upper trace) is moderate and can be considered more or less as the background noise of our environment. Subsequent illumination of the same material generally leads to significant increase of the radical concentration indicating the production of an oxidant as described elsewhere [6]. In order to separate the signal intensity produced by illumination of the photosynthetic organs from those present in the dark, we evaluated the difference spectra light-dark (s. Fig. 2, lower trace).

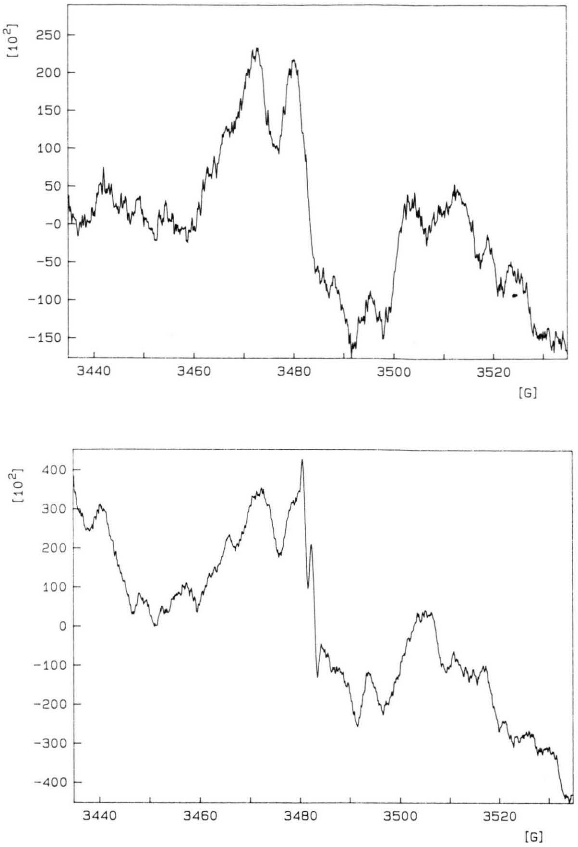

Fig. 1. EPR signal of dark adapted spruce needles average of 50 scans; range 100 G. Upper trace: reference tree Rottenburg/Tübingen; lower trace: tree 5, Jägerwiese-Wien (Vienna).

By comparison of both spectra, Fig. 2 clearly shows remarkable differences of the radical concentrations. Furthermore, large variations are observed for the light induced ascorbic acid radical intensity which does not correlate with the concentrations of trace gases registered. Obviously, other pollutants are responsible for the impact indicated, probably hydrocarbons emitted by the refinery.

At the location Jägerwiese (north of downtown Vienna), a quite different picture is observed. The concentration of the trace gas ozone shows a significant enhancement in summer whereas SO_2 and NO_2 have a minimum at this time.

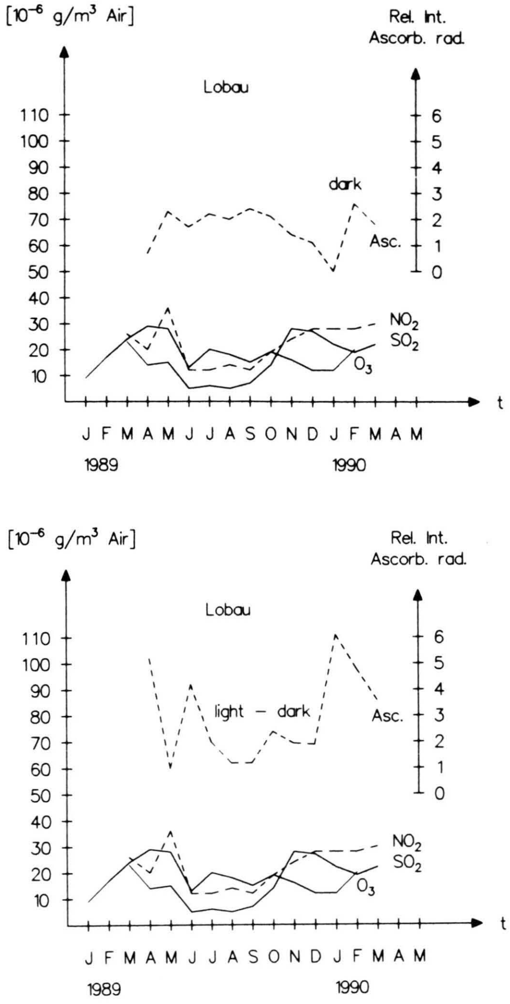

Fig. 2. Concentrations of trace gases and the intensity of the ascorbic acid radical of a tree grown in Lobau-Wien over a period of 12 months. Upper trace: dark, lower trace: light-dark.

