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Reviews

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Extracellular biochemical markers of photochemical oxidant air pollution damage to Norway spruce

by F. J. Castillo*, P. R. Miller[1] and H. Greppin

Laboratoire de Physiologie Végétale, Université de Genève, CH-1211 Genève 4 (Switzerland) and USDA Forest Service, Riverside (California 92507, USA)

Summary. Peroxidase and superoxide dismutase activities, and ascorbic acid content, were measured in both intercellular fluid and cell material of current and 1-year-old needles of Norway spruce saplings treated with ozone, ambient air and activated carbon-filtered air in outdoor fumigation chambers. Ethylene evolution was also compared. Plants from carbon-filtered air treatments had significantly lower enzyme activities and higher ascorbic acid content. These changes were more marked in intercellular fluid than in cell material. Significant changes were noted at ozone levels typical of ambient air quality in a typical urban area. These results suggest the need for simultaneous screening of several biochemical markers as a way of overcoming the lack of specificity of any single marker for the identification of a perturbation by a particular stress, such as ozone.

Key words. Peroxidase; superoxide dismutase; ascorbic acid; ethylene; periplasmic space; ozone; photochemical oxidants; *Picea abies;* forest decay.

Introduction

Recent assessments of the possible causes of the decline and death of forest tree species in Central Europe have focused on the many uncertainties about the possible causes of this complex problem[21]. Circumstantial evidence suggests that the photochemical oxidant, ozone, should be investigated as one of the causal factors[19]. Visible symptoms of ozone injury are useful for diagnostic purposes, but additional evidence is needed to evaluate and verify the invisible injury. For example, ozone injury may also be manifested by biochemical changes in needle tissue. For forest trees, the peroxidase activity of extracts from macerated leaf tissue has been investigated as a possible marker of stress due to air pollutants[15] but the physiological meaning of measurements made from such extracts is subject to discussion[6]. In addition to peroxidases, superoxide dismutase and ascorbic acid play an important role in the maintenance of an adequate redox potential in leaf cells and may protect the cell membrane from active oxidants arising from exogenous sources[10]. Protection could be increased in the periplasmic space, the first compartment of leaves which pollutants enter before reacting with the plasma membrane. The purpose of this study was to investigate the possibility of measuring the presence of these biochemical markers of ozone stress in the extracellular space of current and 1-year-old needles of Norway spruce *(Picea abies)*. This may allow the investigation of a more dynamic aspect of the role of these extracellular biochemical markers of ozone stress by means of their application to a natural population of trees.

Materials and methods

Four-year-old saplings (*Picea abies,* 71.EA 84 Mornens MRC), planted in clay pots containing a standard greenhouse soil mix, were exposed – six in each treatment – to ambient air, carbon-filtered air plus ozone (300 $\mu g \cdot m^{-3}$), and carbon-filtered air in semi-open-top chambers at the Botanical Garden in Geneva. Ozone, generated by passing a stream of oxygen through a high voltage electric discharge, was administered for 7 $h \cdot d^{-1}$ during 28 sunny days in August 1985. Ozone concentrations in the chambers and in ambient air were measured by the chemiluminescent method (Beckman Model 950A). A fourth group consisted of saplings exposed to ambient air nearby and outside the chambers. A complementary observation was made of additional saplings which were exposed to filtered air for 2 weeks, after which one-half were given the

same ozone treatment for only 2 days. Plants were transported to the laboratory immediately prior to the harvest of needles from both the current year and from 1-year-old whorls. Needles were cut from the stems and 2-g aliquots were vacuum infiltrated (−65 kPa) with the appropriate buffer, surface dried with absorbent tissue, and centrifuged (1,000 × g for 10 min) in nested tubes (the upper tube, containing the needles, had a perforated bottom). The intercellular fluid, collected in the lower tube, was maintained in an ice bath and analyzed immediately for enzyme activities, ascorbic acid, and protein content. The remaining leaf tissue was stored in liquid nitrogen and extracted the following day for the determination of enzyme activities, protein, ascorbic acid, and chlorophyll content. All subsequent procedures for enzyme extraction, peroxidase assay, and protein determination were conducted in the same manner as earlier experiments with *Sedum album*[8].

Infiltration medium for ascorbic acid determination in the intercellular fluid contained Na-acetate buffer (40 mM, pH 4.5), 2.5 mM EDTA and 0.1 M KCl. Ascorbic acid from cell material was extracted with cold 0.4 M metaphosphoric acid, 2.5 mM EDTA. Ascorbic acid was determined in the supernatant obtained after centrifuging the crude extract at 20,000 × g for 15 min.

Ascorbic acid was determined by HPLC (Varian Model 5000). Separation was achieved on a 15 cm × 4 mm analytical MicroPak MCH-5-N-CAP column protected by a guard column. The eluting solvent was aqueous 2% $NH_4H_2PO_4$ adjusted with concentrated phosphoric acid to pH 2.8, passed through the column at a flow rate of 1 $ml \cdot min^{-1}$. The column pressure was 120 atm and the detector was set at 254 nm.

Superoxide dismutase activity was assayed by measuring the inhibition of the photochemical reduction of NBT using the method of Beauchamp and Fridovich[3]. The 1-ml reaction mixture contained phosphate buffer (50 mM, pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, and 0–50 µl enzyme extract. Riboflavin was added last and the tubes were placed in fluorescent light. The reaction was allowed to run for 10 min or until the absorbance at 560 nm in the control tubes without enzyme reached 0.2. One unit of enzyme activity was defined as the volume of extract required for 50% inhibition of the level of absorbance produced in control tubes[3]. No interference was observed by the presence of ascorbic acid during the SOD assay.

