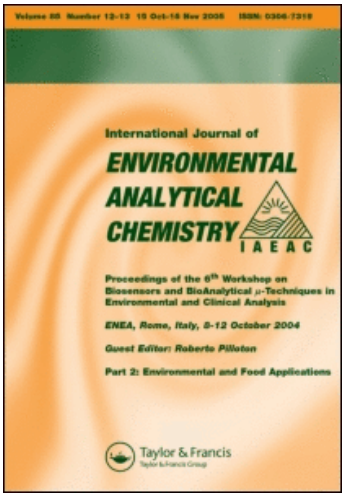

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Single and Combined Effects of Continuous and Discontinuous O_3 and SO_2 Immission on Norway Spruce Needles: I. Histological and Cytological Changes

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Single and Combined Effects of Continuous and Discontinuous O_3 and SO_2 Immission on Norway Spruce Needles

I. Histological and Cytological Changes

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For 22 months young Norway spruces (*Picea abies* Karst.) were separately and simultaneously exposed to a fumigation program, which was selected after the natural pollution values of O_3 and SO_2, both in clean-air areas and in industrial urban areas, had been determined. In all fumigation experiments we observed decreases in all histological parameters studied. Reductions in tissue areas and cell numbers of spruce needles were antagonistic after simultaneous exposures, simulating the clean-air area; those representing the industrial urban area were less than additive.

Discontinuous fumigation with O_3 separately and low constant-concentration exposure to SO_2, overlapped by occasional peak concentration of SO_2, had stronger reducing effects on vascular tissues than continuous fumigation with O_3 of the same concentration or continuous fumigation with SO_2, even with a higher concentration.

KEY WORDS: Forest die-back, ozone, sulphur dioxide, needle tissue alterations.

INTRODUCTION

In the vicinity of urban and industrial areas forest trees are generally exposed to mixtures of gaseous pollutants. As permanent measurements have pointed out, clean-air areas are polluted by mixtures of gaseous compounds too.[1] The main components of air pollutants, which are discussed to cause forest die-back, are SO_2 and O_3. Numerous studies on single and combined effects of ozone and sulphur dioxide on herbaceous plants have been published.[2–6]

Short-term exposures of a mixture of SO_2 and O_3 on *Pinus strobus* showed synergistic effects on the chlorotic dwarf syndrome.[7] Greater than additive effects of both pollutants on clonal stocks of *Pinus strobus* have been reported.[8] More than additive effects were also observed on sensitive clones of *Populus tremuloides*.[9]

The photosynthetic rates of Acer and Fraxinus subjected to combinations of SO_2 and O_3 were reduced synergistically.[10] Sequential exposures to the two gases one day prior to exposure to the mixture predisposed *Pinus strobus* to greater injury.[11] In two growth studies with a hybrid poplar, the combination of SO_2 and O_3 was found to have less than an additive effect in reducing foliar and shoot growth.[12,13] Long-term exposures of low concentrations of O_3 and SO_2 on *Populus* × *euramericana* and *Populus maximowiczii* showed nearly additive effects on loss of dry weight.[14]

In investigations on the resistance of forest trees against air pollutants there are three points of particular importance:

1) the pollutant concentration or dose,
2) the duration of exposure, and
3) the continuity or discontinuity of the pollution process.

As the concentrations of special pollutants change constantly in nature, experiments in fumigation chambers have to simulate the natural process of pollution. For this reason the data, found for the Ruhr area by the LIS* in Essen (FRG) serve as a basis for a simulation experiment with respect to an industrial urban area emitting pollutants. Clean-air areas, far away from emission sources, in Northrhine-Westfalia (FRG) show other patterns of pollutant

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concentrations. The measuring stations of the LIS in the Egge mountain and in the Eifel (FRG) reveal detailed information about these patterns. It is characteristic of industrial urban areas that O_3 is formed only discontinuously during the daylight period, whereas SO_2 is emitted continuously.

In clean-air areas, however, relatively constant O_3 concentrations are to be found, whereas there is an elevated background concentration of SO_2, which is occasionally overlapped by peak concentrations. According to the survey of the forest decline in Northrhine-Westfalia in 1986, 65–85% of the forest area are damaged in the industrial urban areas of the Ruhr whilst only 35–50% of the forests in the clean-air areas of the Eifel and the Egge Mountain have suffered damage.[15] Thus, fumigations either single or combined with the main pollutants O_3 and SO_2, containing the natural concentrations of either an industrial urban area or a clean-air area can give information about which effects the pollutant dose or the sequential exposures (continuity/discontinuity) have on forest trees.

Exemplified by Norway spruce needles (*Picea abies* Karst.), separately and simultaneously exposed to ozone and sulphur dioxide, it is the objective of this part of our study to compare the histological changes of the spruce needles quantitatively, to document ultrastructural changes of the chloroplasts, and to find out the accumulation of phenolic compounds in the vacuoles of mesophyll cells.

EXPERIMENTAL

Fumigation experiments

The fumigation chambers used in the experiment were small exposition glass houses of the LIS in Essen-Kettwig, placed in the open air and sheltered from direct sunlight. Potted young trees were located onto a rotating stage, filled with 6 pots each, that made it possible for all trees to get equal light qualities and quantities. The temperature inside the exposition houses was changing, following the temperature outside the houses. During the summer months the inside temperature was elevated by 2–3 °C; the relative humidity decreased correspondingly.

The exposition houses received charcoal-filtered air. The fumigations were performed with O_3 and SO_2, either separately or in combination, according to the program of Table 1. For these experiments 3–5 year old Norway spruces (*Picea abies* Karst.) were obtained from a tree nursery (Hanses Koering, Münster, FRG) and exposed after the above mentioned program from 23/1/1984 to 18/11/1985. All trees were potted in ED-73 (Fruhstorfer Einheitserde). The fumigation experiments were performed by the LIS in Essen-Kettwig (Dr. B. Prinz and Dr. G. H. M. Krause) and the histological, histochemical and cytological investigations by our group at the University of Paderborn.

Biological methodology

Preparation of spruce needles was as follows: Sections out of the middle of the needle of 2 mm length were fixed at room temperature for 2 h in 2% glutardialdehyde,[16] followed by a terminal fixation for 1 h in a 1% solution of OsO_4.[17] Alternatively needle pieces were fixed for 3 h at room temperature in a solution of 1% OsO_4 and 2.5% $K_2Cr_2O_7$ buffered by 0.1 M phosphate.[19] After a dehydration sequence in ethanol, the fixed sections were embedded in styrene-methacrylate.[19] Slices of 1 and 0.3 μm thickness were cut with a LKB

Table 1 Experimental exposure program

No.	*Pollutant concentration*	*Duration of exposure*	*Abbreviation*
Reference series			
1	Charcoal-filtered air	continuously	R
Clean-air area			
2	$60 \pm 7\ \mu g\ SO_2/m^3$	continuously	
	$+500\ \mu g\ SO_2/m^3$	2 h with 14-day intervals	CA (SO_2)
3	$130 \pm 8\ \mu g\ O_3/m^3$	continuously	CA (O_3)
4	$60 \pm 8\ \mu g\ SO_2/m^3$	continuously	
	$+500\ \mu g\ SO_2/m^3$	2 h with 14-day intervals	
	$+130 \pm 28\ \mu g\ O_3/m^3$	continuously	CA (SO_2+O_3)
Industrial urban area			
5	$99 \pm 9\ \mu g\ SO_2/m^3$	continuously	IU (SO_2)
6	$132 \pm 24\ \mu g\ O_3/m^3$	5 h/day	IU (O_3)
7	$98 \pm 7\ \mu g\ SO_2/m^3$	continuously	
	$+125 \pm 30\ \mu g\ O_3/m^3$	5 h/day	IU (SO_2+O_3)

Ultrotome III and a Reichert Ultracut E, respectively. The cuts were coloured by Toluidinblue.[20] Preparation for scanning electron microscopy consisted of fixation, breaking in liquid nitrogen, dehydration in ethanol, critical-point drying and coating with gold by sputtering. In an alternative preparation semithin sections were extracted from resin by xylene, dried, mounted and coated with gold by sputtering.

A Leitz-Orthomat was used for light microscope studies. Quantitative analysis of ten microscope pictures per experimental series were made with the aid of a digitizer tablet, connected to a microcomputer. Electron microscopic studies were performed with a Hitachi H 3010 scanning electron microscope.

RESULTS

Needle transverse sections

In the needle transverse section a longer vertical ray axis can be distinguished from a shorter horizontal cambium axis. In the needles of all the fumigation series the ray axes are reduced. The cambium axes are less reduced. No difference can be observed in the cambium axis of needles fumigated simultaneously with O_3 and SO_2, simulating the clean-air area. The highest reduction of the cambium axis we observed in needles exposed to O_3 and SO_2 simultaneously, simulating the industrial urban area (Figure 1).

