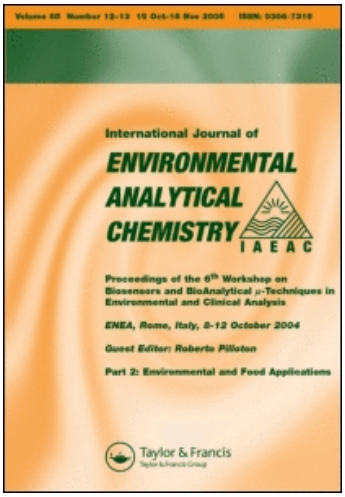

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Effects of H_2O_2-Containing Acidic Fog on Young Trees

Georg Masuch[a]; Antonius Kettrup[a]; Ronald K. A. M. Mallant[b]; Jacob Slanina[b]
[a] Fachbereich Chemie, Universitäat-GH, Paderborn, Federal, Republic of Germany [b] Netherlands Energy Research Foundation ECN, ZG Petten, The Netherlands

To cite this Article Masuch, Georg , Kettrup, Antonius , Mallant, Ronald K. A. M. and Slanina, Jacob(1986) 'Effects of H_2O_2-Containing Acidic Fog on Young Trees', International Journal of Environmental Analytical Chemistry, 27: 3, 183 — 213
To link to this Article: DOI: 10.1080/03067318608079815
URL: http://dx.doi.org/10.1080/03067318608079815

Intern. J. Environ. Anal. Chem., 1986, Vol. 27, pp. 183–213
0306-7319/86/2703-0183 $18.50/0

Printed in Great Britain

Effects of H_2O_2-Containing Acidic Fog on Young Trees

GEORG MASUCH and ANTONIUS KETTRUP

Fachbereich Chemie, Universität-GH, D-4790 Paderborn, Federal Republic of Germany

and

RONALD K.A.M. MALLANT and JACOB SLANINA

Netherlands Energy Research Foundation ECN, P.O. Box 1, 1755 ZG Petten, The Netherlands.

(*Received April 24, 1986*)

The influence of air pollution on forest die-back in Central Europe is not clarified satisfactorily. Both the 'acidification-theory' and the 'ozone-theory' as well cannot satisfactorily explain the die-back to its full extent. Some researchers try to link damage patterns to occurrence of fog and ground-based clouds, but laboratory experiments have failed to show serious effects of fog water on trees caused by acidic compounds. The role of reactive compounds such as H_2O_2, has been overlooked so far.

In this work, the effect of hydrogenperoxide-containing fog on young trees is studied. Young spruces and beeches were exposed for three hours per day to acidic fog with concentrations of 1–5 ppm H_2O_2. After six weeks, serious effects on the internal structure of leaves and needles of H_2O_2-exposed trees were found. The observed symptoms point towards a decreased resistance to drought and a decreased ability to transport assimilates, which in turn may lead to insufficient nourishment of root elements.

KEY WORDS: Forest die-back, hydrogenperoxide, oxidants, fog, intercepted clouds, acid rain.

INTRODUCTION

The causes of the forest die-back in the central part of Europe are not yet clear. The original theory in which deposition of acid and subsequent acidification of the soil is considered to be the principal cause, is now doubted by many scientists for a number of reasons:

— Forest die-back has started at fairly remote locations (Black Forest, Bayerischer Wald). The total deposition of acid at these locations is certainly less than encountered near areas with high emissions, both in Germany and The Netherlands. This is particularly the case if the large contribution of dry deposition in these areas is considered.
— Forest die-back also occurs at locations where the soil contains sufficient calciumcarbonate to neutralize deposited acid (the Jura, Kalkalpen, Schwäbische Alb, etc.).

It could be argued that the interception of cloud water by forests situated on the German "Mittelgebirge" is an extra source (of about 40%) of acidic wet deposition, but it is doubtful whether these areas come near the total load of 6000 equivalents of H^+ per year per hectare which is calculated for the central part of the Netherlands by some investigators.[1] Yet the general condition of the forests in The Netherlands is much better than in the mentioned German areas.

So it was not surprising that another hypothesis for the forest die-back is favoured by Arndt,[2] Prinz,[3] Rehfuess,[4] Zöttl,[5] and co-workers. They proposed that ozone affects the cell membranes, followed by leaching of nutrients (calcium and magnesium ions) and small proteins under the influence of acidic precipitation (including intercepted cloud water).

This hypothesis has a number of strong points:

— Although not many data are available, the small number of long term ozone measurements which have been reported suggest a clear increase of the atmospheric ozone concentration over the last 20 years,[6,7] while emissions of acid-precursors in western Europe have decreased during the same period.[8]
— At high elevations mean ozone concentrations generally are higher than those found in valleys and plains. Main causes are stratospheric input and ozone scavenging by low altitude nitro-

TABLE I

Comparison of ozone concentrations in the Black Forest and The Netherlands (microgr/m^3)

Maximum of	Schauinsland Black Forest FRG	The Netherlands
1-Hourly mean	380	430
24-Hourly mean	240	200

genoxide sources. This gradient in ozone concentration could explain why forest die-back tends to start at higher elevations.

Strong arguments can be raised against this hypothesis:

— Ozone levels in the north-western part of The Netherlands are higher than those encountered at e.g. Schau-ins-Land (Table I),[9] one of the severely affected regions in the Black Forest. No tree damage is observed in this part of The Netherlands.[10] In the eastern and south-eastern part of this country forest die-back occurs, but the mean ozone concentrations are clearly lower than in the north-western part. Peak levels (95 percentiles of hourly mean values) are of the same order in both parts.[11]

— Ozone gradients do not show large spatial differences. Forest die-back however occurs, at least at the initial stages, very locally. Affected trees and healthy ones are found in the same areas. This phenomenon cannot be explained easily by local differences in ozone concentrations. The same argument is valid regarding the acidification hypothesis. No sharp gradients are observed in the total deposition of acid in remote areas, where most of the acid is deposited by rain.

Of course, one can find other explanations for the observed difference in the degree of damage in The Netherlands and Germany and the altitude effect, such as relative humidity during ozone exposure[3] or temperature.

However, atmospheric research has shown that as far as air-pollution is concerned, there is more in the air than just SO_2 and NO_x, more in rain, fog and clouds than just H^+, and there are more photo-oxidants than ozone. More general recent investigations of

rain, fog and dew have revealed that the atmospheric liquid phase frequently contains concentrations in the ppm range of reactive species.[12-14] Hydrogenperoxide is only one of those. Another is sulfite, a component known for its phytotoxicity. One reason that these components were not taken into account might be that many chemists, and even more biologists, have not been aware of the presence of these species. Either they did not have the analytical facility, or the component was removed by reactions before analysis took place. So there is enough reason to be unsatisfied with todays' theories dealing with forest die-back.

The general observation that isolated trees are heavily affected, points to dry deposition of easily deposited species such as gases with high deposition rates (e.g. ammonia), hypermicrone sulfate or nitrate particles or the interception of cloud water. Particle and precursor concentrations however are not very high at locations such as the Black Forest area. Yet, trees are frequently exposed to fog or clouds. The frequency of fog occurrence is 5 times higher at elevations of about 700 meters than at the coastal plains. The occurrence of fog shows an increase above 350 to 400 meters. The frequency is about 120 days per year at 60 meters and goes up to more than 200 days per year at locations over 800.[15,16] Fog is not uniformly distributed, and the interception of droplets particularly is of importance at inhomogeneities such as edges, clearings and isolated or high trees.

These observations have led to the suggestion that forest die-back is strongly connected to the fact that many trees are in frequent contact with fog or cloud water. One explanation could be that these trees are more often exposed to acidic water than those that are only wetted by rainfall. Fog and cloud water are more polluted than rainwater[12,17] and evaporation effects may lead to even higher concentrations of pollutants.[18] However, effects of acidic fog on plants were not clear at pH values above 3.[19-25] These low values do occur in German forests,[12,17] but not enough data are available to determine typical values. In some fog exposure experiments, it is not clear whether precautions were made to prevent partial evaporation of the fog. In others, leaves were not always completely wetted. These problems make it difficult to extrapolate the experimental results to the situation in the German forests. The role of dissolved oxidants in fog- or cloud water has not been taken into account,

mainly because the ozone concentration in water is very low, due to its poor solubility in water. No other dissolved oxidants were investigated in relation to tree damage.

Hydrogenperoxide was mentioned in a more general discussion on damaging photooxidants present in the atmosphere,[26] but up till now, it has not been allotted an important role in the forest die-back, and no effect studies involving H_2O_2 have been reported. Measurements of H_2O_2 have only been performed in studies of sulfur oxidation rates in clouds. They indicated concentrations of 0.1 to ever 5 ppm.[27-32] These reports prompted ECN and the University of Paderborn to investigate the effects of H_2O_2 containing acidic fog on trees.[33,34] If such an effect would exist, it could give a good explanation for the forest die-back, in view of what has been discussed so far. Gasphase concentrations of H_2O_2 in the ambient air are low. Some initial measurements indicate concentrations of 0.03 to a few ppb, so the effect of gaseous H_2O_2 in the ambient air would probably be negligible.

