SILVA FENNICA 1983, vol. 17 n:o 3: 279–287

EFFECTS OF AIR POLLUTION ON SCOTS PINE NEEDLES

I Effects on total peroxidase activity

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Seloste

ILMAN SAASTEIDEN VAIKUTUS MÄNNYN NEULASIIN

I vaikutukset kokonaisperoksidaasiaktiivisuuteen

Saapunut toimitukselle 10.10.1983

The effect of meteorological factors, the total sulfur content of the needles, and SO_2 concentrations in the ambient air on total peroxidase activity of Scots pine needles was investigated in material obtained from southern Finland. The correlation between temperature and total peroxidase activity was highest during the most active growing period. Linear correlation between relative humidity and total peroxidase activity was low and varied inconsistently. The relationship between the total sulfur content of the needles and the total peroxidase activity appears to be low. The correlation between atmospheric SO_2 concentrations and total peroxidase activity was also low and varied inconsistently. The detected low association between the sulfur dioxide pollutant and the total peroxidase activity was assumed to be related to the sensitivity of peroxidase activity to many ecophysiological factors and to the genetic variation in conifers. It is difficult to separate a response due to this pollutant from environmental and genetic factors in a complex coniferous forest. Using total peroxidase activity as a routine indicator of air pollution seems to be unsuitable because of the large sample size required in order to obtain a reliable measurement of the pollutant's effect under low pollution levels.

1. INTRODUCTION

Sulphur dioxide is one of the major gaseous air pollutants capable of causing visible injury and invisible physiological changes in conifers (Kozlowski 1980, Heath 1980). Malhotra and Khan (1976), for example, have shown that SO_2 affects the structure and permeability of cellular membranes, thus interfering with normal cellular electron transport systems (see also Malhotra 1976). Malhotra (1976) has also found that gaseous SO_2 causes chlorophyll breakdown in pine needles. Further studies have revealed that SO_2 affects the composition of needle glycolipids by inhibiting lipid biosynthesis (Malhotra and Khan 1978). Glycolipids are involved in the structural integrity of the chlorophyll membranes, and Malhotra and Khan (1978) have suggested that the ultrastructural disorganization of pine needle chloroplasts is brought about by SO_2 interfering with glycolipidsynthesis.

Biochemical studies on pollution effects have revealed that pollutants affect several enzyme activities in plants. For example, it has been recently detected that SO_2 can cause changes in acid phosphatase activity (Malhotra and Khan 1980). It has been observed that in both young and old needles exposure at 1 000 $\mu g/m^3$ for 24 hours causes marked inhibition in the activity of this entzyme (Malhotra and Khan 1980). The enzyme peroxidase has also been studied as an indicator of air pollution and Keller and Schwager (1972) have carried out experiments on the suitability of using total peroxidase activity for this purpose.

Peroxidase is in fact the most often used plant enzyme in air pollution studies. The idea behind this method is the postulation that changes in total peroxidase activity describe pollution stress (Keller 1974). Since the enzyme has been found to react sensitively to some pollutants, it has been assumed that measurements of the changes in total peroxidase activity will detect any hidden injuries caused by low pollutant concentrations (Keller 1974). Hence, several studies have been made using the peroxidase method, and some wide practical applications for mapping and monitoring purposes have been carried out.

Most of the research carried out on peroxidase enzymes has been based on the use of self-pollinating plants. Far less is known about the biology of peroxidase enzymes on cross-pollinating plants. However, in the last decade forest geneticists have largely studied the genetic structure of conifer populations, and these studies show that enzyme polymorphism exists in Scots pine (Chung 1981). Rudin (1977) has found genetic variation of isoperoxidase patterns within and between stands of Scots pine. Moreover, it is well known that there are complex ecological and meteorological conditions which cause changes temporally and spatially in heterogenous conifer ecosystems (Kozlowski 1980). In such a complex environment the deposition and turbulence of air pollutants, such as SO_2, into a forest and a canopy are dependent on several meteorological and ecophysiological factors and these are only partly understood (Petit et al. 1980, Bache 1977, Garland and Branson 1977, O'Dell et al. 1977).