The outline of the needle transverse sections is long-rhombic in the reference needles; under the influence of O_3 it is wide-rhombic, under the influence of SO_2 high-rhombic. Fumigated needles do not show a difference in comparison to the reference needles in the case that the fumigation simulated the clean-air area. Needles from the experimental series, that simulated the industrial urban area, do not obtain a different outline compared with reference needles, but the transverse section area is distinctly smaller.

Mesophyll

The tissue areas of the needle transverse sections are reduced in all fumigation series compared with the reference series (Figures 2 and 3). The lowest reduction rate was observed in the combined SO_2/O_3 fumigation series, which simulates the clean-air area, the highest

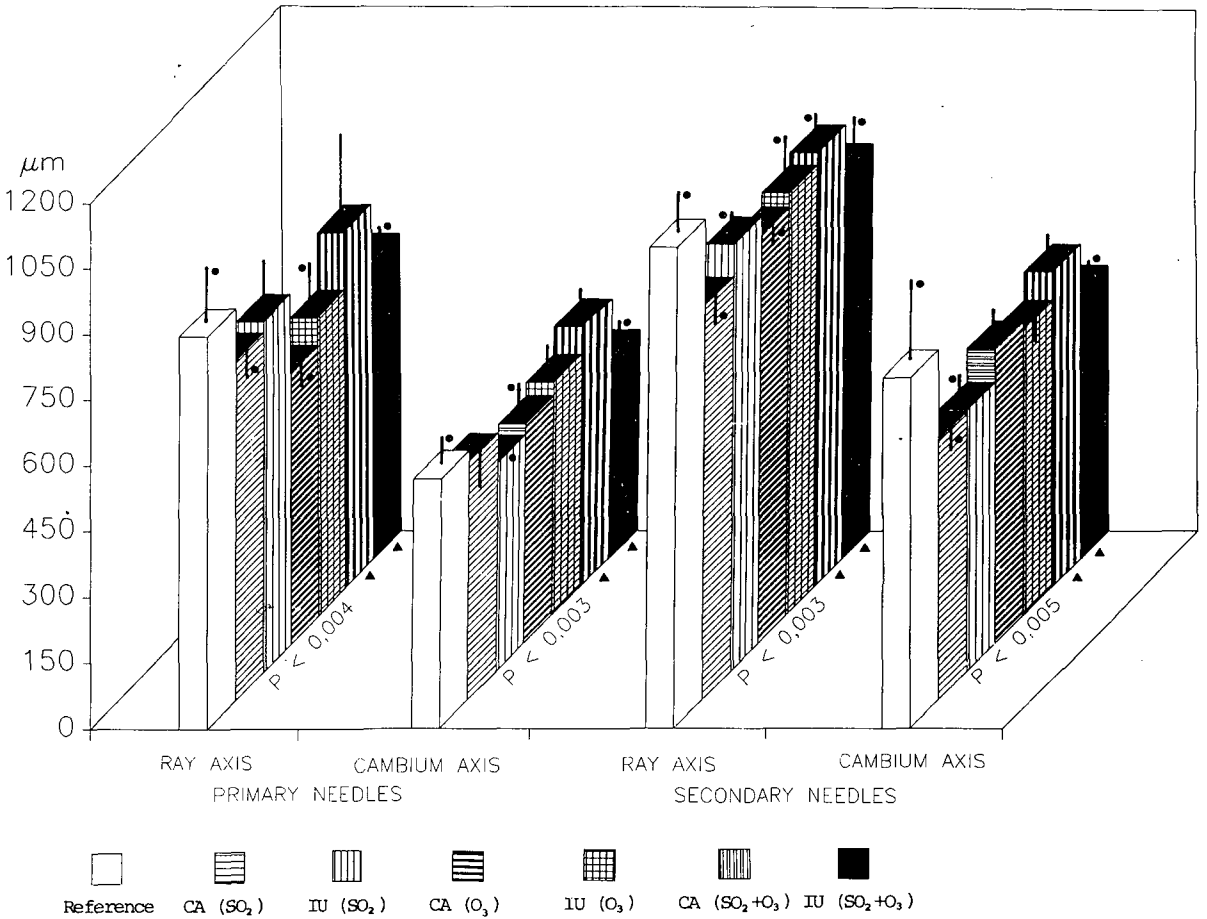

Figure 1 Transverse diameters of Norway spruce needles. Significant differences from references are shown with an * at the top of the vertical bars for standard deviation at the 0.005 level according to the Duncan test. Triangles (▲ or △) at the bottom of the columns indicate significant differences between two experimental series representing the clean-air area or the industrial urban area.

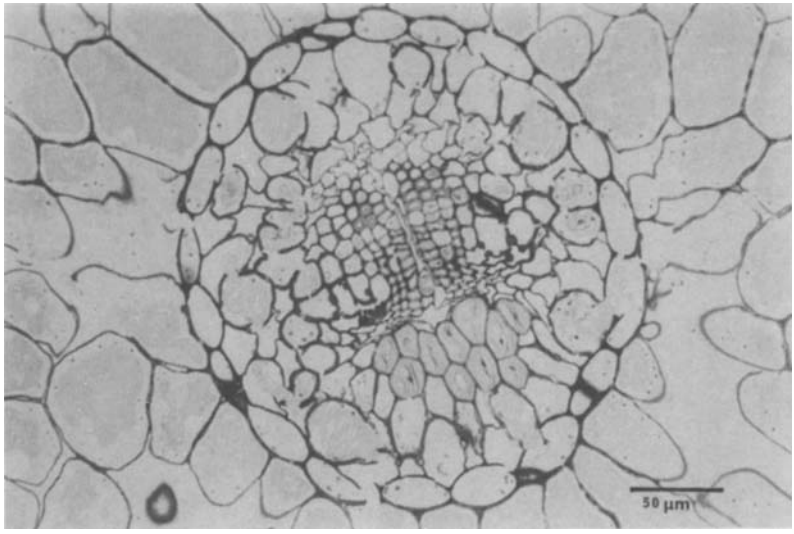

Figure 2 Transverse section of a vascular bundle of a secondary reference series.

reduction rate was found in the combined SO_2/O_3 fumigation series, that represents the industrial urban area. The transverse section areas of needles exposed either to O_3 or SO_2 separately, in concentrations simulating the clean-air area, are more reduced than those exposed simultaneously to SO_2 and O_3. This seems to be an antagonistic effect between both pollutants.

On the other hand, the transverse section areas of needles exposed to continuous fumigation with SO_2 or discontinuous fumigation with O_3 separately, which simulates the industrial urban area, show less reducing effects than a combined fumigation with both pollutants. This effect of a simultaneous fumigation is less than additive.

Under the influence of O_3 emitted continuously we observed the highest decrease of the transverse section area and mesophyll cell area. The intercellular spaces or the mesophyll cells occupy a certain portion of the total transverse section area. If these portions are compared with each other for the different fumigation series, then the tissue changes become evident (Table 2). Influenced by O_3, the areal percentages of the cellular mesophyll decrease whilst those of the intercellular spaces increase compared with the data of the reference needles. Similar changes can be observed under the influence of SO_2, but the data are less striking. Combined fumigation