EXPERIMENTAL

Apparatus

Exposure chambers The fog chambers used in the experiment were 1 m^3 glass boxes, placed in the open air and sheltered from direct sunlight. They were ventilated at a rate of 20 m^3/hr with unfiltered air (Figure 1). Conducting experiments in which fog is involved, requires preconditioning of the air for temperature and humidity. Humidification is important, since air of 20°C and 50% RH can take up about 9 g/m^3 of water vapour before reaching saturation. Spraying artificial fog in unsaturated air leads to more or less complete drying of the droplets as typical liquid water concentrations used in our experiments were about 2 g/m^3. At the same time, preconditioning of temperature is needed to minimize heat fluxes between the fog chambers and the surrounding air, since minor temperature changes have drastic effects on relative humidity. Therefore, the air was passed through a pipe filled with 0.8 cm Raschig rings. During fog exposure, water was poured over the rings. This water was kept at the temperature of the open air by means of a microcomputer controlled thermostatic bath. Due to evaporation

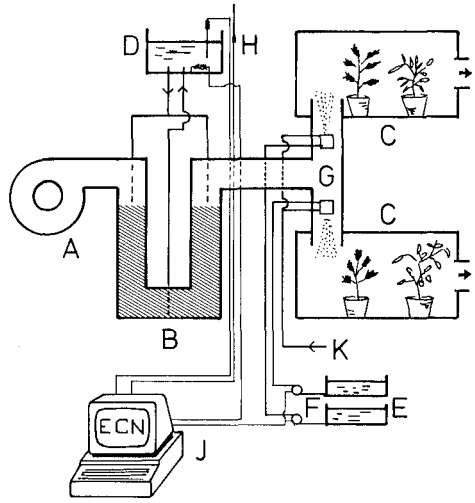

FIGURE 1 Schematic drawing of set-up as used to expose trees to fog. A = blower, B = humidifier, C = glass boxes, D = thermostatic bath, E = spray liquid container, F = peristaltic pump, G = nebulizer, H = Pt 100 temperature sensor, J = minicomputer including interfacing, K = pressurized air.

of water the temperature of the air is lowered when entering the humidifier. But given a long contact time, the air is humidified completely and it attains the water temperature (which in turn equals the open air temperature).

Fog generation and sampling Fog was created by a nebulizer consisting of two steel capillaries (hypodermic needles) arranged perpendicularly.[35] Pressurized air at 4 atm blown through one needle, nebulizes the water which was pumped through the second needle by means of a peristaltic pump at a rate of a few ml/min. Stock solutions were stored in a polyethylene bottle and a glass bottle in the case of the H_2O_2 solution. The bottles were kept in a shelter together with the pumps, microcomputer and humidifier. The H_2O_2 was added to the spray solution at a point a few centimeters from the nebulizer. This was done by mixing two liquid flows. One solution contained artificial fog water without H_2O_2, the second artificial fog water with H_2O_2 in a concentration of 1180 micromoles/liter. By mixing these flows in a 9:1 ratio, we obtained the

desired concentration of 118 micromoles/liter. We have chosen this configuration because a concentrated H_2O_2 solution has a greater stability both in the stock solution and in the tubing leading to the nebulizer. We used silicone tubes of 0.2 cm ID for the stock solution and 0.1 cm ID for the H_2O_2 containing solution. The length of both tubes was about 5 meters. Partial evaporation of water from the droplets will cause the composition of fog water to deviate from the composition of the sprayed solution. To see if this was the case in our experiment, fog was sampled from the boxes using small glass cyclones. These cyclones had a modified Stairmand design.[36] The performance of this type of cyclones is calculated using the Barth theory.[37] The inner diameter of the cyclone is 4 cm, and the calculated 50% cut-off diameter at a flow of 50 l/min is 4 micrometers.

Analytical methodology

Two methods were employed to measure H_2O_2 in water samples; an adapted version of the luminol method, as reported by Kok,[38] and an electrochemical method developed by ECN. The first method differs from the original description given by Kok at two points. The sample is pumped through a column filled with cation exchanger or a mixed bed exchanger to get rid of the heavy metals which interfere in the luminol method. No loss of H_2O_2 by interaction of the ion-exchange materials was observed. The layout as a flow injection analysis method was chosen because it ensures good sample throughput and sensitivity. The selectivity of the method has been enhanced by the use of catalase as a specific enzyme for H_2O_2. The sample is first injected without catalase. Next it is mixed with catalase resulting in a catalase concentration of 4 ppm in the sample. After a reaction time of 30 seconds to remove all H_2O_2, it is injected again. The difference of the two signals is due to the contribution of H_2O_2 only. The method gives a good check on the presence of other (organic) compounds that can induce chemoluminescence. They cause a background signal to remain after addition of the catalase. For the amperometric determination of H_2O_2 a platinum working electrode at a potential of 250 mV versus a silver/silverchloride reference electrode is used. This method is quite sensitive, but it is not very selective. We use it in the same fashion as described for the luminol method (Figure 2). The sample is pumped through a column

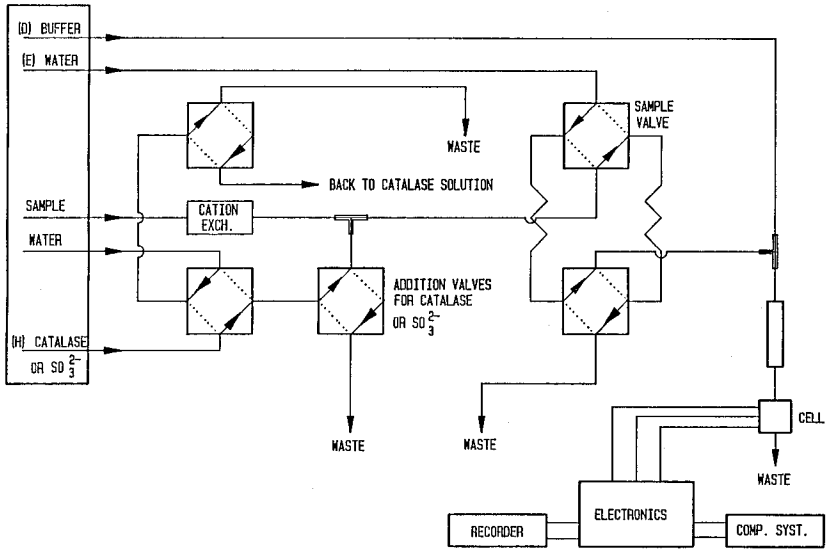

FIGURE 2 Schematic drawing of set-up for H_2O_2-analysis.

filled with cation exchanger to avoid interference by heavy metals. The sample is injected, first without catalase, and a second time after mixing with catalase, again after a reaction time of 30 seconds. The difference in signal again is attributed to the H_2O_2. This method can also be used as a screening test for other oxidants after mixing with a sulfite solution followed by injection.

Both methods described have a detection limit of 1 μgr/l. The reproducibility is typically 5% or better at a concentration level of 50 μgr/l H_2O_2. For precipitation and fog samples, both methods generally agree within 10%.

Biological methodology

Preparation of beech leaves was as follows: Leaf sections of $2 \times 2\,mm^2$ were fixed at room temperature for two hours in glutardialdehyde,[39] followed by a terminal fixation for 1 hour in a 1% solution of OsO_4.[40] After a dehydration sequence in ethanol, the fixed sections were embedded in styrene-methacrylate[41] or Epon.[42] Slices of 1 and 0.3 micrometer thickness were cut with a LKB-

TABLE II

Composition of fog water before spraying (micromol/l)

	Experiment box 1	Reference box 2
Na^+	743	743
K^+	45	45
Mg^{2+}	90	90
Ca^{2+}	77	77
NH_4^+	359	359
H^+	99	99
SO_4^{2-}	375	375
NO_3^-	106	106
Cl^-	785	785
H_2O_2	118	

Ultrotome III and a Reichert Ultracut E respectively. The cuts were coloured by Toluidinblue.[43] Preparation for scanning electron microscopy consisted of fixation, breaking in liquid nitrogen, dehydration in ethanol, critical-point drying, and coating with gold by sputtering.

The spruce needles were fixed for three hours at room temperature in a solution of 1% OsO_4 and 2.5% $K_2Cr_2O_7$ buffered by 0.1 molar phosphate.[44] The needles were then embedded in styrene-methacrylate[41] or Epon.[42] After fixation the embedded needles were cut into 1 micrometer slices for light microscopy studies. Preparation of needles for electron microscopy was done in the same way as described for the leaves.

