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Influence of SO_2 and NO_2 Exposure on Antioxidants and Photosynthesis in Needles of *Pinus sylvestris*

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S u m m a r y

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Scots pine (*Pinus sylvestris* L.) trees were exposed from June to October in an open air exposure system to SO_2 and NO_2 for four years in northern Sweden. Low concentrations of SO_2 and NO_2 did not change the levels of Vitamin E or pigments in the needles. The reduced glutathione level in the exposed needles was not significantly different from the controls.

Although, there was a slight increase in mRNA for superoxide dismutase, enzyme activity did not show any concomitant increase. The observed increase in CuZn SOD mRNA, both in chloroplasts and in cytosol, was not due to a general increase in mRNA of the cells.

There were several after-effects even of low concentrations of air SO_2 and NO_2 under natural conditions. This was evident from a lower light- and CO_2-saturated rate of oxygen evolution, a lower photochemical efficiency of photosystem II and a reduced activity of glutathione reductase in needles of exposed trees. The results suggest that the winter might be a critical period for trees exposed to SO_2 and NO_2.

I n t r o d u c t i o n

It is generally presumed that a combination of environmental stress factors and air pollutants such as SO_2 and NO_2 are the cause of reduced growth and die-back of trees in large areas of Europe. The causes and mechanisms of injury are not known. It has been suggested that cellular injury may be caused by free radicals

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which are induced upon exposure to air pollutants (ASADA & TAKAHASI 1987).

Part of the sulfur dioxide (SO_2) taken up by the plants is metabolized. It can be directly reduced to hydrogen sulfide (HÄLLGREN & FREDRIKSSON 1982) and further assimilated (RENNENBERG & LAMOUREUX 1990). However, toxic effects of sulfite can cause changes in activity of enzymes, disturb and inhibit photosynthetic electron transport, and damage membranes and biomolecules by radical reactions (DITTRICH & al. 1992). Sulfite can be oxidized to sulfate and proceed via a radical chain reaction (ASADA & KISO 1973).

Nitrogen dioxide (NO_2) may also be metabolized by the plant (WELLBURN 1988). However, it can also be regarded as a radical itself, and may therefore be involved in free radical reactions in the cells (ELSTNER 1987). The presence of air pollutants can modify the responses of plants to other environmental stress factors (DAVISON & al. 1988, MANSFIELD & al. 1988).

In the present paper the effects of a long-term SO_2 and NO_2 exposure on the antioxidant status (glutathione, Vitamin E, pigments etc.) in needles of Scots pine (*Pinus sylvestris* L.) trees was studied. Superoxide dismutase (SOD) and glutathione reductase (GR), involved in superoxide scavenging and the detoxification of H_2O_2 were also monitored. In addition, measurements of O_2 evolution and chlorophyll a fluorescence of needles from exposed and control trees were made.

Abbreviations: GR, glutathione reductase. GSH, glutathione. SOD, superoxide dismutase.

Materials and Methods

Scots pine trees (*Pinus sylvestris* L.) were exposed to SO_2 and NO_2 in an open-field fumigation system in northern Sweden for four years. A schematic drawing of the system is shown in Figure 1.

The gases were released from vent-pipes during the days from mid June until beginning of October. The mean concentration of SO_2 and NO_2 were low (10-15 ppb each, low-level exposed area) in the middle of the 60 m circle and higher (high-level exposed area) towards the periphery (Fig. 1). The concentration of the pollutants in the high-level exposed area was approximately 5 times that in the low-level area, with peaks up to 200 ppb. Control samples were taken in the same stand ca 50 m from the exposure system. This area showed a background concentration of less than 1 ppb for both SO_2 and NO_2.

The Scots pine trees were around 50 years old and with a mean height of ca 12 m in 1991. The site is a typical podzol, hence the trees were nitrogen limited but had adequate supply of water. The background ozone concentration in this part of northern Sweden is ca 25 ppb during the vegetation period. Climatic conditions and concentration of gases were recorded continuously measured. A more detailed description is given by WINGSLE 1991.

Samples (shoots or needles) were collected during all seasons, killed in liquid nitrogen and stored in liquid nitrogen or -80° C in the laboratory.

Antioxidants and pigments were measured with high-performance liquid chromatography techniques described earlier (WINGSLE & al. 1989, WINGSLE 1991, WINGSLE & al. 1992). GR (E. C. 1.6.4.2) and SOD (E. C. 1.15.1.1) were isolated and partially purified according to WINGSLE 1989, and WINGSLE & al. 1991. The activity of GR and SOD were measured spectrophotometrically and the isoforms of SOD were also determined by active staining on native-polyacrylamide gel electrophoresis.

SOD mRNA was isolated from needles of different year-classes by the technique described by KARPINSKI & al. (1992a,b). Standard molecular biology techniques were used (e.g. Northern hybridization).