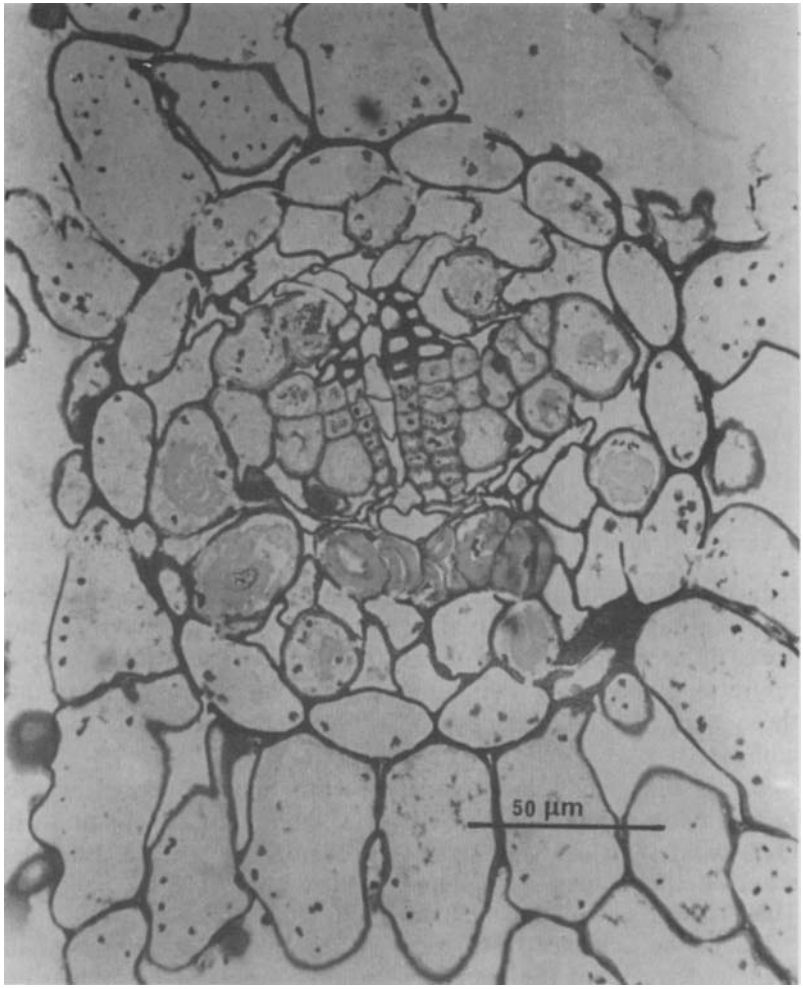

Figure 3 Transverse section of a vascular bundle of a secondary needle of Norway spruce from the SO_2-exposed set, representing the clean-air area.

of O_3 and SO_2 shows values that range between those of separate fumigations with O_3 and SO_2. Percentage decrease in the mesophyll cell area and percentage increase in the intercellular space area can be seen more clearly in the secondary needles than in the primary needles. It has to be pointed out that the secondary needles were

Table 2 Percentages of tissue areas of the related needle transverse section area of Norway spruce

	Primary needles		*Secondary needles*	
	Area of mesophyll cells	*Area of intercellular space*	*Area of mesophyll cells*	*Area of intercellular space*
Reference	60±3.6	17.6±2.9	64±1.2	14.4±2.0
CA (SO_2)	58±5.0	13.8±3.6[a]	55±4.5[a]	20.0±4.7[a]
IU (SO_2)	56±2.5[a]	19.0±3.1	53±2.5[a]	19.4±1.7[a]
CA (O_3)	53±2.7[a]	17.8±3.4	50±2.8[a]	23.7±1.9[a]
IU (O_3)	51±5.3[a]	21.9±4.5[a]	53±7.3[a]	20.5±5.7[a]
CA (SO_2+O_3)	55±4.4[a]	18.5±2.7	52±3.0[a]	22.0±3.0[a]
IU (SO_2+O_3)	56±2.5[a]	18.3±2.4	54±3.0[a]	29.5±2.4[a]
	[a]$P \leqq 0.02$	[a]$P \leqq 0.025$	[a]$P \leqq 0.0001$	[a]$P \leqq 0.0005$

[a]P in comparison to reference needles.

Table 3 Mesophyll cell numbers per needle transverse section area and average area (μm^2) per mesophyll

	Primary needles		*Secondary needles*	
	Number of mesophyll cells	*Average area per mesophyll cell*	*Number of mesophyll cells*	*Average area per mesophyll cell*
R	158±15	1340±240	220±50	1740±270
CA (SO_2)	146±20	1145±260	155±10[a]	1230±210[a]
IU (SO_2)	136±12[a]	1080± 80[a]	200±10	1100± 70[a]
CA (O_3)	125±12[a]	850±140[a]	160±14[a]	1260±170[a]
IU (O_3)	127±12[a]	945±137[a]	140±20[a]	1540±280
CA (SO_2+O_3)	144±25	1190±230	200±25	1190±230[a]
IU (SO_2+O_3)	116± 7[a]	1020±150[a]	150±20[a]	1250±270[a]

[a]$P < 0.005$.

exposed to the pollutants from the beginning of their growth in the first year.

In the primary and secondary needles we observed decreases in the average transverse section area per mesophyll cell and in mesophyll cell numbers in all series exposed to pollutants (Table 3). Separate fumigation with O_3 caused a stronger decline in mesophyll

cell numbers than separate exposure to SO_2. The smallest mesophyll cells we observed in primary needles fumigated with O_3 continuously.

Vascular bundle tissues

The tissues of the vascular bundle in spruce needles decrease after separate treatment with SO_2 or O_3; SO_2 has a more reducing effect than O_3 (Figures 2–4).

Simultaneous exposures, simulating the SO_2 and O_3 concentrations of an industrial urban area, cause a strong decrease in the transverse section areas of the vascular bundle (Figure 5), whereas the experiments simulating the pollutant concentration of clean-air areas, do not affect the total areas of the vascular bundle (Figures 6 and 7). The areas of the vascular bundle do not decrease to the same extent as the transverse section areas of spruce needles (Table 4). Under the influence of SO_2 the percentage of the vascular bundle area decreases more than that of the needle cross-cut. By the fumigation with O_3 a stronger percentage reduction of the area of the total needle cross-cut is obtained than that of the vascular bundle.

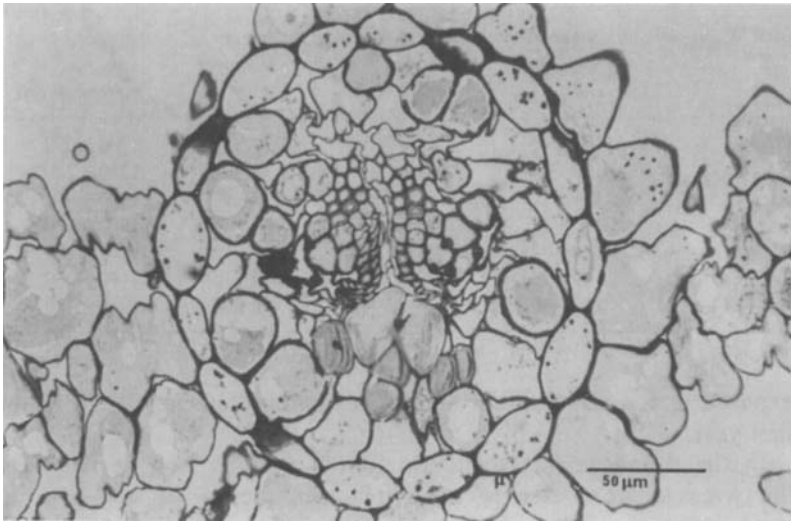

Figure 4 Transverse section of a vascular bundle of a secondary needle of Norway spruce from the O_3-exposed set, representing the industrial urban area.

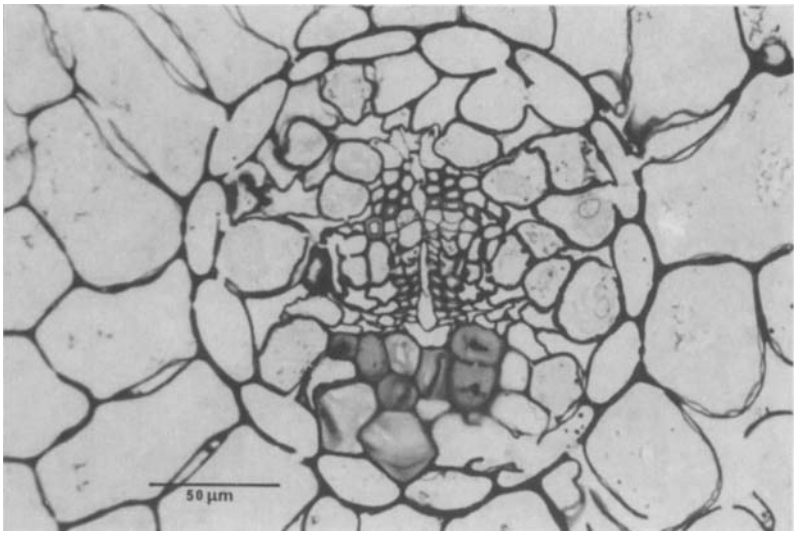

Figure 5 Transverse section of a vascular bundle of a secondary needle of Norway spruce from the set exposed to SO_2+O_3, representing the industrial urban area.

The tissue areas of the xylem and the intact phloem are reduced in the needles of all the fumigation series compared with the needles of the reference trees (Figures 8 and 9, Table 5).

Spruces fumigated with an elevated background concentration of 60 μg SO_2/m^3 and short, occasional peaks (500 μg SO_2/m^3 for 2 h with 14-day intervals) had a stronger decline in the areas of the xylem and the phloem than spruces fumigated with a constant SO_2 concentration of 100 $\mu g/m^3$. Furthermore, the numbers of tracheids, cambial cell rows and intact sieve tubes of secondary needles were significantly reduced.