A Leitz-Orthomat was used for light microscope studies. Quantitative analysis of the microscope pictures were made with the aid of a digitizer tablet, connected to a microcomputer. Electron microscopic studies were performed with a Hitachi H3010 scanning electron microscope.

Experimental procedure

The composition of the fog water before spraying is listed in table II. Note that only one of the boxes received water containing H_2O_2. To

isolate a peroxide effect, both boxes received the same fog water with regard to all other components.

To check whether the fog water was contaminated by metals leaching from the hypodermic needle of the nebulizer, we sampled the fog immediately after spraying by impaction on a polyethylene substrate.

At irregular time intervals, the performance of the humidification section and nebulizer was tested by comparing the composition of the fog water before spraying with the water sampled by the cyclones. The liquid water content of the fog generated is estimated by the amount of water sampled in small glass cyclones. The liquid water content can only be estimated because of the unknown sampling efficiency of the inlet for large water drops.

Three year old Norway spruce trees (Picea abies) were taken from a spruce forest in the Egge Mountains in Germany in July 1984. The trees remained in their original soil, Gault-Sandstone. Three year old beech trees were taken from a Melico-Fagetum in the Egge Mountains and cultivated in the original soil that had developed from shell-lime. Three spruce trees and five beeches were placed in each box. Fog generation started every morning at 6 and lasted for three hours. Relative humidity slowly decreased after the fog generation was stopped. After the water that remained in the humidifier had evaporated, the relative humidity equaled that of the outdoor air. The experiments lasted about six weeks. After treatment, the trees were transported to the University of Paderborn, where the leaves and needles were fixed. Then, two sets of neeedles and leaves were prepared for evaluation, one set from each box. A set of needles was composed as follows: from each of the three spruces in a box three first year needles and three second year needles were taken, so a total of eighteen needles per box. From several transverse sections of every needle, two were taken to be analysed. A set of leaves consisted of one young and one grown-up leaf of each of the five beeches in a box, so ten leaves per box were examined. From every leaf, four sections were taken for further preparation and analysis. The results were subjected to statistical analysis with the Student *t*-test, to determine whether the two sets of needles showed significant differences for the variables measured (cell number, cell area, etc.).

The leaves and needles were analysed for dry weight, the number of various cells and stomata, surface area of needle transverse

sections and cells, as well as the presence of chloroplasts and tannins (in the case of needles).

RESULTS

Fog water analysis and size distribution

The size distribution of the fog droplets as measured by a foreward scattering optical probe is given in Figure 3. The mass median diameter was about 25 micrometers.

The fog water sample obtained by direct impaction of the spray-jet contained no detectable amounts of Fe and Cr while concentrations of Mn and Ni were at the ppb level.

Composition of spray liquid and a typical fog water sample are compared in Figure 4. Hydrogenperoxide concentrations in the spray solution are given in Figure 5. Although the H_2O_2 concentration

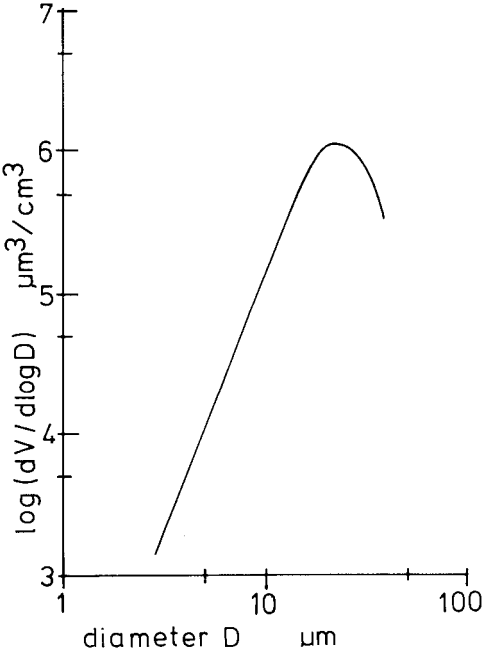

FIGURE 3 Volume distribution of output of nebulizer used.

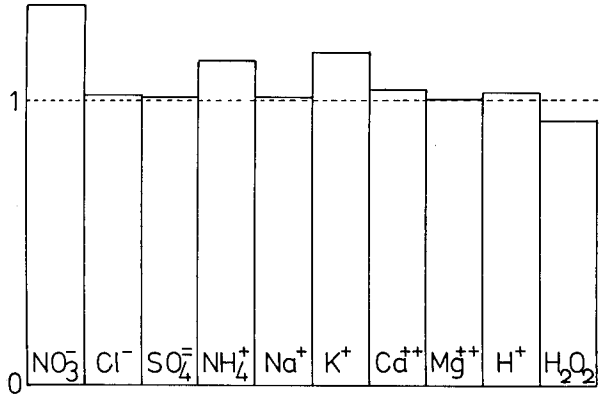

FIGURE 4 Typical concentration ratio of components, sampled fog water/spray liquid.

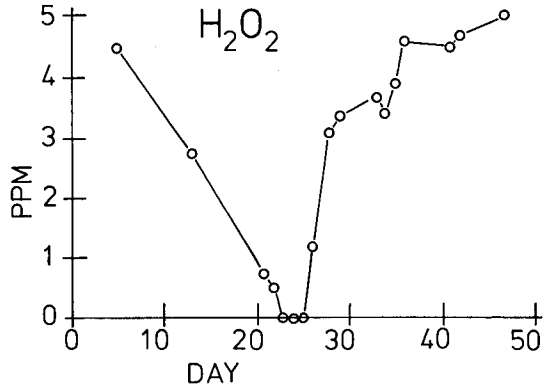

FIGURE 5 H_2O_2 concentration in spray liquid (in container) as function of time. The rise of concentration is a result of renewal of the liquid.

was meant to be constant during the entire experiment, there was a decrease during the first weeks, caused by a decay of H_2O_2 in the bottle which contained the stock solution. In the second half of the experiment, this problem was overcome by frequent replacing of the liquid and cooling of the glass bottle.

Effects on plants

Norway spruce Some of the variables studied (i.e. cell numbers or areas) showed a decrease, others an increase for the plants exposed to H_2O_2. Also, the changes were not always observed in both primary and secondary needles. In the primary needles, we observed a significant (i.e. $P<0.05$) decrease in cell number in the epidermis, the mesophyll and the endodermis (Figures 6 and 7). In the secondary needles, only a decrease of the number of hypodermal cells was observed. Other changes in cell numbers outside the vascular bundle were not significant (Tables III and IV). Significant decrease of cell area of the mesophyll was observed in both primary and secondary needles of H_2O_2-treated plants, whereas the area of the intercellular spaces increased. The area of the vascular bundle decreased significantly in the primary needles only (Table V and VI). In the vascular bundle of both primary and secondary needles of H_2O_2-treated plants, we observed strong deformation of the phloem

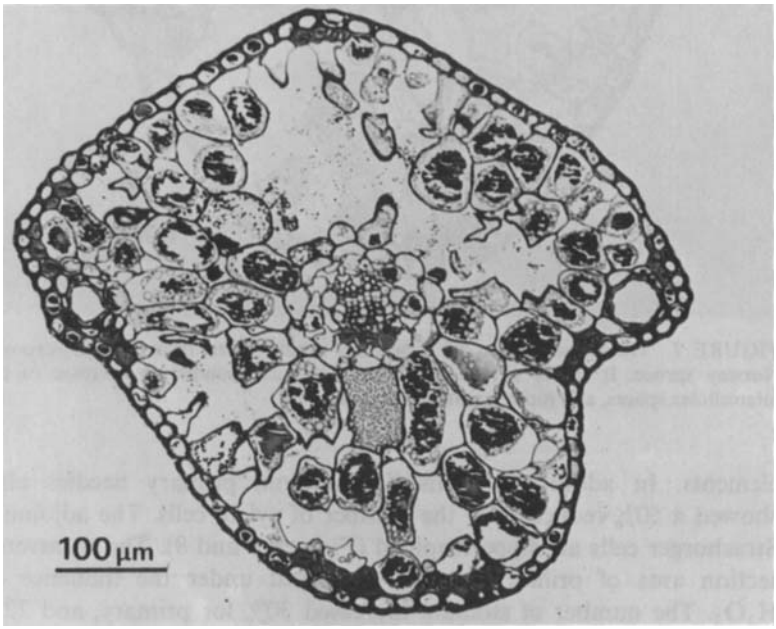

FIGURE 6 Transverse section of a primary needle of a Norway spurce from the reference box.

