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Apoplast: a sensitive site for assessing some biochemical effects of O_3 or SO_2 in Norway spruce needles

G. Ogier, F.J. Castillo and H. Greppin

Laboratory of Plant Biochemistry and Physiology, University of Geneva, CH-1211, Geneva 4, Switzerland

Introduction

The study of the cell wall–plasma membrane interphase is of great importance for the understanding of gaseous air pollutants and leaf cell interactions. In the apoplast liquid phase, the pollutants are solubilized and they can generate oxidative products (Tingey and Taylor, 1982). For example, O_3 or SO_2 could lead to H_2O_2 production (Tingey and Taylor, 1982; Khan and Malhotra, 1982). In order to protect the plasma membrane and the components of the extracellular matrix, cells are believed to dispose of oxidant-scavenging mechanisms. One of the enzymatic systems which could play a protective role against oxidative stresses includes peroxidases (Castillo and Greppin, 1988).

Peroxidase activity, with guaiacol as the electron donor, and protein content were measured in Norway spruce needles (*Picea abies* (L.) Karst) after fumigation (24 h/d) in semi-open top chambers, for 12 wk in summer with O_3 or for 10 wk in winter with SO_2. These parameters were followed in the intercellular washing fluid (IWF) and in the residual cell material (RCM). The plants treated in summer remained 12 wk longer in the chambers in order to assess any visible injury caused by O_3 in autumn.

Materials and Methods

Two groups of 20 clone saplings (4 yr old grafted *P. abies*) were selected from the nursery of the Swiss Federal Institute of Forestry Research (Birmensdorf, CH), one group for each experiment. Prior to fumigation, the plants were distributed randomly into 4 semi-open top chambers (5 individuals per chamber). Current year old needles and 1 yr old needles were analyzed in samples harvested at the end of the fumigation period. The experimental approach is shown in Table I.

The IWF was obtained after infiltration of phosphate buffer (40 mM, pH 4.5), 0.1 M KCl, 3 μM EDTA, and centrifugation (10 000 x *g*, 4°C, 10 min) according to Castillo *et al.* (1987). The RCM extract was obtained from 0.5 g of the remaining needles, which were ground under liquid nitrogen, in the presence of PVP (0.5 g), then solubilized with 3 ml of phosphate buffer (66 mM, pH 7), and centrifuged (10 000 x *g*, 4°C, 10 min).

Table I. Experimental approach for O_3 and SO_2 fumigations.

Treatments	*Experiments*	
	O_3 (summer)	*SO_2 (winter)*
I	filtered air (fa) [a]	filtered air (fa) [a]
II	fa + 100 μg O_3/m^3	fa + 70 μg SO_2/m^3
III	fa + 200 μg O_3/m^3	fa + 200 μg SO_2/m^3
IV	ambient air [b]	ambient air [b]

Picea abies cloned saplings were continuously fumigated (24 h/d) for 12 wk (O_3 experiment) or 10 wk (SO_2 experiment). Samples were harvested at the end of the fumigation period.

[a] O_3 or SO_2 were not detected in filtered air.

[b] Monthly means in ambient air were for O_3: July (67.6 μg O_3/m^3), August (55.8 μg O_3/m^3), September (19.5 μg O_3/m^3); for SO_2: January (60 μg SO_2/m^3), February (23.2 μg SO_2/m^3), March (19 μg SO_2/m^3).

Peroxidase activity was assayed by measuring the oxidation of guaiacol at 470 nm. This activity was carried out using phosphate buffer (66 mM, pH 6.1), 16 mM guaiacol, 3.3 mM H_2O_2 and 0–10 μl of enzyme extract. Protein contents were determined according to Bradford (1976) using a Bio-Rad protein assay (0–20 μl of enzyme extract).

Statistics. The only environmental factor differing between groups was the air composition within the chambers. Considering this factor, plants fumigated with either filtered air (fa) or fa plus added pollutants were under controlled conditions (a); whereas in ambient air chambers, the fumigation conditions were uncontrolled (b). In both experiments, the data of the (a) plant groups were tested by analysis of variance. Means which were significantly different were identified using a *t*-test. The data of the groups which were statistically equivalent were pooled. Then, those of the (b) plant groups were compared to the pooled or unpooled ones of the (a) plant groups using a *t*-test. For these analyses, we chose $P < 0.05$ as significant.

Results

Guaiacol peroxidase activity, (Fig. 1), decreased in the IWF of current and 1 yr old needles of plants treated with 200 μg O_3/m^3 (22 and 24% of the control values, respectively). This enzyme activity was not affected in the IWF by SO_2 treatment. The only noticeable change in the RCM was an increase in 1 yr old needles of the summer ambient air-treated plants (124% of the pooled values of the plants exposed to controlled conditions).