Discontinuous fumigation with 130 μg O_3/m^3 (5 hours daily) has a stronger reducing effect on xylem and phloem tissue areas than continuous fumigation of O_3 containing the same concentration.

The numbers of tracheids, cambial cell rows, and intact sieve tubes are significantly reduced in primary as well as secondary needles (Figures 10 and 11). Combined fumigation with SO_2 and O_3 has an antagonistic effect on the reduction of the xylem and phloem transverse section and related parameters, if simulating the clean-air area, but less than an additive effect if simulating the pollutant

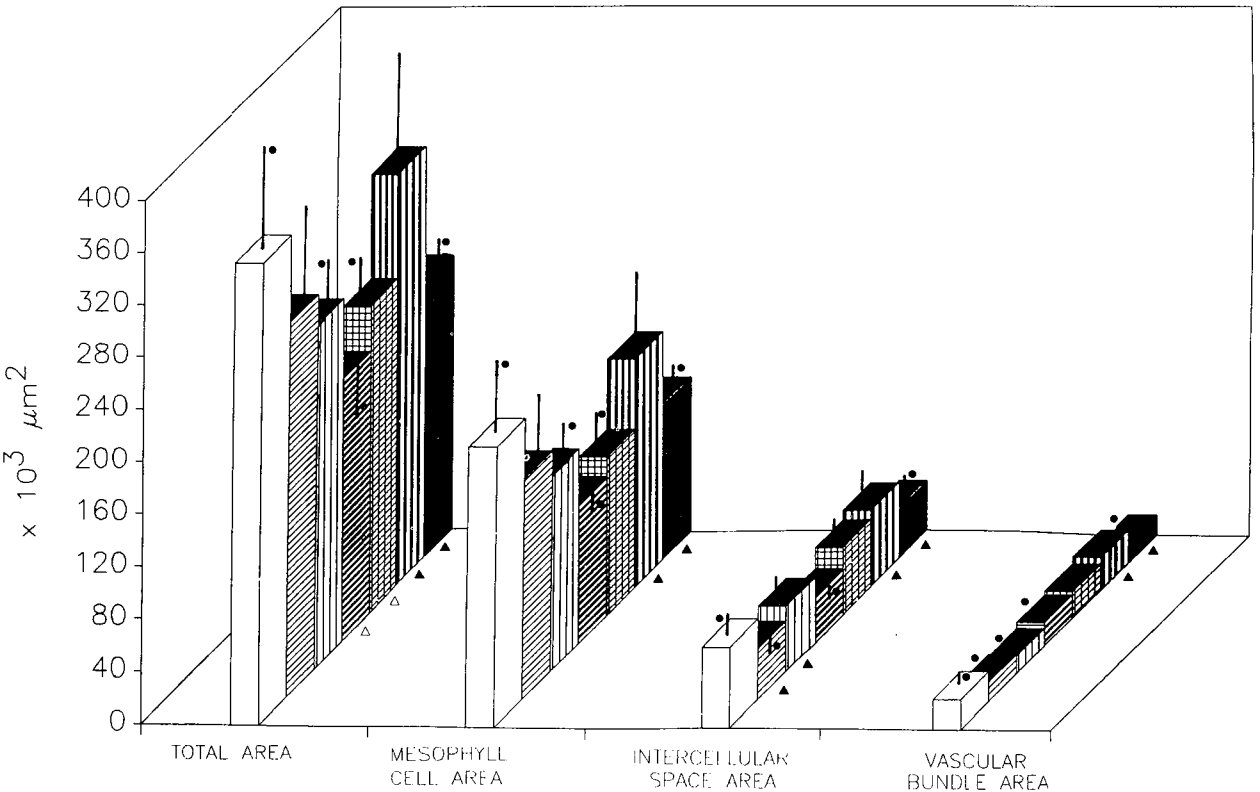

Figure 6 Tissue areas of transverse section of primary needles of Norway spruce. The significance levels are at the 0.05 level. Further explanations, see legend to Figure 1.

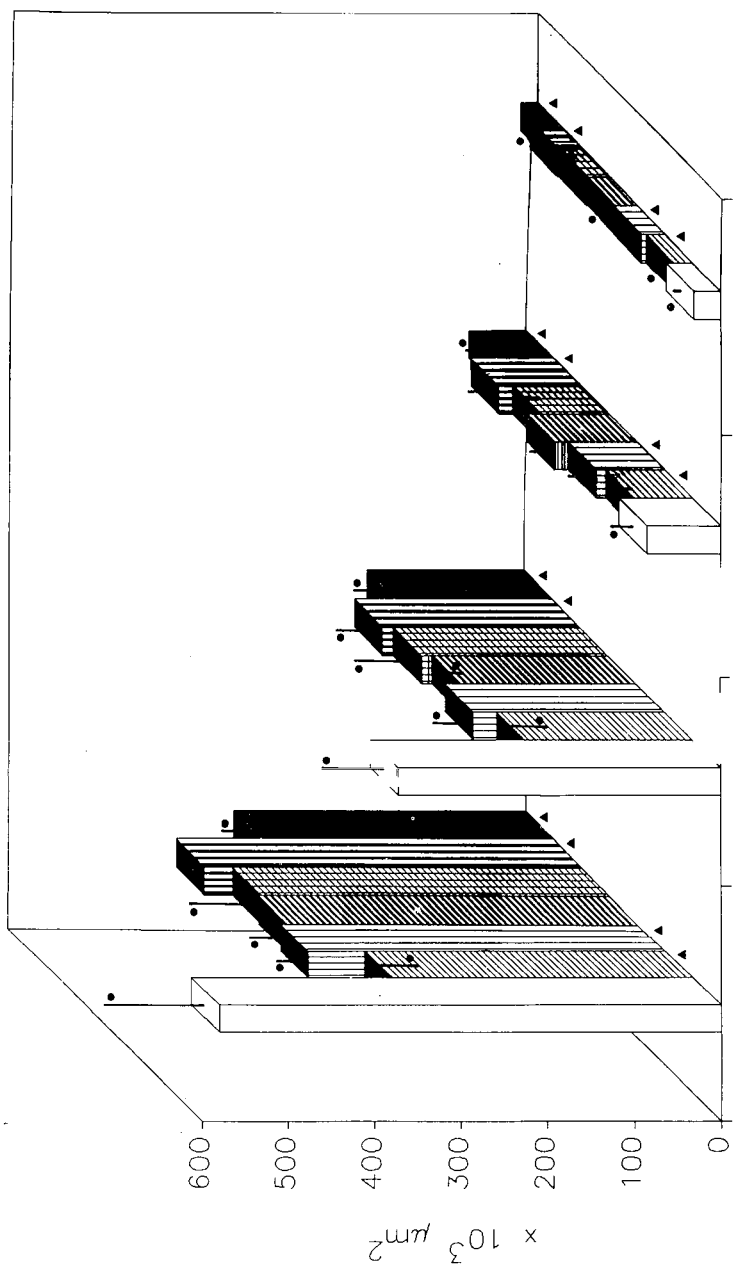

Figure 7 Tissue areas of transverse section of secondary needles of Norway spruce. The significance levels are at the 0.01 level. For further explanation, see legend to Figure 1.

Table 4 Percentage decrease (increase) of tissue areas in comparison to those of the reference needles of Norway spruce

	Primary needles		*Secondary needles*	
	Needle cross-cut	*Vascular bundle*	*Needle cross-cut*	*Vascular bundle*
Reference	353 000 $\pm 77\,000\ \mu m^2$	23 000 $\pm 5000\ \mu m^2$	581 000 $\pm 115\,000\ \mu m^2$	81 000 $\pm 7600\ \mu m^2$
CA (SO_2)	−19	−35	−40	−28
IU (SO_2)	−26	−41	−29	−11
CA (O_3)	−43	−23	−30	−17
IU (O_3)	−33	−11	−30	−12
CA (SO_2+O_3)	−11	+12	−25	− 6
IU (SO_2+O_3)	−41	−30	−42	−35

concentration of an urban industrial area. The symptoms observed are more similar to those caused by separate fumigation with O_3 than that with SO_2.

The more striking effects were observed in experiments of continuous SO_2 fumigation simultaneously with discontinuously emitted O_3 than in those with continuous O_3 fumigation combined with continuous SO_2 fumigation.

The reductions of the transverse section areas of xylem and phloem can be put down to the fact that the cambium layer is less developed. From these cambium cells the rows of xylem and phloem cells develop.

Cytological changes of mesophyll cells

The incorporation of the total phenolic compounds into the central vacuoles of the mesophyll cells had increased in all experimental series fumigated separately and simultaneously with O_3 and SO_2. On SEM pictures the polyphenolics look like reticulated droplets (Figure 12). With increasing amount of total phenolics the diameter of the droplets decreases, the degree of reticulation is increased, and the distances between the droplets become shorter (Figure 13).