Therefore, some fundamental questions arise concerning the interpretation of the factors that cause changes in the total peroxidase activity of Scots pine needles.

First, how can the enzyme activity changes caused by pollutants be reliably separated from genetic and environmental factors on cros-spollinating conifers known to be polymorphous?

Second, how can air pollution effects be separated from a number of interacting ecological factors causing changes on enzyme activities in a complex coniferous forest?

Third, how should experimental material be collected from heterogenous conifer populations in order to meet the statistical requirements of sampling theory?

In the present paper attempts are made to explain the variation of total peroxidase activity on the basis of recent information obtained from the meteorological aspects of SO_2 deposition and turbulence in the forest. The use of total peroxidase activity as a routine test for monitoring air pollution is discussed.

The research was supported by the Finnish Academy of Sciences. We are grateful to Professor S. Kellomäki, Dr P. Pelkonen, H-S. Katainen, M.Sc. and H. Lättilä, M.Sc. for critical reading of the manuscript. Appreciation is expressed to S. Karjalainen, and E. Mäkinen for technical assistance with this study. The English text was kindly revised by Ms. Heather MacKenzie.

2. MATERIALS AND METHODS

The pine needle samples were collected from two study sites; Helsinki (Pitkäkoski, 60° 16'N 24° 54'E), which is affected by emissions from energy production plants and from other urban activities, and Valkeakoski (61° 16'N 24° 01'E), where the environment is affected by emissions from a sulphate pulp and paper mill and rayon manufactoring plant. The number of needle samples is presented in Table 1. In addition, the pine needle samples were collected from a clean area Kuohijoki, 61° 19'N 24° 48'E) during the period XI/78–X/79 at two months intervals.

The collection of sample needles was carried out in Helsinki for one year from 27.11.1978 to 26.11.1979. The sample series consisted of daily observations taken during the following periods 4.12.–20.12., 5.3.–16.3., 23.4.–25.5., 7.6.–15.6., and 1.10.–26.11. During the rest of the periods sampling was carried out twice a week. However, no samples were collected in July. In Valkeakoski the series consisted of sample collections taken at two months intervals during the period 31.1.1978 to 30.5.1979.

Mixed samples of the same amount of needles (0.3 g for analysis)from two trees in Helsinki, and from three trees in Valkeakoski were taken. After cutting the branches and selecting green needles of the previous year, the samples were immediately stored in silicon tubes and frozen with liquid nitrogen for transport to the laboratory. In the laboratory the samples were removed from liquid nitrogen and stored in a freezer at –40°C. Mixed samples were used for analyses in order to minimize the effects due to the local environment of one tree.

The measurement of the total peroxidase activity of pine needles was based on the method developed by Keller and Schwager (1972). The method was slightly modified at the Finnish Meteorological Institute. The temperature of the samples was kept stable during measurement by a cooled cell chamber. The results were obtained using an automatic strip chart recorder. An attempt was made to improve the accuracy of the method by using the Merck product No. 24567 as a standard (100 IU/mg measuring the activities of 0,1, 0,2, and 0,4 IU/samples).

Seasonal variation of the total peroxidase activity of the pine needles was determined from the material obtained at the Helsinki and Valkeakoski sites.

Computations of the effect of the meteorological factors on the total peroxidase activity were based on material from the Pitkäkoski area and on meteorological observations from the Helsinki–Vantaa airport.

Collections of the pine needle samples for the total sulphur measurement were performed at the same time as for the peroxidase analysis. Branches from two trees in Helsinki and from three in Valkeakoski were collected and stored in plastic bags. Mixed samples of the needles from the same growing season were utilized and dried for sulphur analysis.

The measurement of the total sulphur content of the needles was made by the x-ray-fluorescence method and carried out at Oulu University (see e.g. Turunen and Visapää 1972).

The SO_2 content of the ambient air was measured in Pitkäkoski using two pieces of parallel equipment, an automatic monitor and an absorption sampler. In Valkeakoski SO_2 measurements were performed with an automatic monitor and with two absorption samplers. The determination of absorbed SO_2 was based on the Thorin method according to the SFS-standard 3864.

The correlation between the total peroxidase activity and the total sulphur content of the pine needles was based on the Helsinki material.