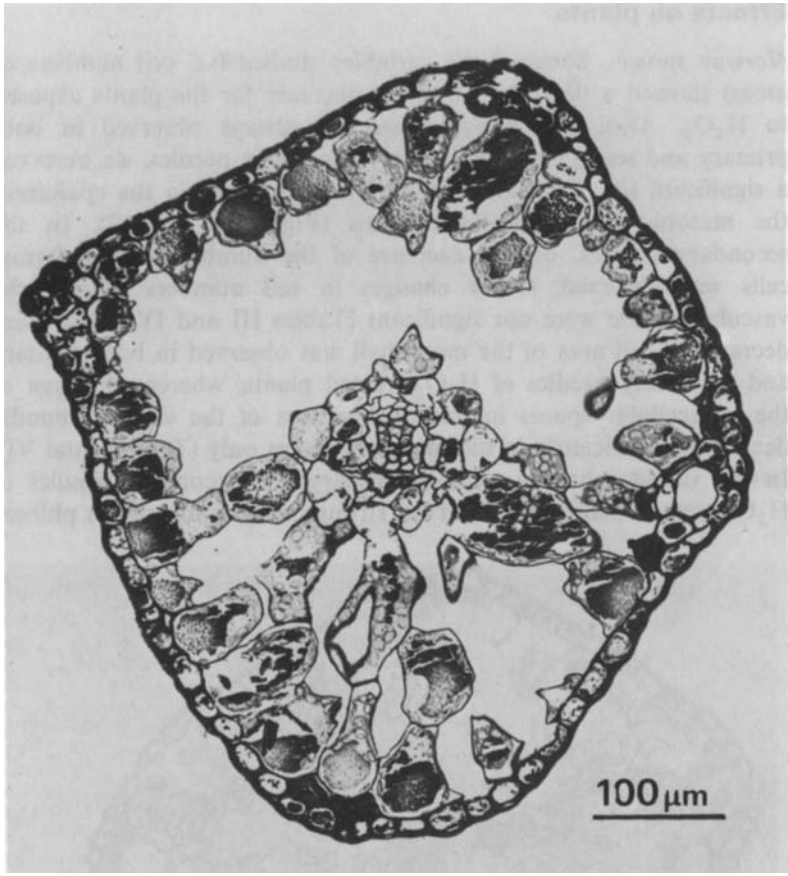

FIGURE 7 Transverse section of a primary needle taken from a H_2O_2-exposed Norway spruce. It shows a reduction of the vascular bundle, an increase of the intercellular spaces, and more tannins in the vacuoles.

elements. In addition to this deformation, primary needles also showed a 50% reduction of the number of xylem cells. The adjoining Strasburger cells are hypertrophied (Figures 8 and 9). The transverse section area of primary needles decreased under the influence of H_2O_2. The number of stomata increased 30% for primary, and 22% for secondary needles (Table VII). The surface density of the contact area between mesophyll cells and the intercellular space gives an

TABLE III

Average number of cells in a transverse section of primary needles of Norway spruce

	Epidermis	Sclerenchyma c	Mesophyll in hypodermis	Endodermis
Ref	80±4.9	18.3±3.4	53.6±6.1	11.7±0.8
H_2O_2	67.9±7.0	16.4±10.6	48.8±4.5	9.8±1.2
P	<0.001	0.52	<0.02	<0.001

TABLE IV

Average number of cells in a transverse section of secondary needles of Norway spruce

	Epidermis	Sclerenchyma c	Mesophyll in hypodermis	Endodermis
Ref	72.6±11.9	25.0±11.1	52.8±6.6	11.5±1.3
H_2O_2	75.8±9.8	14.0±6.8	51.7±5.8	11.5±1.3
P	0.47	0.008	0.67	1

TABLE V

Changes in tissue area of primary needles of Norway spruce as seen in transverse sections (% transverse section area)

	Mesophyll area	Cell area of mesophyll	Intercell.sp. area of mes.	Area of the vascular bundle
Ref	75.5±1.0	55.0±4.8	20.0±4.5	4.0±0.5
H_2O_2	75.5±1.9	44.0±6.3	31.0±6.9	3.0±0.7
P	0.96	<0.001	<0.01	<0.002

TABLE VI

Changes in tissue area of secondary needles of Norway spruce as seen in transverse sections (% of transverse section area)

	Mesophyll area	Cell area of mesophyll	Intercell.sp. area of mes.	Area of the vascular bundle
Ref	75.1±0.7	57.5±2.4	17.6±2.6	3.2±0.3
H_2O_2	74.5±2.0	48.2±5.4	26.2±4.3	3.5±0.4
P	0.33	<0.001	<0.001	<0.075

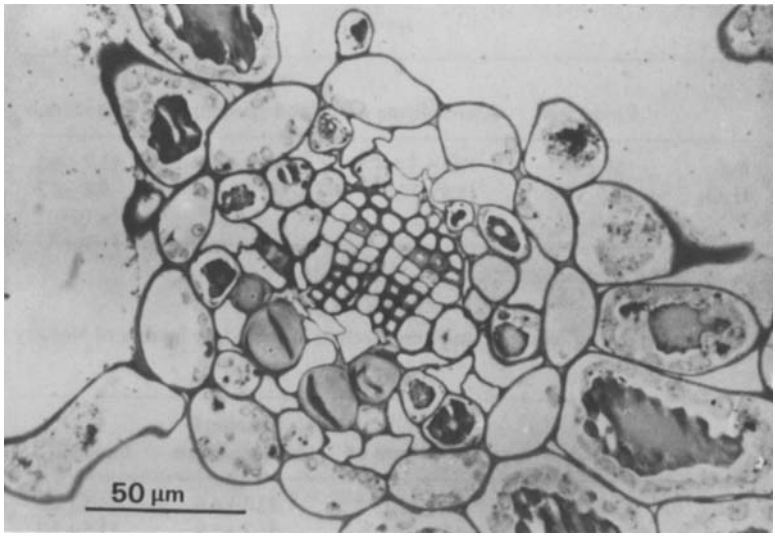

FIGURE 8 Transverse section of a vascular bundle of a primary needle of Norway spruce from the reference box.

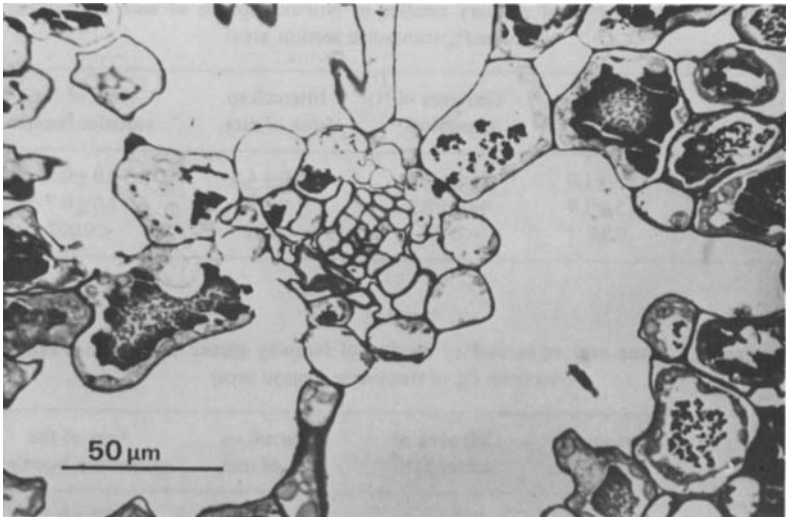

FIGURE 9 Transverse section of a vascular bundle of a primary needle of Norway spruce from the H_2O_2-exposed set. Note the strong reduction of xylem and phloem.

TABLE VII

Transverse section area ($\times 1000\,\mu m^2$) and number of stomata per 700 μm stomatal row in needles of Norway spruce

	Section area		Number of stomata	
	Primary	Secondary	Primary	Secondary
Ref	187±13	144±29	4.0±0.4	4.5±0.5
H_2O_2	126±27	137±32	5.2±0.7	5.5±0.8
P	<0.001	0.58	<0.001	<0.001

TABLE VIII

Surface density ($\mu m^2/\mu m^3$) of needles of Norway spruce

	Primary needles	Secondary needles
Ref	26.6±2.3	32.0±3.4
H_2O_2	31.1±5.9	36.0±4.1
P	<0.001	0.036

indication for gas exchange. Under the influence of H_2O_2 the surface density has increased in both primary and secondary needles, the effect being stronger in the primary needles (Table VIII). The values given in the table are the mean values and standard deviations of cell numbers or surfaces. A summary of results is found in Table IX. We also observed a tremendous amount of tannins in the vacuoles of the mesophyll cells of the H_2O_2-treated trees (Figures 10, 11 and 12), and the chloroplasts showed a considerable increase in starch grains.

Beeches The effect of H_2O_2 on leaves of beeches is dependent on the age of the leaves. Especially, it seems to be of importance whether the plants were exposed before or after tissues started to develop into their ultimate form.