Structural changes of the chloroplasts can be observed in mesophyll cells of all fumigated spruces. Chloroplasts of secondary control needles are filled up with thylakoids, only single dilatations

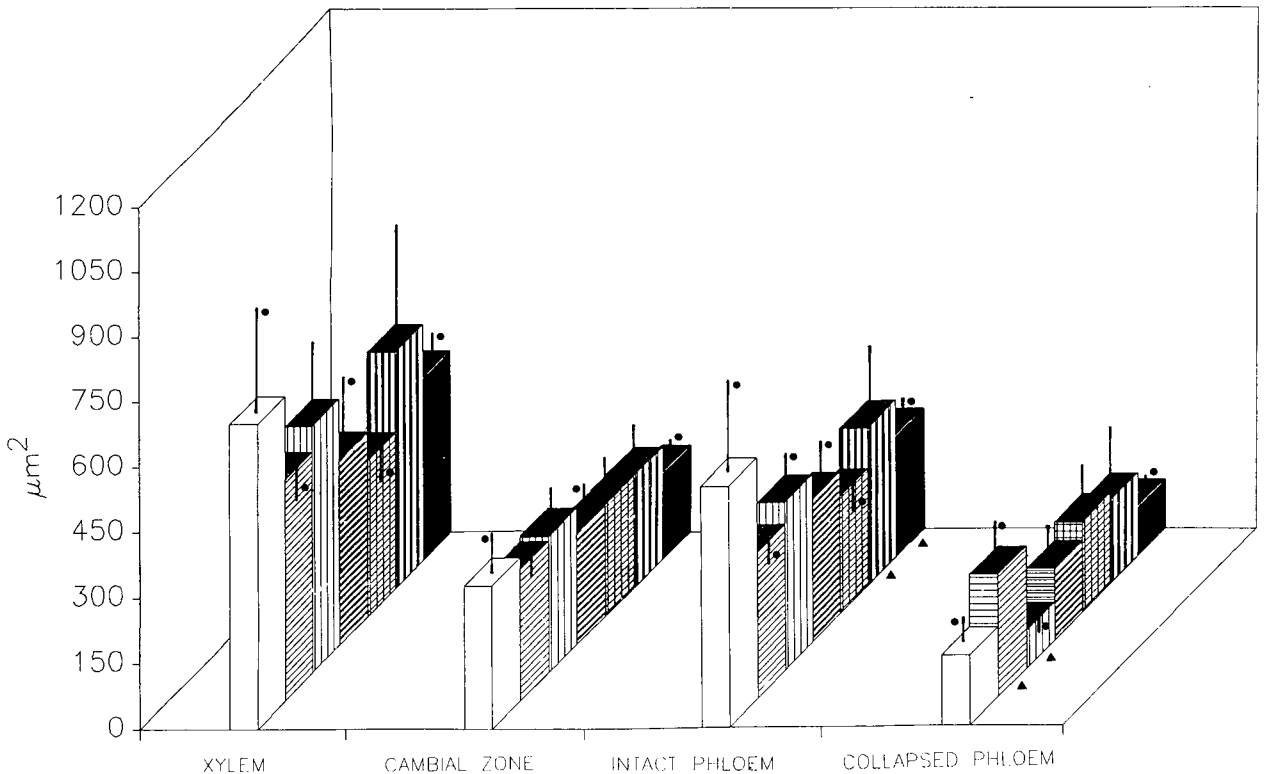

Figure 8 Tissue areas of the vascular bundle of primary needles of Norway spruce as seen in transverse sections. The significance levels are at the 0.05 level. For further explanation, see legend to Figure 1.

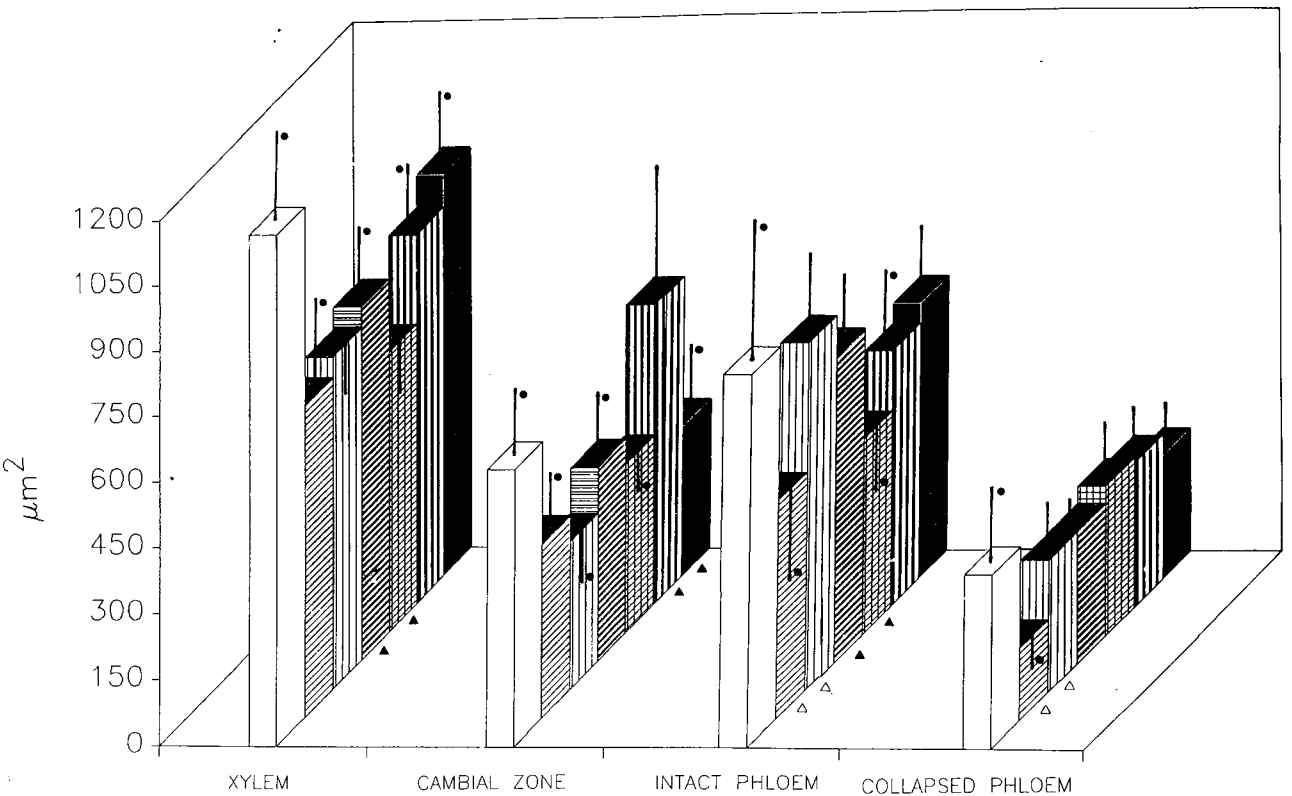

Figure 9 Tissue areas of the vascular bundle of secondary needles of Norway spruce as seen in transverse sections. The significance levels are at the 0.02 level. For further explanation, see legend to Figure 1.

Table 5 Percentage decrease of vascular tissue areas in comparison to reference needles of Norway spruce

	Primary needles		*Secondary needles*	
	Xylem	*Intact phloem*	*Xylem*	*Intact phloem*
CA (SO_2)	−27	−39	−39	−42
IU (SO_2)	−19	−31	−35	− 7
CA (O_3)	−39	−41	−31	−18
IU (O_3)	−49	−51	−45	−48
CA (SO_2+O_3)	−22	−35	−30	−31
IU (SO_2+O_3)	−40	−50	−22	−26

can be seen (Figure 14). Structural changes in chloroplasts after simultaneous and separate fumigation with O_3 and SO_2 are dilatations of thylakoidal membranes and occurrence of plastoglobules. Chloroplasts fumigated continuously with O_3 contained the highest amount of dilated thylakoids (Figure 15).

Discontinuous exposure to O_3 brought about smaller dilated thylakoids. Chloroplasts injured by SO_2 after intermitting peak concentrations and an elevated background concentration were more damaged than chloroplasts continuously exposed to higher SO_2 concentrations ($100\,\mu g/m^3$) (Figure 16). After simultaneous fumigation, simulating the clean-air area, chloroplasts contain some large and a great number of slight dilated thylakoids. The injury pattern of the combined SO_2/O_3 fumigation, representing the industrial urban area, is indicated by accumulations of plastoglobules and dilated thylakoids too (Figure 17).