The correlation between the total peroxidase activity and the SO_2 concentrations was also calculated from the Helsinki material.

Table 1. The variation of total peroxidase activity of pine needles within different months.

Taulukko 1. Männyn neulasten kokonaisperoksidaasiaktiivisuuden vaihtelu eri kuukausina.

Month *Kuukausi*	N	x̄	standard deviation s.d.	coefficient of variation c.v.
January 1979 *Tammikuu*	8	0.290	0.126	43.4
February *Helmikuu*	5	0.381	0.096	25.2
March *Maaliskuu*	15	0.273	0.086	31.5
April *Huhtikuu*	12	0.307	0.099	31.6
May *Toukokuu*	19	0.303	0.090	29.7
June *Kesäkuu*	14	0.215	0.064	29.8
July *Heinäkuu*	1	0.270	–	–
August *Elokuu*	9	0.270	0.046	21.2
September *Syyskuu*	7	0.277	0.093	33.7
October *Lokakuu*	20	0.411	0.083	20.2
November *Marraskuu*	18	0.538	0.114	21.2
December *Joulukuu*	14	0.354	0.012	30.8

3. RESULTS

3.1. Seasonal variation of total peroxidase activity and total sulfur content of pine needles

The annual pattern of total peroxidase activity is presented in Figure 1. The activity is higher during the winter period when the trees are in their dormant stage. The activity levels in unpolluted ambient air conditions are consistently lower than those in polluted areas. The total sulfur content of the needles seems to vary slightly from season to season.

The variation of total peroxidase activity within different months appears to be wide (Table 1). The coefficients of variation ranged from 20,2 to 43,4. There is no detectable consistent trend among the values between different months.

3.2. Effect of temperature and humidity on total peroxidase activity

Peroxidase activity appears to be negatively associated with air temperature (Table 2) and statistically significant correlations ($p<0.05$)were found during the most active period of growth. In winter the coefficients are small but are nonetheless negative in every case.

The correlation between relative humidity and peroxidase activity is presented in Table 3. Generally the coefficients are positive and low during the autumn period. In the winter period the coefficients were also low however the relationship varied inconsistently.

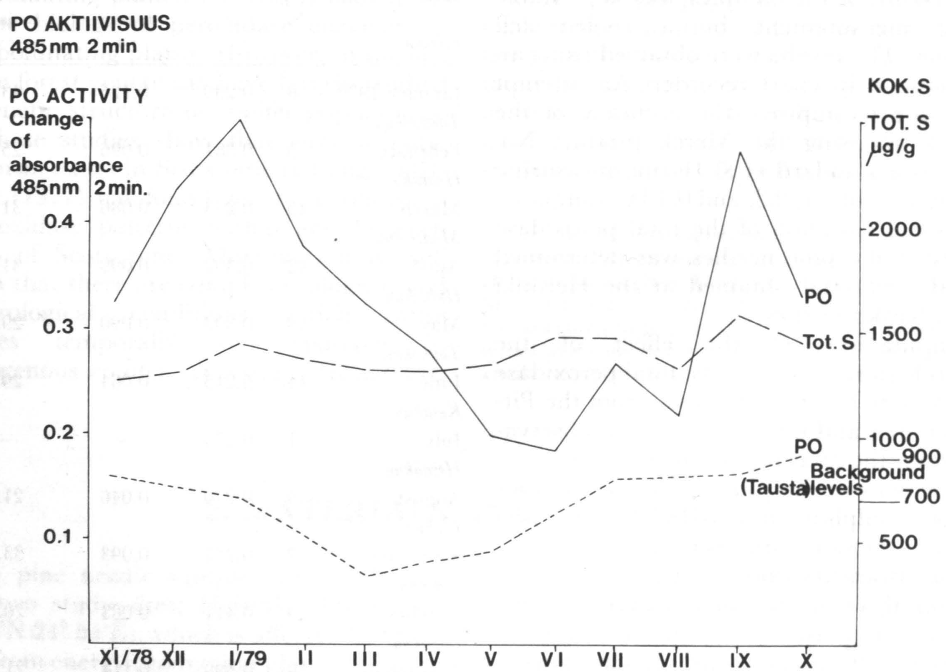

Fig. 1. Seasonal variation of peroxidase activity in a polluted (Pitkäkoski, South Finland) and in a clean area (Kuohijoki, Central Finland) and the sulfur content of pine needles. (PO = peroxidase activity, TOT.S = total sulfur content of the needles).