The young leaves were still growing at the beginning of the experiment. At the moment they were taken for analysis, their average leaf area was 374 mm^2. The dry weight of H_2O_2-exposed leaves was 0.054 mgr/mm^2 versus 0.058 mgr/mm^2 for the reference

TABLE IX

Summary of results for spruce needles; changes in H_2O_2-exposed plants

	Primary needle	Secondary needle
Dry weight	decreased	decreased
Water content	increased	increased
Crossection area	decreased	—[a]
Mesophyll cell tissue	decreased	decreased
Interc. space (mesophyll)	increased	increased
Stomata density	increased	increased
Number of cells per section of:		
Epidermis	decreased	—[a]
Sclerenchyma	—[a]	decreased
Mesophyll	decreased	—[a]
Endodermis	decreased	—[a]
Area of:		
Xylem cells	decreased	—[a]
Phloem cells	decreased	—[a]
Surface density	increased	increased

[a]— denotes: no changes observed.

leaves. The water content showed a more clear effect; 47% for the exposed, 62% for the reference leaves.

The leaf structure of both sets of plants show a development typical for trees growing in the shade; there is one layer of palisade mesophyll, the spongy mesophyll is rich in intercellular spaces (Figure 13), and the stomata density at the lower surface of the leaves is only 116 per mm^2.

The H_2O_2-exposed leaves show histological and cytological changes. Shrunken cells are more abundant in all types of tissue, individual palisade and spongy mesophyll cells are collapsed (Figure 14). The palisade cells in the reference plants are uniformly distributed, have a slightly conical shape, and look turgescent (Figure 15). The H_2O_2-exposed leaves show a rather stocky structure. There is little sideways contact between individual palisade cells, which

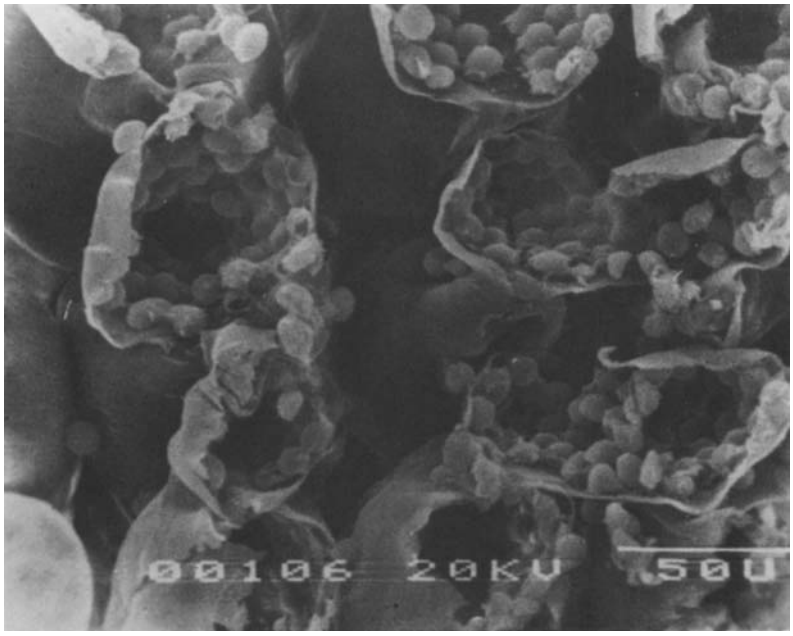

FIGURE 10 Scanning electon microscopic picture of a section of a primary needle from the reference series, showing mesophyll cells. Numerous chloroplasts fill the periphere cytoplasm. The central vacuoles are free of any substance.

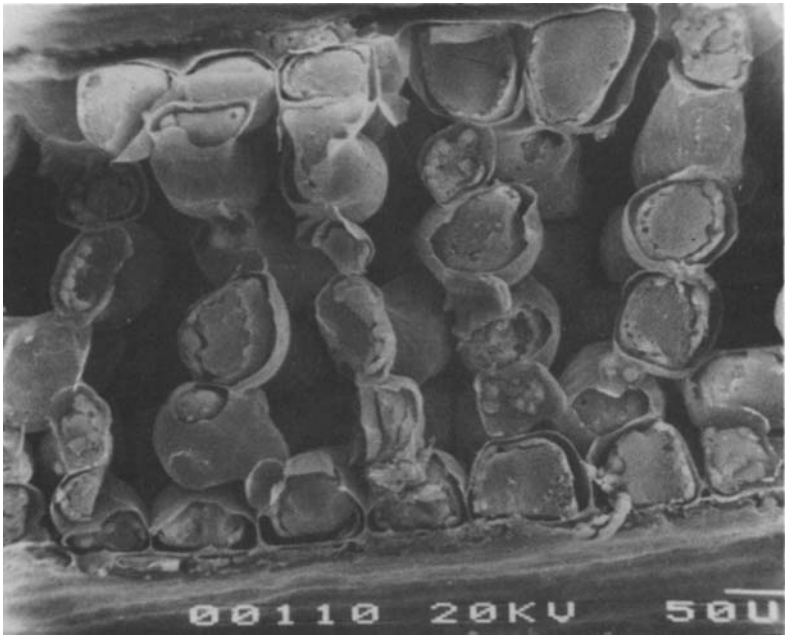

FIGURE 11 Scanning electon microscopic picture of a section of primary needle from the H_2O_2 series, showing mesophyll cells. The central vacuoles are filled with

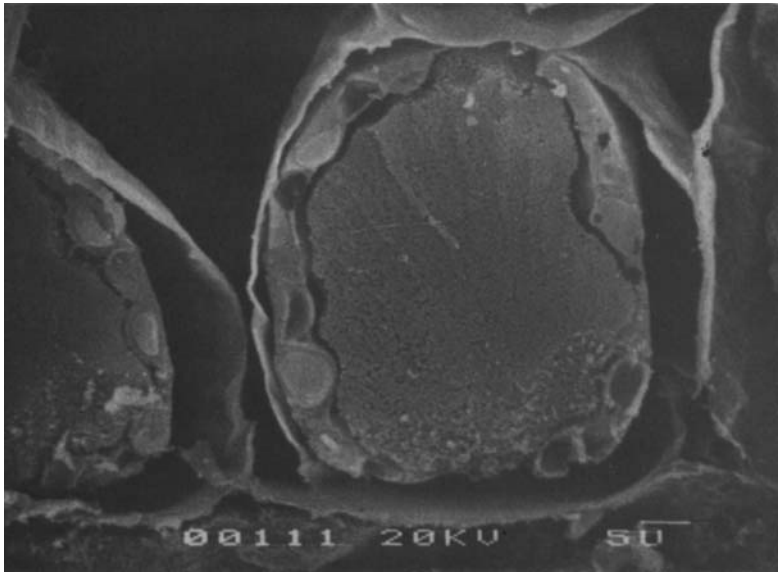

FIGURE 12 Close-up of a single mesophyll cell of a H_2O_2-exposed Norway spruce. The central vacuole is filled with tannins, chloroplasts are found in the periphere cytoplasm.

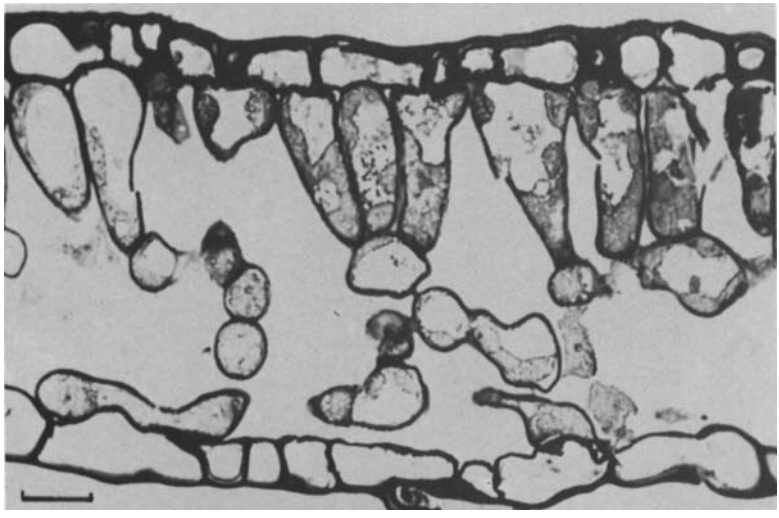

FIGURE 13 Transverse section of a young leaf of Red beech from the reference box. The palisade mesophyll consists of one cell layer, the spongy mesophyll is rich of intercellular spaces (indicated length scale is 10 micrometer).