DISCUSSION OF RESULTS

Although nearly all SO_2/O_3 interaction studies have employed simultaneous exposures to the two pollutants, the potentiation effect of sequential or discontinuous exposures to the pollutants has received little attention. Pretreatment of herbaceous plants with SO_2 has been shown to alter the response of plants to a succeeding exposure to SO_2 and O_3 in combination.[21] As we have demon-

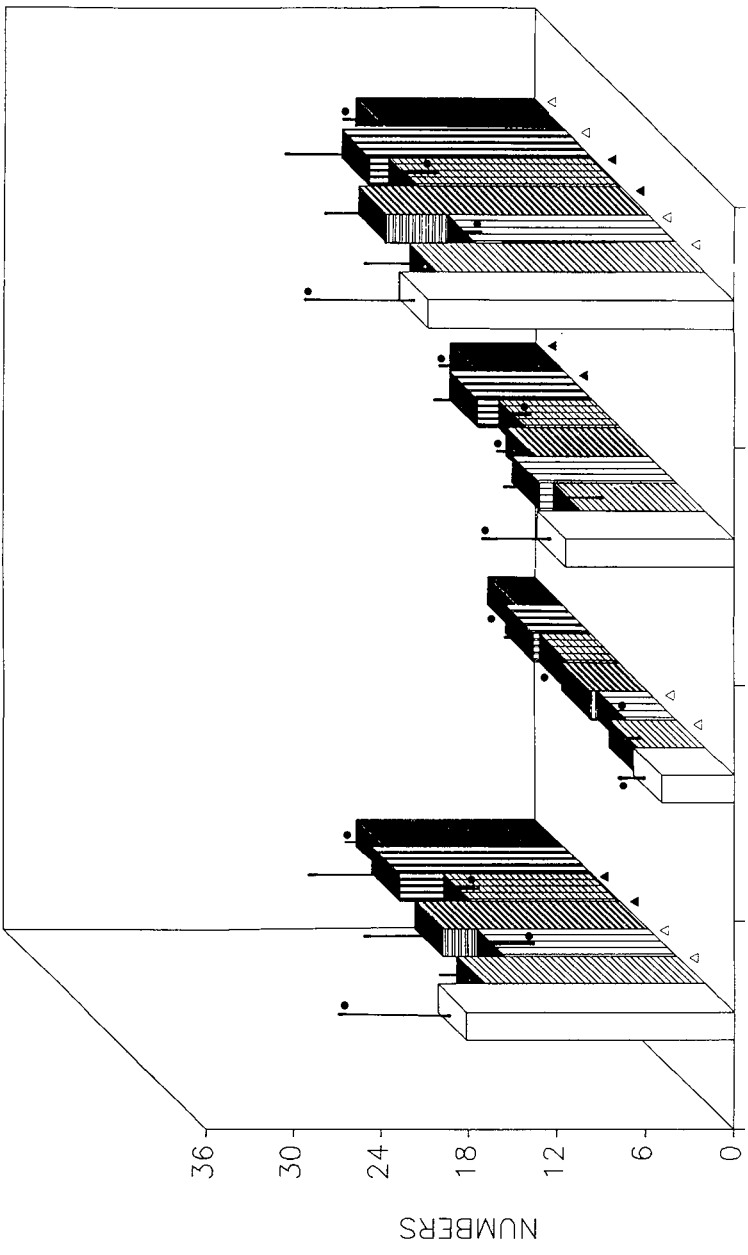

Figure 10 Cell numbers or cell rows of vascular bundle tissues of primary needles of Norway spruce as seen in transverse sections. The significance levels are at the 0.05 level. For further explanation, see legend to Figure 1.

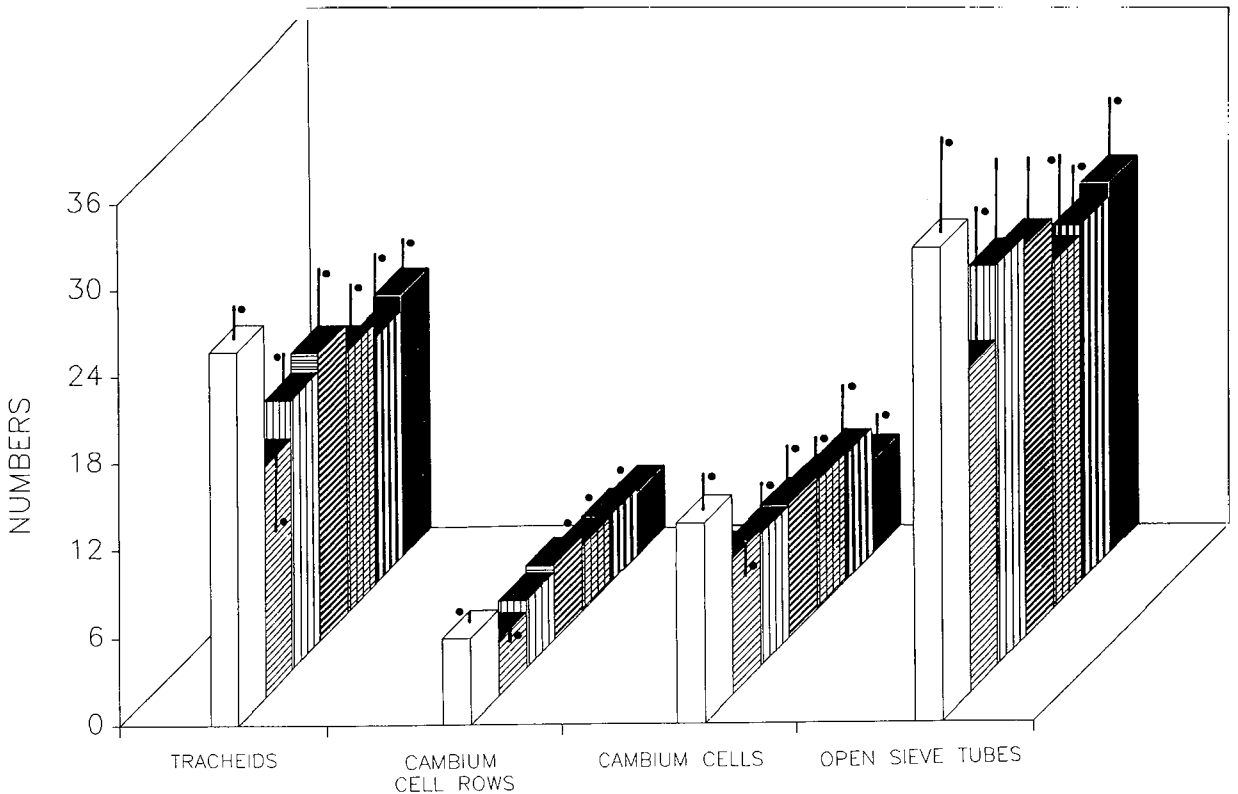

Figure 11 Cell numbers or cell rows of vascular bundle tissues of secondary needles of Norway spruce as seen in transverse sections. The significance levels are at the 0.005 level.

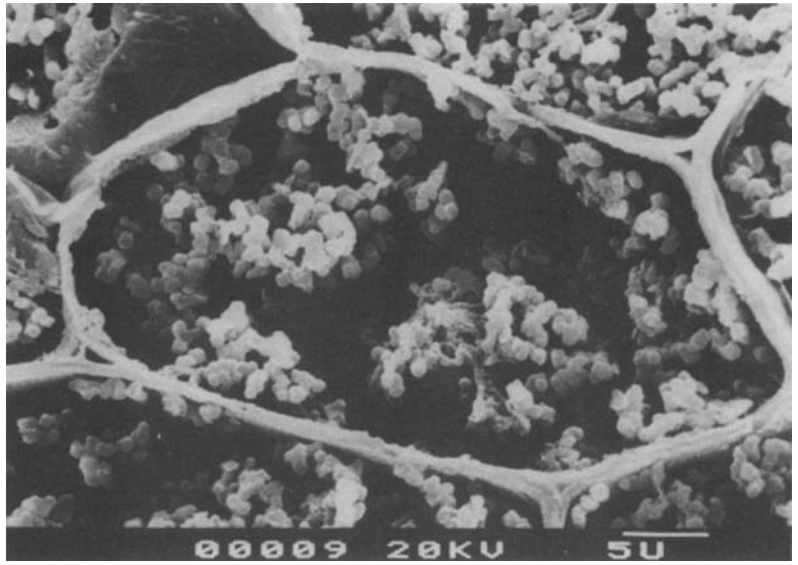

Figure 12 Scanning electron microscopic picture of a mesophyll cell of a primary needle of Norway spruce from the reference series, showing large droplets of phenolic compounds inside the central vacuole.