Kuva 1. Peroksidaasiaktiivisuuden ja neulasten rikkipitoisuuden vuodenaikaisvaihtelu saastuneella ja puhtaalla alueella. (PO = peroksidaasiaktiivisuus, TOT.S = neulasten kokonaisrikkipitoisuus).

Table 2. Correlation between total peroxidase activity and air temperature. The temperature has been measured one to eight days before peroxidase measurements.

Taulukko 2. Kokonaisperoksidaasiaktiivisuuden ja ilman lämpötilan välinen korrelaatio. Lämpötila on mitattu 1–8 vuorokautta ennen peroksidaasimittauksia.

Days *Päivät*	Period *Ajanjakso* XI/78 – III/79	Period *Ajanjakso* IV – VIII/79	Period *Ajanjakso* IX – XI/79
1.	–0.089	–0.379***	–0.277
2.	–0.165	–0.493***	–0.389**
3.	–0.165	–0.437***	–0.396***
4.	–0.186	–0.525***	–0.393***
5.	–0.121	–0.413***	–0.450***
6.	–0.290	–0.404***	–0.443***
7.	–0.089	–0.452***	–0.549***
8.	–0.281	–0.487***	–0.603***

*, **, *** Indicates significance of P 0,05, 0,01 and 0,001 respectively.
*, **, ***: *P:n merkitsevyys vastaavasti 0,05, 0,01 ja 0,001*

Table 3. Correlation between total peroxidase activity and relative air humidity. Humidity has been measured one to eight days before peroxidase measurements.

Taulukko 3. Kokonaisperoksidaasiaktiivisuuden ja ilman kosteuden välinen korrelaatio. Ilman kosteus on mitattu 1–8 vuorokautta ennen peroksidaasimittauksia.

Days *Päivät*	Period *Ajanjakso* XI/78 – III/79	Period *Ajanjakso* IV – VIII/79	Period *Ajanjakso* IX – XI/79
1.	0.046	–0.036	0.381**
2.	0.287	0.236	0.349*
3.	0.143	0.044	0.584***
4.	0.096	0.259	0.302
5.	–0.001	0.026	0.132
6.	–0.119	0.007	0.389**
7.	–0.039	0.096	0.539***
8.	0.154	0.216	0.465***

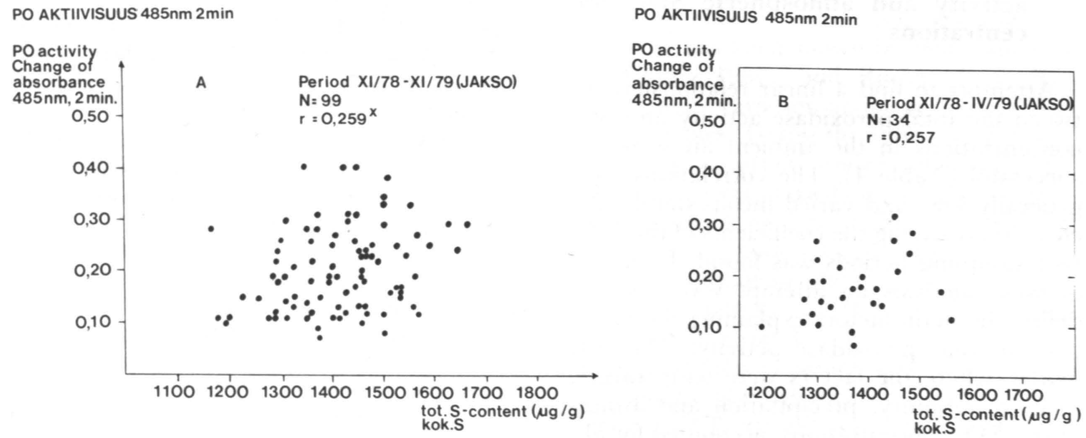

Fig. 2. The relationship between total peroxidase activity and total sulfur content of the pine needles. Samples were taken at the same time for measurements of total peroxidase activity and total sulfur content of the needles from the Helsinki area of Pitkäkoski (A = data from the whole period, B = data from the winter period).