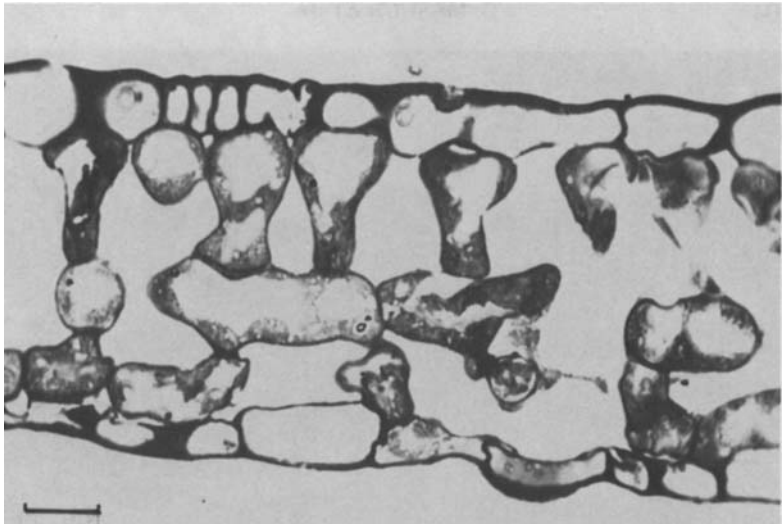

FIGURE 14 Transverse section of a young leaf exposed to H_2O_2-containing acidic fog. The leaf thickness is reduced, shrunken cells are more abundant in all types of tissue (indicated length scale is 10 micrometer).

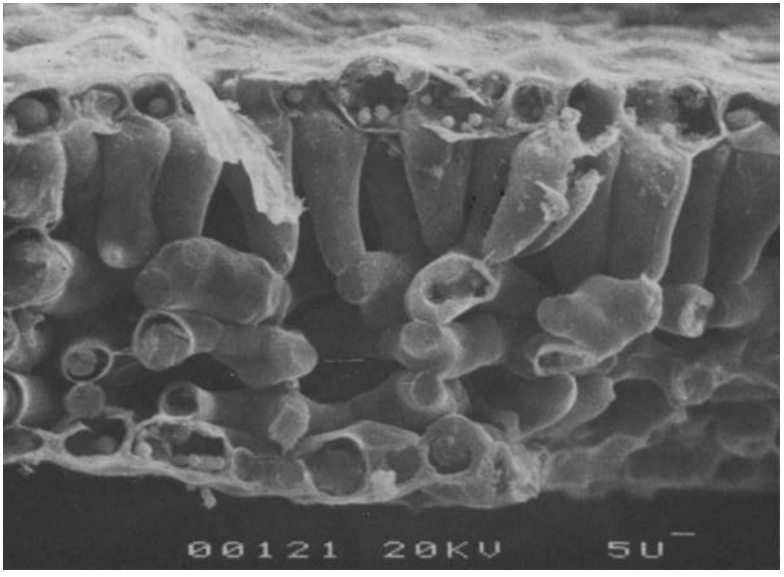

FIGURE 15 Transverse section of a mature leaf of Red beech from the reference box. The palisade cells are turgescent, and rejuvinate going into the direction of the spongy mesophyll.

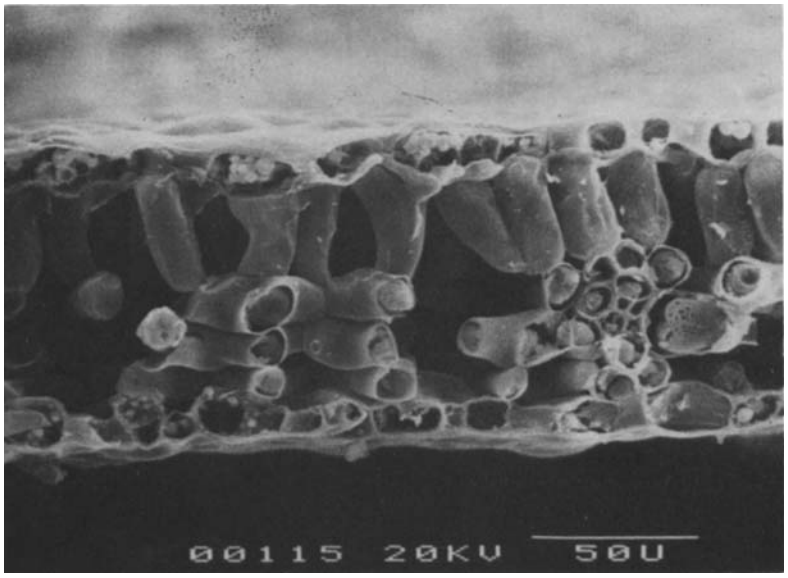

FIGURE 16 Transverse section of a mature leaf exposed to H_2O_2-containing acidic fog. The palisade cells are less turgescent and rejuvinate hardly.

reduces the transport of water. The cells are less uniformly distributed, and rejuvinate hardly in the direction of the spongy mesophyll. The number of palisade cells per 100 micrometer of section width is significantly lower in the H_2O_2 exposed leaves: 7.5 ± 0.8 versus 8.7 ± 1.2 ($p = 0.002$). The spongy mesophyll is more compact in the case of the H_2O_2-exposed leaves (Figure 16).

All types of tissue in the H_2O_2-exposed leaves have a reduced thickness (Table X), although the percentual decrease is not constant. Consequently, the leaf thickness is also reduced: 55.8 ± 2.6 versus 63.4 ± 1.3 micrometer ($p < 0.001$).

The area of most of the tissue types when measured over 100 micrometer section width has significantly decreased. The reduction amounts 12% for the upper epidermis, 22% for the lower epidermis, 12% for the intercellular spaces and 16% for the palisade mesophyll. Only the area of the spongy mesophyll shows an increase of 13%. Therefore, there is no difference in the total mesophyll area, only the partitioning has changed at the cost of palisade mesophyll. The

TABLE X

Changes in tissue thickness of young Red beech leaves as seen in transverse sections (in micrometers)

	Upper epidermis	Palisade mesophyll	Spongy mesophyll	Lower epidermis
Ref	6.7±0.4	22.0±1.0	27.5±2.0	6.8±0.7
H_2O_2	6.3±0.6	19.5±0.6	23.2±1.9	6.2±0.5
P	0.003	<0.001	<0.001	0.003

absolute area of the intercellular spaces in the spongy mesophyll has decreased, but the contribution of these intercellular spaces relative to the area of the transverse section has not changed. Also, the ratio internal/external surface area has not changed for the total mesophyll. The net effect for the plant is a relative increase of internal surface area under influence of H_2O_2.

The stomata density at the lower surface of the leaf is 116±12 per mm^2 for the reference plants; for the H_2O_2-exposed plants, it is 140±14 ($P=0.03$).

The size of the stomata was not different. The ratios of length and width of both stomacells and apertures were the same for both series of plants. The stomata therefore were in the same stage of development.

In the median vein of the leaf the xylem is surrounded by phloem. The vascular bundles of the large veins are enclosed by a sclerenchyma bundle-sheath (Figure 17). The relative portions of xylem and phloem change under influence of H_2O_2 in favour of the xylem (Table XI). The sclerenchyma bundle-sheath is enlarged (Table XII, Figure 18).

In the case of the grown-up leaves the situation is somewhat different. The mean area of the leaves was 1060 mm^2. There was no considerable growth of these leaves during the experiment. Parameters such as dry weight, water content, number of stomata, leaf thickness and thickness of several tissue types did not change. There was a tendency for the upper epidermis and the intercellular space area to decrease when expressed as area per 100 micrometer transverse section area. The internal surface area and the ratio internal/external surface area decreased under the influence of H_2O_2 (Table XIII).

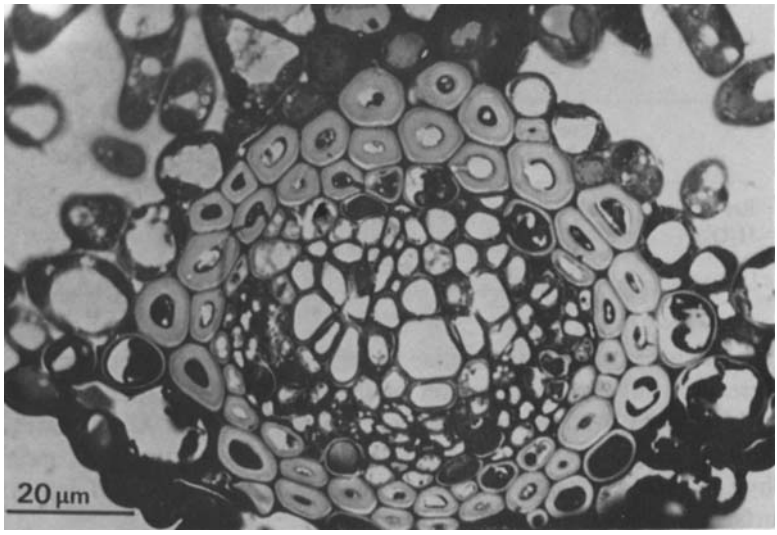

FIGURE 17 Transverse section of the median vein of a young leaf of a reference plant.