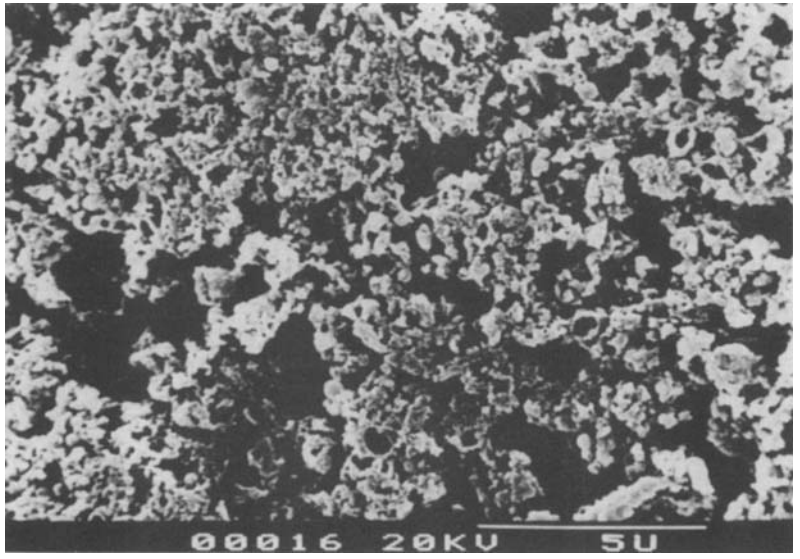

Figure 13 Densely located small phenolic droplets out of the central vacuole of a mesophyll cell of a primary spruce needle for the combined SO_2/O_3 fumigation series, simulating the industrial urban area.

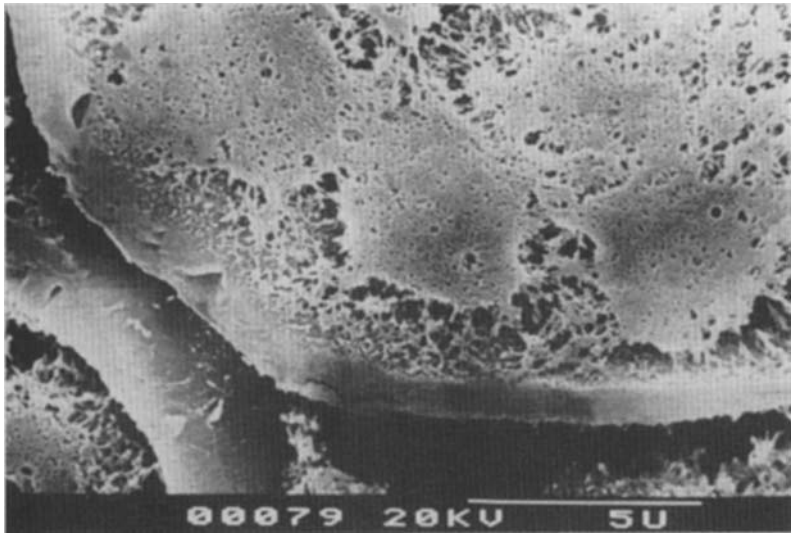

Figure 14 Scanning electron microscopic picture of a section of a mesophyll cell of a secondary spruce needle from the reference series, showing intact chloroplasts.

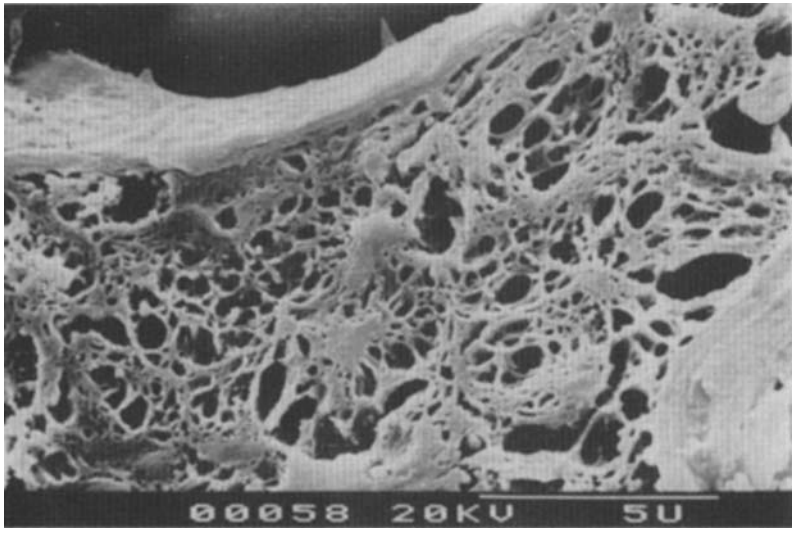

Figure 15 Close-up of some chloroplasts with strongly dilated thylakoids of a secondary needle from the ozone series, continuously fumigated with 130 μg O_3 (SEM picture).

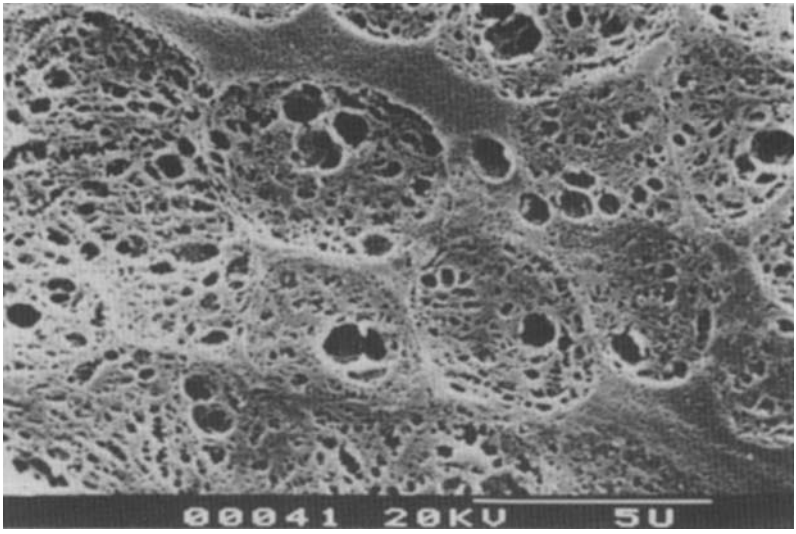

Figure 16 Mesophyll cell chloroplasts of a secondary spruce needle, continuously exposed to 60 μg SO_2/m^3 and, intermittently, to peak concentrations of 500 μg SO_2/m^3 for 2 h with 14-day intervals. Thylakoids are dilated (SEM picture).

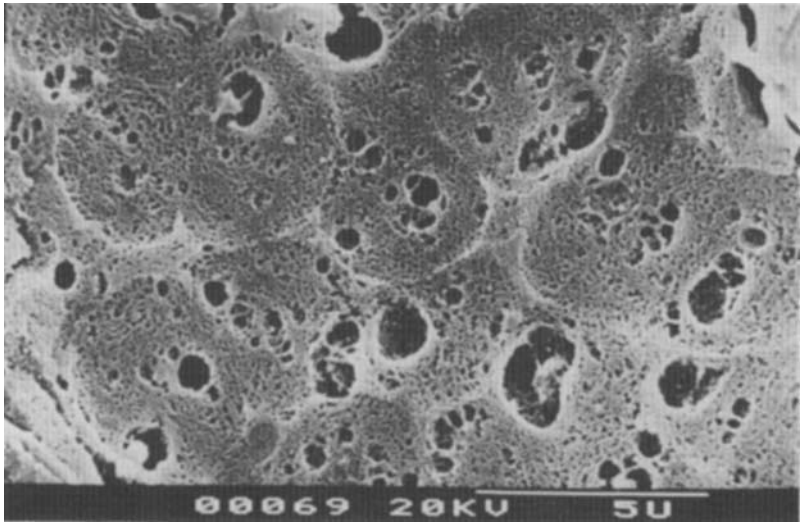

Figure 17 Mesophyll cell chloroplasts of a secondary spruce needle, simultaneously fumigated with SO_2 and O_3, representing the industrial urban area. Thylakoids are dilated and to some extent vesiculated (SEM picture).

strated, discontinuous exposure to SO_2 or O_3 (Tables 6 and 7) and to combinations of SO_2 and O_3 (Table 8) cause greater decreases in tissue areas and cell numbers than continuous fumigation. There are more decreases in the tissue areas of the vascular bundle than in that of the needle mesophyll. Imbalances of water transport via xylem vessels and assimilate transport via phloem vessels are effects and causes of tissue alterations. Many injuring symptoms can be related to the deficit of water and dissolved nutrients. Ozone attacks the cellular membrane system and supports the weathering of the cuticle of the needle.[22] Cellular membranes of leaf organs are the primary