Kuva 2. Kokonaisperoksidaasiaktiivisuuden ja männyn neulasten kokonaisrikkipitoisuuden korrelaatio. Näytteet peroksidaasiaktiivisuus- ja rikkimäärityksiä varten kerättiin samanaikaisesti Helsingin Pitkäkoskelta (A = koko näytekeräysjakson aineisto, B = talvijakson aineisto).

3.3 Correlation between total peroxidase activity and the total sulfur content of Scots pine needles

The results from the Helsinki material are presented in Figure 2. The detected correlations were low and only during the winter period did the coefficients show nearly significant correlation (Fig. 2). During the most active growth period there seems to be no relationship between the peroxidase activity and the total sulfur content of the needles. The observed correlations were also weak for the material taken from the Valkeakoski area. The coefficients varied from r=0.051 to r=0.200 (Fig. 3).

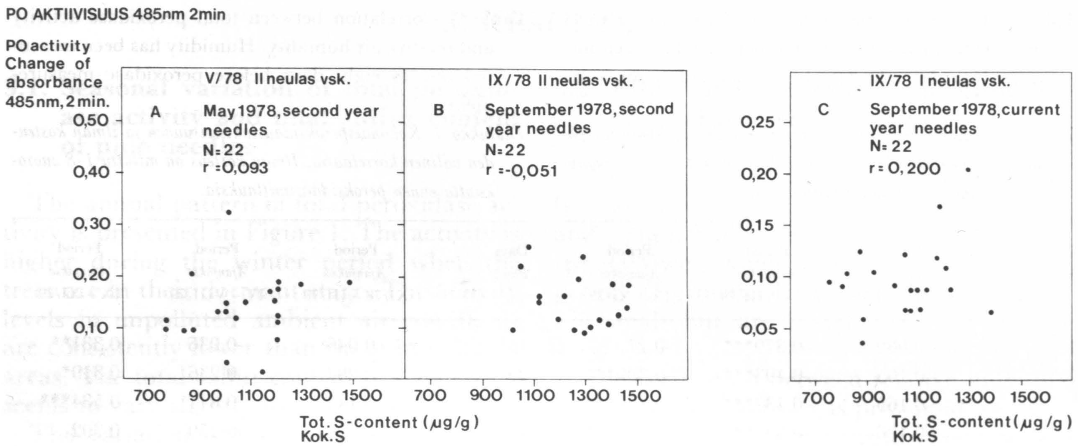

Fig 3. The relationship between total peroxidase activity and total sulfur content of the pine needles measured at the Valkeakoski area on certain days at 22 sample sites.

Kuva 3. Kokonaisperoksidaasiaktiivisuuden ja männyn neulasten kokonaisrikkipitoisuuden korrelaatio yhden päivän aikana 22 pisteestä kerätyistä näytteistä.

3.4. Correlation between total peroxidase activity and atmospheric SO_2 concentrations

Attempts to find a linear relationship between the total peroxidase activity and SO_2 concentrations in the ambient air were not successful (Table 4). The correlations were generally low, and varied inconsistently. No clear trend among the coefficients of the different sampling periods was found. Using regression analysis an attempt was made to define the main factors explaining the variation in total peroxidase activity. The best model, where the factors were temperature, relative humidity, precipitation and atmospheric SO_2 concentrations, accounted for about 50 % of the variation in peroxidase activity. It seems that the linear regression model is not able to reveal the underlying relationships of these variables, that is if such a relationship exists.

Table 4. The relationship between total peroxidase activity and atmospheric SO_2 concentration during the periods XI/78–III/79, IV–VIII/79 and IX–XI/79. Atmospheric SO_2 concentration having been measured one to eight days before peroxidase measurements.

Taulukko 4. Kokonaisperoksidaasiaktiivisuuden ja ilman rikkidioksidi pitoisuuden korrelaatio aikaväleillä XI/78–III/79, IV–VIII/79 ja IX–XI/79. Rikkidioksidipitoisuus mitattiin 1–8 päivää ennen peroksidaasimääritystä.