Measurements of O_2 exchange of needles were made with a leaf-disc oxygen electrode (Hansatech, U.K.) as described by DELIEU & WALKER 1983.

Results and Discussion

The S/N ratio was significantly higher in one- to three-year-old exposed needles but other minerals did not show any major significant differences between exposed and control samples (data not shown). Low concentrations of SO_2 and NO_2 did not affect the levels of Vitamin E, chlorophyll or carotenoids in the needles (data not shown). The GSH level in the exposed needles was not significantly different from the controls (Table 1). The sulfur content in current-year needles ranged from 7 to 10 mg g dry weight^{-1} in both the exposed and the control plot, indicating that the "threshold" for GSH accumulation had not been reached.

In spite of a slight increase in mRNA for SOD (Fig. 2), enzyme activity did not show any concomitant increase, rather a decreased activity was noticed on other occasions (data not shown). The reason for this is not known but may be due to inhibition of CuZn SOD by H_2O_2. Several mechanisms of regulation may exist. The observed increase in CuZn SOD mRNA, both in chloroplasts and in cytosol, was not due to a general increase in mRNA of the cells. This result may suggest that an increased *de novo* synthesis of SOD is needed to maintain its activity in needles of exposed trees.

Metabolic alterations in needles emerged of exposed trees after the fumigation had stopped. These after-effects were evident from measurements of GR activity and photosynthesis. There was a decrease in GR activity at the beginning of shoot growth in June, and an increase during autumn and winter (Fig. 3), which was to be expected from other reports (e.g. SMITH & al. 1990). GR activity was significantly lower in needles of exposed trees than in control trees during autumn and winter ($p < 0.05$). This suggests that the natural increase in the GR activity during this period was lower due to the exposure of SO_2 and NO_2.

After storage at -19° C of samples collected in January, the light- and CO_2-saturated rate of O_2 evolution in needles of trees exposed to SO_2 and NO_2 was lower than in needles of trees exposed to natural clean air (Fig. 4). Furthermore, the photochemical efficiency of photosystem II as indicated by the ratio of variable to maximum fluorescence (FV/FM) was lower for needles of exposed trees (data not shown). Photosynthetic capacity after storage at -19° C was not significantly different from that obtained before storage (data not shown). Hence, there was no evidence for a greater sensitivity to prolonged freezing stress in needles of exposed trees.

The general hypothesis is that GR and SOD protects the cells from damages by radicals. This experiment showed that these enzymes may be affected by relatively low concentrations of SO_2 and NO_2 under natural conditions in the

field. However, it is tentatively suggested that the enzyme capacity in the cells might be high enough since no visible injuries was detected. The observed decreases in GR activity and photosynthetic capacity may be triggered by environmental conditions during autumn and winter.

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Table 1. Reduced glutathione content in one year old *Pinus sylvestris* needles exposed to low- and high-levels of SO_2 and NO_2. The values from 12 August 1988 to 11 September 1989 represent the mean of 3 (1988) and 6 (1989) measurements (± SE). The values from 13 October represent pooled samples from five trees and the high-level value represent the mean of 7 measurements from different sections (± SE). - not determined. The values are given as µmol g dry weight^{-1}.

Date	Control	Low-level	High-level
12 August, 1988	0.62 ± 0.01	0.64 ± 0.03	-
26 September, 1988	0.94 ± 0.05	0.96 ± 0.14	-
18 January, 1989	2.39 ± 0.11	2.16 ± 0.09	-
11 September, 1989	0.68 ± 0.07	0.68 ± 0.16	-
13 October, 1989	0.68	0.73	1.10 ± 0.19

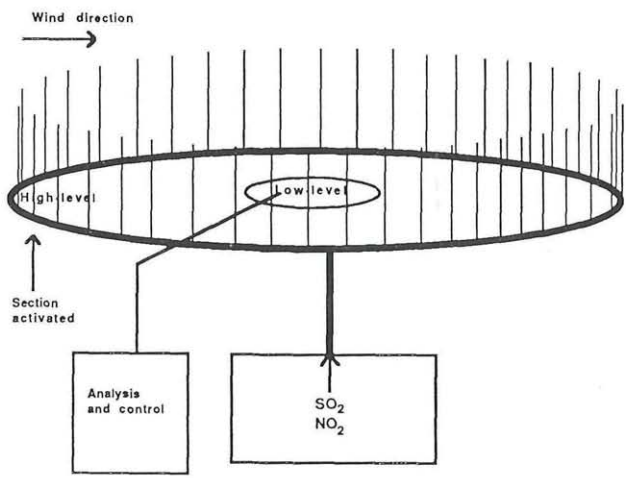

Fig. 1. A schematic drawing of the open-air exposure system located at Svartberget, Vindeln.