TABLE XI

Comparison of the relative areas of xylem and phloem in the vascular bundle in young Red beech leaves (% of bundle area)

	Xylem	Phloem
Ref	63 ± 1	37 ± 1
H_2O_2	68 ± 4	30 ± 3
P	< 0.001	0.008

TABLE XII

Comparison of the tissue areas of the vascular bundle of young beech leaves per 100 micrometer section width (μm^2)

	Sclerenchyma	Vascular bundle-sheath	Xylem bundle	Phloem
Ref	2917 ± 357	2289 ± 520	1430 ± 322	858 ± 205
H_2O_2	3579 ± 151	2836 ± 446	1922 ± 236	857 ± 191
P	0.005	0.11	0.02	1

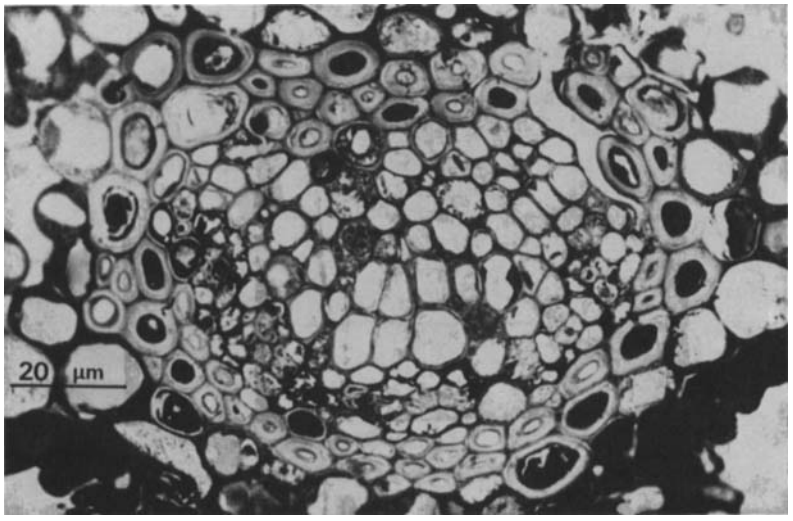

FIGURE 18 Transverse section of the median vein of a H_2O_2-exposed young leaf.

Changes in the vascular bundle of grown-up H_2O_2-exposed beech leaves are much the same as for the young leaves, but not significant ($0.09 < P < 0.31$ depending on parameter involved).

Table XIV gives an overview of observed changes.

DISCUSSION OF RESULTS

Observed effects on plants

Norway spruce Since the primary needles were still developing at the time they were exposed to the fog, they are probably more susceptible to H_2O_2 compared to the secondary needles. The primary needles showed a deformation of cells and a reduced amount of certain types of cells. This reduction was not found, except for the hypodermal cells, in secondary needles. Primary and secondary needles show deformation of the existing cells, most clearly in the phloem. The Strasburger cells, adjoining the sieve tubes of the phloem, are hypertrophied. Collapse of phloem cells and hypertrophy of Strasburger cells are also observed in needles of dying

TABLE XIII

Internal surface area of grown-up Red beech leaves as seen in 100 micrometer transverse sections

	Internal surface area (μm^2)	Internal/external surface area
Ref	732 ± 58	3.7 ± 0.3
H_2O_2	674 ± 63	3.4 ± 0.3
P	<0.05	<0.005

TABLE XIV

Summary of results for Red beech leaves; changes observed in H_2O_2-exposed leaves

	Young leaf	Grown-up leaf
Leaf area	decreased	(decreased)
Leaf thickness	decreased	—
Upper epidermis	decreased	—
Palisade mesoph.	decreased	—
Spongy mesoph.	decreased	—
Lower epidermis	decreased	—
Water content	decreased	—
Int. surf. area	increased	decreased
Tissue area per 100 μm section width:		
Upper epidermis	decreased	(decreased)
Palisade mesoph	decreased	—
Spongy mesoph.	increased	—
Interc. space	decreased	(decreased)
Lower epidermis	decreased	(decreased)
Transverse area of:		
Vascular bundle	(increased)	(increased)
Xylem	increased	(increased)
Phloem	(decreased)	—
Scl. bundle sh.	increased	—

( ) denotes: no significance.

spruce trees in the south of the Black Forest, and the effect is discussed to have more connection to SO_2-pollution than to ozone exposure.[45] Spruces, exposed to 292 micrograms SO_2/m^3 for two months showed the collapse of phloem cells and hypertrophy of Strasburger cells. Spruces treated with ozone (194 microgram/m^3), showed reduced amounts of sieve tubes (though they were not collapsed), while Strasburger cells were also hypertrophied.[46] Since SO_2 concentrations in the Black Forest are low[9] it is likely that the collapse found there will have other causes. The observed high starch content may be caused by the fact that collapsed phloem cells can no longer transport assimilation products out of the needle.[45] This in turn can lead to a decay of the root system.

The observed increase in tannins was also found in spruces from polluted areas of Finland.[47] Tannins are polyhydroxyphenolic compounds with high molecular weight, and represent a substantial portion of the carbon reserves of the leaf.[48] Increase in tannins was also observed in the mesophyll cells after SO_2 exposure.[46] Production of tannins increases the plant's resistance to transpiration.[49] The observed high tannin and starch grain content in the H_2O_2-treated needles in our experiment, differs from earlier observations where high concentrations of both products did not occur at the same time.[50]

The plants in our experiment showed an increased stomata density. This is one of the symptoms of water-stress.[51,52] Also, the observed increase of the internal surface area is an indication of water-stress. The plant increases its ability for gas exchange in periods of less stress. Also the high starch grain content may be connected with water-shortage; hydrolysis of starch will not take place.[53,54] A possible cause of the water-stress may be the observed 50% reduction of xylem cells.[55,56]

Beeches Young beech leaves that were still growing during fog exposure show serious effects of H_2O_2. Grown-up leaves show less, insignificant changes. These changes, however, show the same trend as in the case of the young leaves. The lower degree of damage can be attributed to the higher resistance of mature leaves for pollution.[57]

The following symptoms that we observed in young leaves exposed to H_2O_2 lead to the conclusion that these leaves were in a

situation of water-stress:

— the decrease of water content,
— the increased stomata density,
— the large fraction of xylem in the vascular bundle,
— shrunken cells in the palisade tissue,
— a higher content of fine oildrops in the upper epidermis.

In mature leaves water-stress is less clear in tissue changes than in reduced water content, plasmolysis of the cells of the palisade mesophyll and the presence of oildrops in the cells of the upper epidermis and the spongy and palisade mesophyll. The presence of oildrops can be regarded as xeromorphic adaption of the leaves. In this respect, the oildrops have the same function as the tannins in the spruce needles.

The following symptoms also point towards a decreased rate of photosynthesis of the young leaves:

— the somewhat reduced specific weight,
— the reduced leaf thickness,
— the reduced leaf surface,
— less palisade cells,
— the reduced fraction of palisade cells in the mesophyll
— the reduced fraction of phloem in the vascular bundle.

The reduction in phloem and the partially collapsed sieve tubes reduce the transport of assimilates. A hold-up of these products reduces the rate of photosynthesis.[58] The hypertrophied Strasburger cells indicate an increased loading with assimilates.

Exposure facility

The set-up chosen for this type of experiments was found to give a good and reproducible fog. The fact that the liquid water content is not exactly known, is not very important as long as one does not try to copy natural fog water deposition rates. Very little is known about this rate, and there will be a large natural variation, depending on liquid water content, wind speed, turbulence and position of needles and leaves within the canopy.

Adding a new component to the list of pollutants which are possibly responsible for forest die-back, requires a discussion why the component has become active in the last decade. The emissions of NO_X in the Federal Republic of Germany have increased in the last decades, hydrocarbon emissions remained constant. This may be in favour of an increased production of oxidants (including ozone). The emissions of SO_2 have decreased since 1970.[4,59] These decreased emissions probably have resulted in a decreased consumption of those oxidants that are rapidly scavenged by SO_2, such as H_2O_2 in the liquid phase. So, all arguments concerning the temporal development that are in favour of a role of ozone, also apply to H_2O_2, with an additional argument being the decrease of SO_2 emissions.

CONCLUSIONS

The first results of experiments in which young spruce and beech trees are exposed to H_2O_2-containing acidic fog show that the internal structures of needles of Norway Spruce and of leaves of Red Beeches are affected by H_2O_2-containing acidic fog. There are indications that the plants have a reduced capacity for transport of water and assimilation products due to major changes in the vascular bundle. These changes are also observed in spruce trees in the Black Forest. Macroscale visible effects have not been observed after six weeks of exposure.