The ascorbic acid radical intensity in the dark is much larger compared with those of the tree grown at Lobau (near Vienna), and follows in good approximation the ozone curve (s. Fig. 3). The light-dark signals are comparable in intensity and, therefore, the generation of additional ascorbic acid radicals in comparable concentrations by light is indicated. This means the antioxidative system is activated as well by pollutants already present in the dark as from those generated by illumination. Furthermore, the light-dark curve shows an interesting feature which was observed with other trees, too. This is the sharp decrease of the ascorbic acid radical intensity in August, though the ozone concentration increases slightly. A reasonable interpretation of this behaviour may be an overload of the antioxidative system or at least a decrease of the ascorbic acid concentration in consequence of a continuous oxidative stress. Such a situation will be very harmful for the plant. Consistent with this interpretation are the EPR signals of spruce needles (not shown) investigated at the end of the growing period. In those spectra, the

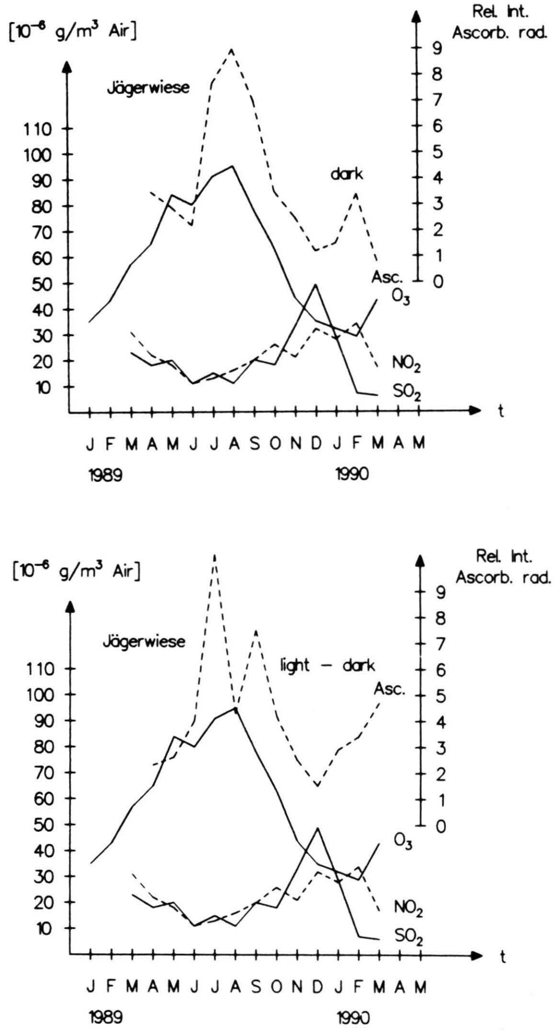

Fig. 3. Concentration of trace gases and the intensity of the ascorbic acid radical of a tree grown at Jägerwiese-Wien over a period of 12 months. Upper trace: dark; lower trace: light-dark.

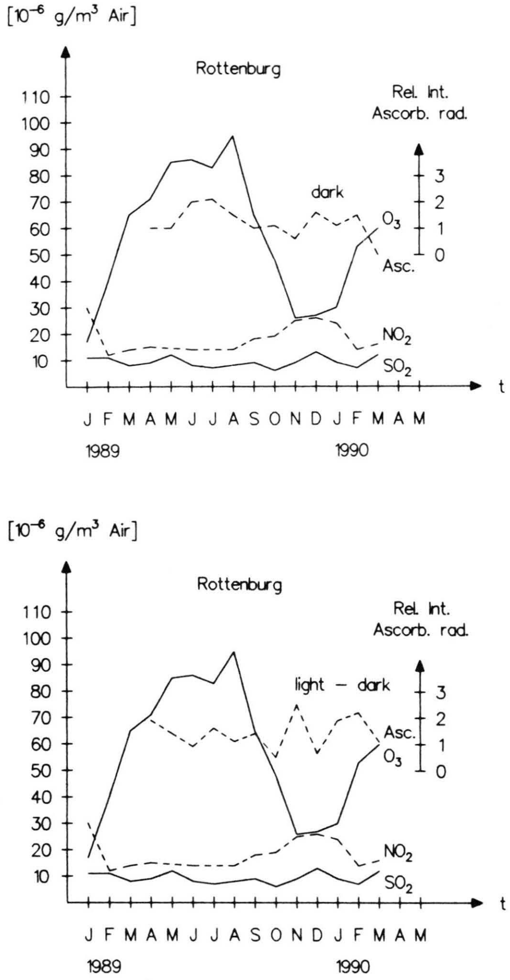

Fig. 4. Concentration of trace gases and the intensity of the ascorbic acid radical of a tree grown in Rottenburg/Tübingen over a period of 12 months. Upper trace: dark, lower trace: light-dark.

usual pattern of PS II can hardly be detected whereas the background signals, probably due to oxidation products, govern the signals observed.

By comparison of the results obtained at the location Jägerwiese with the data obtained in Rottenburg/Tübingen, it is shown that the concentration dependences of the trace gases O_3, SO_2 and NO_2 are almost comparable (s. Fig. 4).

However, the signal of the ascorbic acid radical is much smaller as well in the dark as under illumination. This means, ozone is not the hazardous agent which is monitored by the change of the concentration of the ascorbic acid radical observed. Obviously, further pollutants or synergistic effects are necessary in order to produce harmful substances and result in the phenomena observed. Probably, hydrocarbons and chlorinated species play an important role in this connection as indicated by the investigations in Lobau. But this assumption has to be confirmed by further investigations and additional informations of the air quality in the regions investigated.

Despite of the lacks of knowledge concerning the synergistic compounds or effects the results obtained clearly show that ozone plays a key role and can be used as a monitor of noxious situations for green plants. The variation of ascorbic acid radical concentration reflects the actual state of one part of the oxidative defense system and provides an early and easy diagnosis of interactions of air pollutants with the photosynthetic apparatus of big trees. Thus, EPR spectroscopy is not merely a powerful method for investigations of the primary processes of photosynthesis but, furthermore, it has the capability of detection of the impact of air pollutants on the photosynthetic organs in an early stage.

Acknowledgements

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