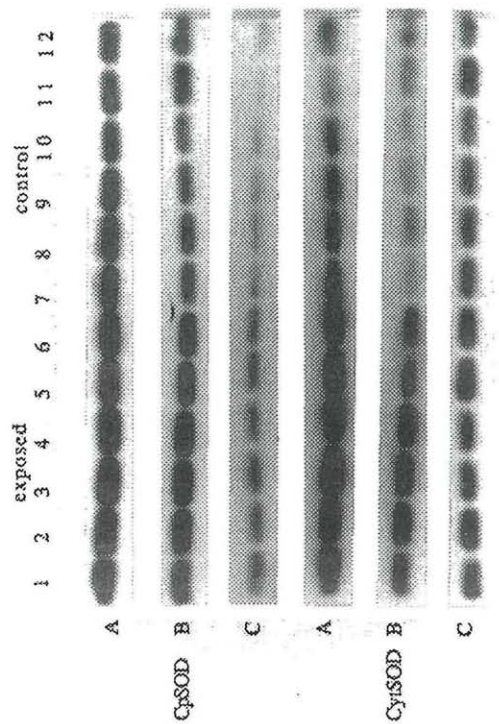

Fig. 2. Northern blot hybridization to ^{32}P labelled chloroplastic CuZn-SOD cDNA probe (CpSOD) and cytosolic CuZn-SOD cDNA probe (cysSOD). Lines 1 to 6 represent RNA isolated from needles collected from six different SO_2 and NO_2 low-level exposed trees, lines 7 to 12 represent RNA isolated from needles collected from six different control trees. A, B and C represent current, one- and two-year old needles.

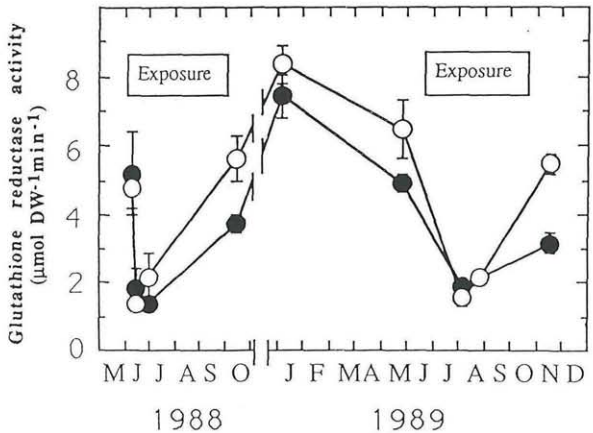

Fig. 3. Glutathione reductase activity in one-year-old Scots pine needles from a low-level area exposed to SO_2 and NO_2. Control (o) and exposed (O). Each value represents mean of 6 measurements (± SE)

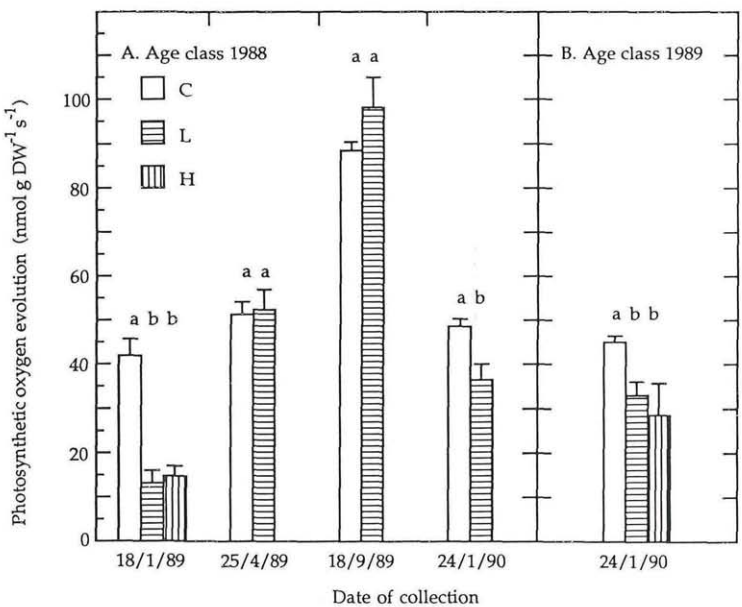

Fig. 4. Rate of oxygen evolution near light saturation (1030-1090 µmol photons m^{-2} s^{-1}) of Scots pine needles (age class 1988, A, age class 1989, B) collected at different dates from control trees (C), and trees exposed to low-level (L) and high-level (H) concentrations of SO_2 and NO_2. Samples collected in January 1989 and January 1990 were stored at -19 °C for 1 to 2 weeks and 3 to 4 weeks, respectively. Measurements were made at a CO_2 concentration of 5% and temperature of 20 °C. The data represent the mean of 3 to 5 measurements (± SE). Bars which are designated by the same letter are not different at the 0.05 level of significance.