The protein content (Fig. 2) in the IWF of young needles was 1.3–2.3 times greater after low and high ozone exposure, and 1.6–1.7 times greater after low and high SO_2 concentrations, respectively, as compared to the control values. On the other hand, the protein content of the RCM was only affected by the high ozone exposure and was lower. In the 1 yr old needles, the only change observed was an increase of the protein content in the IWF after high ozone exposure.

No visible damage could be noted when the plants were sampled either in September (O_3) or in March (SO_2). However, in November, the needles of the plants treated with 200 μg O_3/m^3 began to show a 'dirty grey' aspect and to fall. By the end of the experiment (late December) most of the current year and some of the 1 yr old needles were dead. This phenomenon was only observed in plants exposed to 200 μg O_3/m^3.

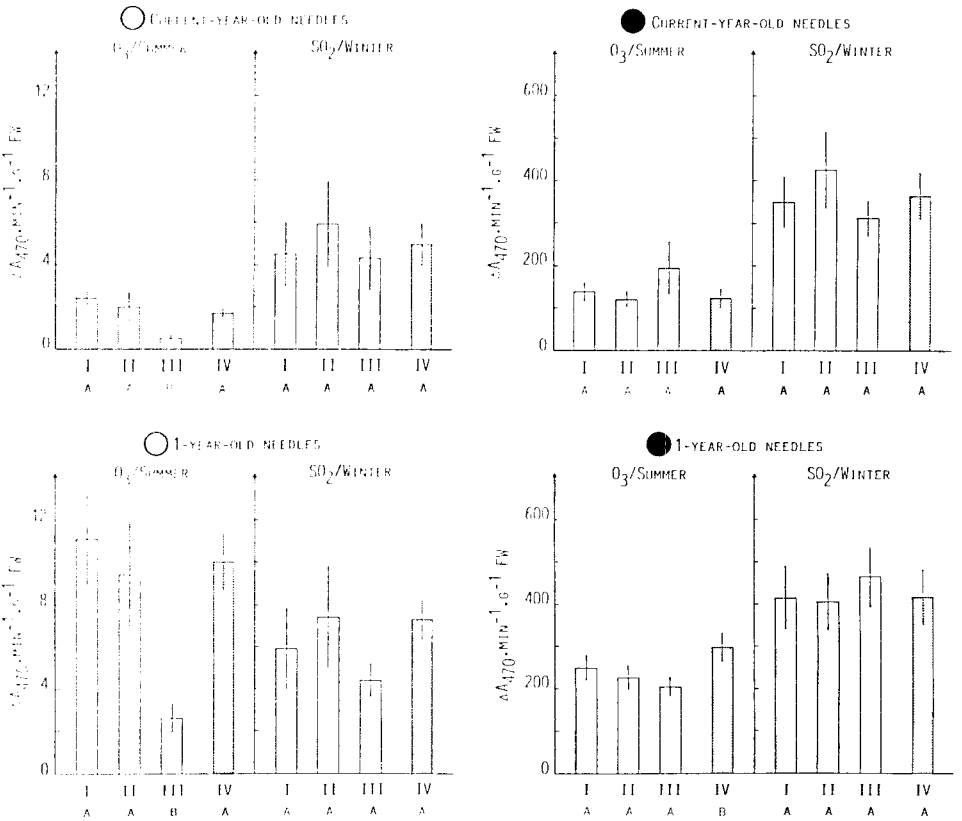

Fig. 1. Peroxidase activity ($\Delta A_{470} \cdot min^{-1} \cdot g^{-1}$ fresh weight) in the intercellular washing fluid (O) and in the residual cell material (●) from Norway spruce needles after long-term O_3 or SO_2 exposure. The data represent the means of 5 measurements ± SD. Statistical analyses were performed using $P < 0.05$ as the significance threshold.

Discussion and Conclusion

This type of experiment does not allow us to know the actual leaf pollutant uptake, which is controlled in part by the thickness of the boundary layer, the opening of the stomata and the transpiration rate of the cells. However, according to the marked differences of responses between young and old needles, one can assume that young needles take up more pollutants than old ones (Tingey and Taylor, 1982).