Day Päivät	Period Ajanjakso XI/78–III/79	Period Ajanjakso IV–VIII/79	Period Ajanjakso IX–XI/79
1.	0.088	0.068	–0.053
2.	0.134	0.125	0.059
3.	0.015	0.081	0.181
4.	0.048	0.346*	0.278
5.	0.170	0.079	0.272
6.	0.144	0.296	0.247
7.	–0.002	0.211	0.354*
8.	0.239	0.162	0.353*

* Indicates significance of P<0.05
* P:n merkitsevyys 0.05

4. DISCUSSION

Attempts were made, using *Pinus sylvestris* as an indicator, to assess whether the total peroxidase activity of pine needles is associated with the measured total sulfur content of the needles or with the SO_2 concentrations in the ambient air. The present results reveal that there appears to be a weak relationship between the sulfur dioxide pollutant and the total peroxidase activity under low pollution concentration conditions. Consequently, it is suggested that environmental and genetic factors may mask the effects caused by low pollutant concentrations in a complex coniferous forest.

The above suggestions are difficult to explain critically, particularly because part of relevant ecophysiological information is unknown. However, several speculative explanations may be presented. The first explanation comes from recent forest meteorology work, which has clarified the complex phenomenon of SO_2 deposition into a forest and the turbulence of SO_2 within a forest ecosystem (Garland and Branson 1977, Murphy et al. 1977).

Murphy et al. (1977) have shown that the change in vertical SO_2 concentration is small above the canopy, where the flux density is nearly constant and diffusivity is high. In the canopy the diffusivity decreases rapidly vertically. The uptake rate of the canopy sink may also be higher in the upper part of the canopy than in the lower one. Garland and Branson (1977) noticed that the deposition velocity for SO_2 into a pine forest canopy varies from about 0,1 cms^{-1} at night to a daytime maximum value of 0,6 cms^{-1}. The rate of uptake may be a magnitude faster when the canopy is wet. They have also detected that the rate of uptake may vary in a ratio of 2:1 when simultaneous measurements between trees are taken. The range of variation between the upper canopy and the lower canopy was about the same with higher values measured at the top. These recent results and the knowledge of the nature of meteorological parameters in the forest ecosystem may provide quite a good explanation for the detected weak relationship between the sulfur pollutant and the total peroxidase activity obtained in this study.

During the most active growing period (see e.g. Pelkonen 1981) the enzyme activity seems to be particularly affected by temperature and atmospheric humidity, suggesting that peroxidase activity is sensitive to environmental factors. The above results confirm the recent results by Endress et al. (1980), who stressed the extremely sensitive nature of total peroxidase activity to environmental stress. In the spring time especially peroxidase activity may be strongly affected by temperature because at that time the temperature of pine needles fluctuates greatly within a short time interval (Christersson and Sandstedt 1978). Hence it seems that during the spring time temperature stress might easily mask the pollution response. The ecophysiological explanation of peroxidase activity and temperature remain open to discussion, and further research is necessary.

The existence of enzyme polymorphism in Scots pine has been shown by many authors (e.g. Chung 1981), and Rudin (1977) has found genetic variation amongst the peroxidase isoenzymes. To test the above assumption of "internal variation" some limited sampling experiments were carried out. The first experiment consisted of two samples to illustrate the variation within a forest stand. The preliminary experiment showed that the total peroxidase activity of the needles varied widely within a stand at the same time. From the point of view of sampling procedure, the minimum sample size was calculated (see e.g. Mäkinen 1976) considering the requirement that a difference of 10 % between the obtained means and the new sample means would be detected with a probability of 0,95 (10 % risk level). The calculated sample sizes varied from between 100 to 200 observations. In addition, sampling experiments were carried out to study the variation of the total peroxidase activity within a canopy. The detected variation was wide, and the calculated statistical sample sizes required from 35 to 200 observations in order to fulfill the statistical criterion of a 10 % difference between means.