Table 6 Summary of results; discontinuous exposure of O_3 ($100\,\mu g$ O_3/m^3, 5 h daily) has more decreasing effects on transverse tissue areas (a) or cell numbers (n) than continuous exposure

Primary needles	*Secondary needles*
—	intercellular space (a)
xylem (a)	xylem (a)
tracheids (n)	tracheids (n)
cambial cell rows (n)	cambial cell rows (n)
intact phloem (a)	intact phloem (a)
sieve tubes	sieve tubes

Table 7 Summary of results; discontinuous exposure of SO_2 ($60\,\mu g\,SO_2/m^3$ constantly; $500\,\mu g/m^3$ for 2 h with 14-day intervals) has more decreasing effects on transverse tissue areas (a) or cell numbers (n) of spruce needles than continuous exposure ($60\,\mu g$ SO_2/m^3 continuously)

Primary needles	*Secondary needles*
—[a]	total cross-cut (a)
mesophyll cell (n)	mesophyll cell (a, n)
intercell. space (a)	intercell. space (a)
—[a]	vascular bundle (a)
xylem (a)	xylem (a)
—[a]	tracheids (n)
intact phloem (a)	intact phloem (a)
—[a]	sieve tubes (n)

—[a]; no changes observed.

Table 8 Summary of results; simultaneous exposure of SO_2+O_3 simulating the industrial urban area has more decreasing effect on transverse tissue areas (a) or cell numbers (n) of spruce needles than that simulating the clean-air area

Primary needles	*Secondary needles*
total cross-cut (a)	total cross-cut (a)
mesophyll cells (a)	mesophyll cells (a)
mesophyll cells (n)	mesophyll cells (n)
intercell. space (a)	intercell. space (a)
vascular bundle (a)	vascular bundle (a)
(xylem (a))	—[a]
(tracheids)	—[a]
cambial zone (a)	cambial zone (a)
(cambial cell rows (II))	(cambial cell rows (n))
intact phloem	—[a]
sieve tubes	—[a]

—[a]; no changes observed.

( ); no significance.

site of ozone action, resulting in an increased water and ion loss.[23,24] Membrane damage may occur without visible injury[25] and is followed by cation efflux,[26] so that essential nutrients including anions can be leached from the needles by fog and/or rain.[27]

Water deficit causes changes in metabolism of spruce needles, which leads to an premature senescence with increasing catabolic and decreasing anabolic metabolisms. These can be observed in starch congestion in the chloroplasts on one side[28] and decay of starch corresponding with an increased synthesis of phenolic compounds on the other side.[29] There are some accompanying phenomena to water deficit in leaves and needles: structural degeneration of chloroplasts,[30] decay of chlorophylls[31] and decrease in the rate of photosynthesis.[32] Some histological changes refer to chronic water deficit: reductions in xylem vessels and sieve tubes, originating from reduced divisions of the cambium cells. The rate of mitosis as well as the growth of cells can be hindered by water stress.[33,34]

When the water content in a leaf is minimized and its turgor is reduced, the downward transport of assimilates in the phloem can be stopped.[35] The translocations of assimilates out of the leaf can be more reduced by water stress than the rate of photosynthesis.[36] The incorporation of phenolic compounds into the central vacuoles of

the mesophyll is suited to prevent a dehydration of the spruce needles.[37]

CONCLUSIONS

The results of experiments in which young spruce trees are exposed to SO_2 and O_3, separately and simultaneously, show that the tissues of needles are more affected by discontinuous fumigation, causing an interactive air pollution pattern of O_3, SO_2, SO_3^{2-} and SO_4^{2-}, which corresponds to the simulation model of an urban industrial area, than by continuous fumigation experiments. There are indications that the young trees have a reduced capacity for transport of water and assimilates due to reduction of the tissues of the vascular bundle. These changes are also observed in spruce trees of the Egge Mountain in Northrhine-Westfalia.

It is emphasized that the changed histological and cytological pattern can be attributed to water stress. More information is needed about water potential, rate of photosynthesis, loss of nutrients by leaching, metabolic parameters, and quantitative identification of phenolic compounds.

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References

1. H.-V. Pfeffer, *Staub-Reinhalt. Luft* **45,** 287 (1985).
2. H. A. Menser and H. E. Heggestad, *Science* **153,** 424 (1966).
3. J. B. Mudd and T. T. Kozlowski, *Responses of Plants to Air Pollution* (Academic Press, New York, 1975).
4. D. T. Tingey and R. A. Reinert, *Environ. Pollut.* **9,** 117 (1975).
5. R. Kohut. In: *Sulfur Dioxide and Vegetation* (W. E. Winner, H. A. Mooney and R. A. Goldstein, eds.) (Standford Univ. Press, Stanford, 1985), pp. 296–312.
6. K. Vogels and G. Masuch. In: *The Evaluation and Assessment of the Effects of Photochemical Oxidants on Human Health, Agricultural Crops, Forestry, Materials and Visibility*, Proceedings Intern. Workshop, Göteborg 1984 (P. Grennfelt, ed.), Swedish Environ. Res. Inst., Göteborg, 1984.

7. L. S. Dochinger, F. W. Bender, F. O. Fox and W. W. Heck, *Nature* **225,** 476 (1970).
8. D. B. Houston, *Can. J. Forest Res.* **4,** 65 (1974).
9. D. F. Karnosky, *Can. J. Forest Res.* **6,** 166 (1976).
10. R. W. Carlson, *Environ. Pollut.* **18,** 159 (1979).
11. A. C. Costonis, *Eur. J. For. Path.* **3,** 50 (1973).
12. R. D. Noble and K. F. Jensen, *Am. J. Bot.* **67,** 1005 (1980).
13. K. F. Jensen, *Environ. Pollut.* **26,** 243 (1981).
14. J. Mooi, *Forst- und Holzwirt* **39,** 438 (1984).
15. Umweltbundesamt, Daten zur Umwelt 1986/87, Erich Schmidt Verlag, Berlin, 1986.
16. D. D. Sabatini, K. Bensch and R. J. Barnett, *J. Cell. Biol.* **17,** 19 (1963).
17. G. E. Palade, *J. Exp. Med.* **95,** 285 (1952).
18. M. Senser, F. Schötz and E. Beck, *Planta* **126,** 1 (1975).
19. H. Kushida, *J. Electron Microsc.* **10,** 16 (1961).
20. B. F. Trump, E. A. Smuckler and E. P. Benditt, *J. Ultrastruct. Res.* **5,** 343 (1961).
21. G. Hofstra and D. W. Beckerson, *Atmos. Environ.* **15,** 383 (1981).
22. S. Huttunen and K. Laine, *Ann. Bot. Fen.* **20,** 79 (1983).
23. J. T. Perchorowicz and P. Ting, *Amer. J. Bot.* **61,** 787 (1974).
24. B. Prinz, G. H. M. Krause and H. Stratmann, *LIS-Berichte* (Essen, FRG, 1982) No. 28.
25. A. Keitel and U. Arndt, *Angew. Bot.* **57,** 193 (1983).
26. L. S. Evans and I. P. Ting, *Amer. J. Bot.* **60,** 155 (1973).
27. G. H. M. Krause, B. Prinz and K.-D. Jung, *VDI-Bericht* (Düsseldorf, FRG, 1983), No. 500, p. 257.
28. K. Vogels, R. Guderian and G. Masuch. In: Acidification and its Policy Implications (T. Schneider, ed.) (Elsevier, Amsterdam, 1986), pp. 171–186.
29. D. Grill, D. Esterbauer and H. Beck, *Phytopatz. Z.* **82,** 182 (1975).
30. K. Vogels and G. Masuch, *Verh. Ges. Ökol.* **XIV**, 373 (1986).
31. N. O. Adedipe, R. A. Fletcher and D. P. Ormrod, *Atmos. Environ.* **7,** 357 (1973).
32. P. R. Miller, J. R. Parmeter, B. H. Flick and C. W. Martinez, *J. Air Poll. Contr. Ass.* **19,** 435 (1969).
33. D. Doley and L. Leyton, *New Phytol.* **67,** 579 (1968).
34. T. C. Hsiao, *Ann. Rev. Plant Physiol.* **24,** 519 (1973).
35. W. F. Pickard, P. E. H. Minchin and J. H. Troughton, *J. Exp. Bot.* **30,** 307 (1979).
36. J. F. Wardlaw, *Austr. J. Biol. Sc.* **22,** 1 (1969).
37. A. Fahn, *Plant Anatomy* (Pergamon Press, Oxford, 1982).