The long lasting period of high O_3 concentration (200 μg O_3/m^3, 12 wk in summer + 12 wk in autumn) together with subzero temperatures during autumn could be responsible for the drop of the needles (Brown *et al.*, 1987; Barnes and Davison, 1988). These authors have reported that 1 yr old needles from 3 out of 10 and 3 out of 8 clones were sensitive to frost injuries due to long-term O_3 fumigations (≥ 200 μg O_3/m^3). Despite the fact that in our case both current and 1 yr old needles were injured, their fall after

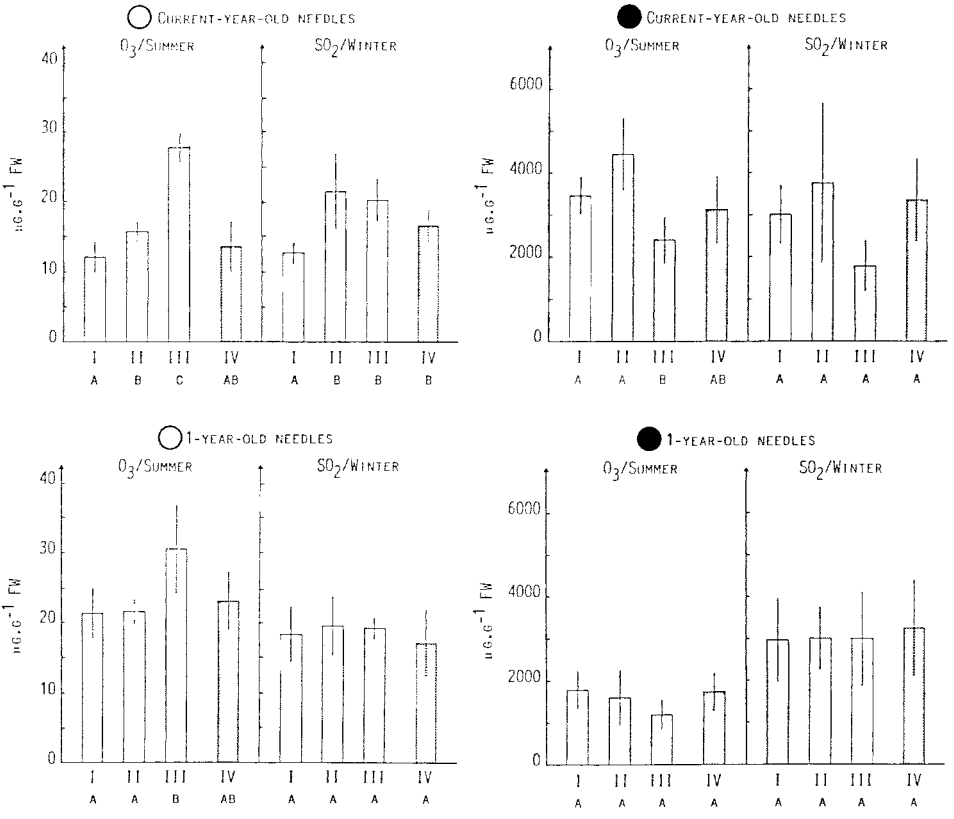

Fig. 2. Protein content ($\mu g \cdot g^{-1}$ fresh weight) (see Fig. 1 for explanations).

exposure to a high O_3 concentration indicates the sensitivity of our clone to this pollutant. Moreover, this sensitivity is probably revealed by frost events in late autumn.

The enhancement of the protein contents in the IWF promoted by both pollutants in current-year needles and by O_3 alone in 1 yr old needles, could be attributed to the alteration of protein secretion. Whether this change is a consequence of an increased secretion or leakage of stored or newly synthesized proteins is currently under investigation.

The decreased peroxidase activity in the IWF after high O_3 exposure could result from either altered enzyme secretion or direct enzyme denaturation by O_3 or its by-products. In an earlier study (Castillo *et al.*, 1987), an increase of extracellular peroxidase activity in needles of *Picea abies* saplings fumigated with 300 μg O_3/m^3, 7 h/d for 4 wk was observed. The apparent contradictory response of extracellular peroxidase between both experiments is probably due to different experimental conditions. In the previous paper (Castillo *et al.*, 1987), the experiment was carried out with a heterogeneous popula-

tion of saplings and the total dose for that short-term O_3 fumigation was 30 ppm/h. In this report, the data were obtained from grafted saplings originating from the same clone and the total dose for this long-term O_3 fumigation was 200 ppm/h. Apparently, extracellular peroxidase responds in a different way depending upon the level and length of pollutant exposure and/or on the genetic characteristics of the plant material.

In the case of high O_3 exposure, the decreased extracellular enzyme activity and the increased protein content in the IWF of young needles could be explained by the high O_3 concentration applied (200 μg O_3/m^3, 24 h/d, for 12 wk), which is probably above the threshold value that the plant can tolerate without disruption of homeostasis.

Based on these observations, it appears that the apoplast of Norway spruce needles is a sensitive site for the detection of stresses induced by gaseous pollutants.

Acknowledgments

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