The above findings, although based on limited material, confirm the previous ideas on the sensitivity of total peroxidase activity to environmental and genetic factors (Jokinen et al. 1981, Endress et al. 1980). Thus some implications from the above can be made regarding sampling procedure in order to obtain reliable results concerning the levels of total peroxidase activity of Scots pine needles. It seems evident that large sample sizes are required to separate a response due to the pollutant from the environmental and genetic factors affecting the total peroxidase activity.

Gaseous pollutants usually occur as mixtures in the ambient urban air. Empirical evidence has recently been obtained indicating that pollutant interactions may significantly affect the enzyme activities of the plant, and the effects may be more than additive (Wellburn et al. 1981, 1980, Horsman and Wellburn 1975). Especially in the Helsinki area, but also in Valkeakoski, the environment may be affected by pollutant interactions. Association of SO_2 with ozone and NO_2 may provide more information concerning the detected weak correlations between total peroxidase activity and atmospheric SO_2 concentrations.

Pollution stress is influenced by many environmental stress factors when measured under field conditions. The sensitivity of a plant to SO_2 damage has been shown to be dependent on, for instance, water stress, soil nutrient conditions, day lenght and radiation (Davies 1980, Smith 1980, Jäger and Klein 1976), and on attacks by several fungi and pests (Kozlowski 1980, Smith 1980, James et al. 1980). Until now the interactions between plant diseases or pests and pollution effects on conifers are poorly known but some studies refer to their possible importance involving increased pollution damage in the urban environment (Laurence 1981, Kozlowski 1980, Smith 1980, Weidensaul and Darling 1979, Gryzwacz and Wazny 1973).

The idea of conifer trees in the northern environment being sensitive to pollution damage caused by low gaseous pollution concentrations has generated studies to find a method for monitoring hidden injuries (e.g. Huttunen et al. 1980). So far, it seems that peroxidase method is unsuitable for this purpose for many reasons, particularly because peroxidase activity is greatly affected by environmental factors such as temperature and humidity. Moreover, the sampling experiment contributes to the assumption that a reliable sampling procedure might become too laborious to be used as a routine method. Critical comments on the use of total peroxidase activity as a bioindicator of latent injury caused by air pollution have recently been made by Endress et al. (1980), and our results substantiate those comments.

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SELOSTE

ILMAN SAASTEIDEN VAIKUTUS MÄNNYN NEULASIIN

I Männyn neulasten kokonaisperoksidaasiaktiivisuuteen vaikuttavat tekijät

Tutkimuksessa on tarkasteltu säätekijöiden, ilman rikkidioksidipitoisuuksien ja neulasten kokonaisrikkimäärien vaikutuksia männyn neulasten kokonaisperoksidaasiaktiivisuuteen. Aineisto (neulasnäytteet) on kerätty Helsingin Pitkäkoskelta ja Valkeakoskelta. Ilman lämpötilojen ja neulasten peroksidaasiaktiivisuuksien korrelaatio on suurin aktiivisen kasvukauden aikana (Taulukko 2). Suhteellisen kosteuden ja peroksidaasiaktiivisuuden välinen korrelaatio on alhainen ja vaihtelee epäsäännöllisesti (Taulukko 3). Neulasten kokonaisrikkimäärien ja peroksidaasiaktiivisuuksien välinen korrelaatio oli heikko (Kuva 2). Rikkidioksidipitoisuuksien ja neulasten kokonaisperodsidaasiaktiivisuuksien välinen korrelaatio oli myös alhainen ja vaihteli epäsäännöllisesti (Taulukko 4). Peroksidaasiaktiivisuuksien ja ulkoilman rikkidioksidin välisen heikon yhteyden oletetaan selittyvän paitsi havupuiden geneettisen vaihtelun perusteella myös sille, että peroksidaasiaktiivisuus reagoi herkästi moniin ekofysiologisiin tekijöihin. Saastevaikutuksen erottaminen geneettisten ja ympäristötekijöiden vaikutuksista osoittautui vaikeaksi havupuuekosysteemissä. Kokonaisperoksidaasiaktiivisuuden käyttö ilman saasteiden vaikutusten rutiiniseurannassa voi tulla liian työlääksi, koska luotettava tulos edellyttää suurta otoskokoa.