Hydrogenperoxide cannot be ruled out as an important factor in the forest die-back, although more refined experiments will be needed to give more information about to what extent H_2O_2 affects trees. Also, more information is needed about the occurrence of H_2O_2-containing fog in the affected regions. Furthermore, it is desirable to extend the examination of H_2O_2-exposed plants to determine if growth rate, evaporation, and photosynthesis are affected and to see if leaching occurs.

References

1. Verzuring door atmosferische deposite; evaluatierapport, ministerie van landbouw en visserij, ministerie van volkshuisvesting, ruimtelijke ordening en milieubeheer, The Hague, The Netherlands, (1984).

2. U. Arndt, G. Seufert and W. Nobel, *Staub-Reinhalt. Luft* **42,** 243 (1982).
3. B. Prinz, G.H.M. Krause and H. Stratmann, *Forest Damage in the Federal Republic of Germany,* Landesanstalt für Immisionsschutz des Landes Nordrhein-Westfalen, Essen, F.R.G., Report No. 28 (1982).
4. K. E. Rehfuess. In: *Was wir über das Waldsterben wissen* (E. Niesslein and G. Voss, ed.) (Deutscher Instituts-Verlag, Köln, F.R.G., 1985) pp. 124–130.
5. H. W. Zöttl. In: *Was wir über das Walsterben wissen* (E. Niesslein and G. Voss, ed.) (Deutscher Instituts-Verlag, Köln, F.R.G., 1985) pp. 121–141.
6. R. Guderian, *Air Pollution by Photochemical Oxidants* (Springer-Verlag, Berlin, F.R.G., 1985), pp. 25–54.
7. J. A. Logan, *J. Geophys. Res.,* **90**(D6), 10,463 (1985).
8. J. Schmölling and K.E. Jörss, VDI-Berichte 500, Kolloquium Lindau 1983, Verein Deutscher Ingenieure, Düsseldorf, F.R.G., pp. 13–19.
9. H. Stratmann, *Wirkungen von Luftverunreinigungen auf die vegetation,* Landesanstalt für Immisionsschutz des Landes Nordrhein-Westfalen, Essen F.R.G., Report No. 49 (1984).
10. W. M. J. den Boer, *Bossterfte, samenhang met zure deposite,* paper presented at the Conferentie Energie en zure regen, Utrecht, The Netherlands, March 19, 1985.
11. Nationaal Meetnet voor Luchtverontreiniging, NML-RIVM nr. 26, Report nr. 228216020, Rijksinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven, The Netherlands, (1984).
12. R.K.A.M. Mallant, Unpublished results (1984).
13. J. Slanina, R.K.A.M. Mallant, G. Masuch and A. Kettrup, paper presented at the Cost Action 61Abis meeting, Petten, The Netherlands, December 9–10, 1985.
14. F. G. Römer, paper presented at the Cost Action 611 meeting, Bilthoven, The Netherlands, September 23–25, 1985.
15. Monatlicher Witterungsbericht 1984, Deutscher Wetterdienst, Offenbach am Main, F.R.G., 32(13), (1985).
16. H. W. Georgii and G. Schmitt, *Staub-Reinhalt. Luft* **45**(6), 260 (1985).
17. E. Schrimpff, *Staub-Reinhalt. Luft* **43**(6), 240 (1983).
18. M. H. Unsworth, *Nature* **312,** 262 (1984).
19. T. Wood and F. H. Bormann, *Environ. Pollut.* **7,** 259 (1974).
20. T. Wood and F. H. Bormann, *Ambio* **4**(4), 169 (1975).
21. J. B. Wedding and M. Ligotke, *Environ. Sci. Technol.* **13**(7), 875 (1979).
22. I. J. Hindawi, J. A. Rea and W. L. Griffis, *Amer. J. Bot.* **67**(2), 168 (1980).
23. T. Scherbatskoy and R. M. Klein, *J. Environ. Qual.* **12**(2), 189 (1983).
24. A. L. Granett and R. C. Musselman, *Atmos. Environ.* **18**(4), 887 (1984).
25. R. A. Skeffington and T. M. Roberts, *Oecologia* **65,** 201 (1985).
26. J. S. Gaffney, *Adirondac* **XLVIII**(4), 8 (1984).
27. J. Kadleck, S. McLaren, N. Camarota, V. Mohnen and J. Wilson, Precipitation Scavenging, Dry Deposition and Resuspension, Proceedings of the Fourth International Conference, Santa Monica, California, 29 November–3 December, 1982 (Elsevier Science Publishing Co., Inc. 1983) pp. 103–113.
28. G. L. Kok, *Atmos. Environ.* **4,** 653 (1980).
29. G. L. Kok, *Measurements of Hydrogen Peroxide in the OSCAR Program,* Final Report, Harvey Mudd College, Claremont, CA (1982).

30. P. H. Daum, S. E. Schwartz and L. Newman, Precipitation Scavenging, Dry Deposition and Resuspension, Proceedings of the Fourth International Conference, Santa Monica, California, 29 November–3 December, 1982 (Elsevier Science Publishing Co., Inc. 1983).
31. F. G. Römer, J. W. Viljeer, L. van den Beld, H. J. Slangewal, A. A. Veldkamp and H. F. R. Reijnders. In: *Acid Deposition, Proceedings of the CEC workshop Physico-Chemical Behaviour of Atmospheric Pollutants* (S. Beilke and A. J. Elshout ed.) (D. Reidel Publishing Co., Berlin, F.R.G., 1983), pp. 195–203.
32. F. G. Römer, J. W. Viljeer, L. van den Beld, H. J. Slangewal and A. A. Veldkamp, *Atmos. Environ.* **19**(11), 1847 (1985).
33. R.K.A.M. Mallant, J. Slanina, G. Masuch and A. Kettrup, paper presented at the 2nd U.S.–Dutch symposium: AEROSOLS, Williamsburg (VA), U.S.A., May 1985.
34. G. Masuch, A. Kettrup, R.K.A.M. Mallant and J. Slanina, proceedings of the VDI-Kolloquium Waldschäden, VDI-Berichte 560, VDI Verlag Düsseldorf F.R.G., 1985, pp. 761–776.
35. A. Plomp, proceedings of ECLASS 85: 3rd. Int. Conf. on Liquid Atomisation and Spray systems-85, Imperial College London, July 8–10, pp. IIB/4/1–9, (1985).
36. J. M. Hochstrasser, The investigation and development of cyclone dust collector theories for application to miniature cyclone presamplers. Ph.D. Thesis, Univ. of Cincinnati (1976).
37. W. Barth, *Brennstoff-Warme-Kraft* **8,** 1 (1956).
38. G. L. Kok, T. Holler, M. Nachtrieb and M. Yuan, *Environ. Sci. Technol.* **12,** 1072 (1978).
39. D. D. Sabatini, K. Bensch and R. J. Barrnett, *J. Cell Biol.* **17,** 19 (1963).
40. G. E. Palade, *J. Exp. Med.* **95,** 285 (1952).
41. H. Kushida, *J. Electron Microsc.* **10,** 16 (1961).
42. A. R. Spurr, *J. Ultrastruct. Res.* **26,** 31 (1969).
43. B. F. Trump, E. A. Smuckler and E. P. Benditt, *J. Ultrastruct. Res.* **5,** 343 (1961).
44. M. Senser, F. Schötz and E. Beck, *Planta* **126,** 1 (1975).
45. S. Fink, *Allg. Forst Zeitschr.* 660 (1983).
46. G. Masuch, K. Vogels and R. Guderian, Unpublished results (1985).
47. S. Soikkeli, *Ann. Bot. Fennici* **18,** 47 (1981).
48. J. F. Chabot and B. F. Chabot, *Can. J. Bot.* **53,** 295 (1975).
49. A. Fahn, *Plant Anatomy* (Oxford, 1982), p. 42.
50. S. C. Chafe and D. J. Durzan, *Planta* **113,** 251 (1973).
51. W. Zalenski, *Abh. Tech. Hochsch. Kiew* **4,** Teil 1,1 (1904).
52. E. J. Salisbury, *Philos. Transact. Roy Soc. London* ser B. 216,1 (1927).
53. J. F. Wardlaw, *Austr. J. Biol. Sci.* **22,** 1 (1969).
54. E. W. R. Barlow and L. Boersma, *Agronomy Journ.* **68,** 923 (1976).
55. B. Huber, *Jahrbuch wiss. Bot.* **67,** 877 (1928).
56. W. R. Müller-Stoll, *Zeitschr. für Botanik* **29,** 161 (1935).
57. P. J. Kramer, *Water relations of plants,* (Academic press, New York, 1983).
58. H. Mohr and P. Schopfer, *Lehrbuch der Planzenphysiologie* (Springer Verlag, Berlin, Heidelberg, F.R.G., 1978).
59. B. Prinz, *J. Air Pollut. Control Assoc.* **35**(9), 913 (1985).