Intercellular fluid was also assayed for G6PDH, acid phosphatase, and ribulose 1,5 diphosphate carboxylase as enzyme markers to detect any contamination from the cytoplasm, vacuole, or chloroplast, respectively. No contamination by these enzymes was detected in the intercellular fluid obtained at −65 kPa vacuum.

For ethylene determination the severed end of stem segments with either current or 1-year old foliage were placed in vials containing 1 mM ACC. These units were placed in a glass bottle (150 cm^3) hermetically closed by a butyl rubber plug. Three samples of 1 cm^3 air were taken from the bottles with a syringe after 24 h. Ethylene was determined by gas chromatography using a stainless steel column (3 m × 1.5 mm) filled with Porapack R (80–100 Mesh). Column, injector, and flame ionization detector temperatures were 60, 90, and 90 °C, respectively. The electrometer sensitivity was 1×10^{-12} A/mV. N_2 was used as carrier gas (55 $cm^3 \cdot min^{-1}$).

All results of the 30-day treatment were tested by analysis of variance. Means which were significantly different ($p < 0.05$) were identified by the least significant range (LSR)[22]. Only means and standard deviations are presented for the 2-day treatment. There were only 2 plants per treatment and thus replication was insufficient for analysis of variance.

Foliage was also inspected for the presence of visible ozone symptoms.

Results and discussion

The average ½-h ozone concentrations, measured in the carbon-filtered air plus ozone and in the ambient air of Geneva during the experiment, are shown in figure 1. The number of fumigation hours per day are shown in figure 2. The weather was mostly warm and sunny with only occasional interruptions by rain.

Two of six plants in the ozone treatment showed very slight ozone injury symptoms to sun-exposed needles near the base of the current year whorl. No symptoms were visible on plants in any of the other treatments.

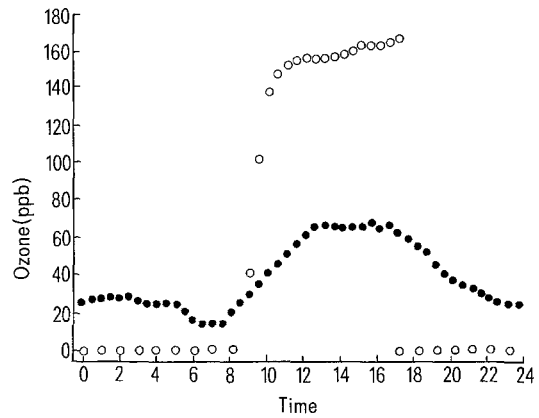

Figure 1. Average ½-h ozone concentrations measured in the chamber with carbon-filtered air plus ozone (○) during 26.5 days of a 30-day fumigation experiment (rain prevented fumigation during 4.5 of the 30 days) and in ambient air during the same period (●). No ozone was detected in the chamber supplied with filtered air.

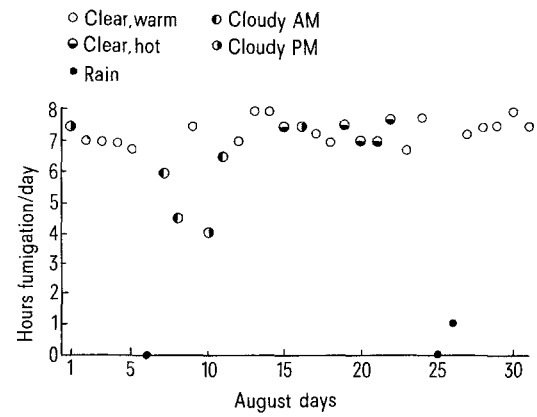

Figure 2. Record of weather conditions and number of hours fumigation in the chamber with carbon-filtered air plus ozone.

The distribution of peroxidase activity between intercellular fluid and residual cell material from current-year and 1-year-old Norway spruce needles were influenced by treatments (table 1). Saplings exposed to filtered air always exhibited lower values of enzyme activity, whereas ozone-treated saplings showed higher values in both current and 1-year-old needles. Peroxidase activity in the intercellular fluid of 1-year-old needles was significantly ($p = 0.05$) greater in plants exposed to ambient air in the chamber (210%), ambient air outside the chamber (236%), and in the filtered air plus ozone (316%) compared to that of saplings maintained in filtered air. The 30-day exposure also affected the level of peroxidase activity in the cell material. The increase was significantly higher in ozone treated plants compared to those in the ambient-air and filtered-air chamber treatments. Current-year and 1-year-old needles responded in a similar way. Extracellular peroxidase activity represents only a minor part (0.7%) of the total activity in current-year needles, whereas it attains 6% in 1-year-old needles (calculated on a gram fresh-weight basis). This extracellular peroxidase activity is about 80 times higher in 1-year-old needles than in current-year needles. Specific activity (mg protein basis) of cell material from 1-year-old needles exhibits seven times higher activity than that of current-year needles (table 1). This increase of peroxidase activity with aging has already been observed in other plant tissues[12]. The short-term experiment with saplings exposed to filtered air for 2 weeks prior to ozone exposure for 2 days agreed with the results of the 30-day treatment. Peroxidase activity was enhanced in both fractions (extracellular fluid and cell material) from current-year and 1-year-old needles. The effect of ozone is much more pronounced on extracellular activity, which is increased by 2.2–3 times, than on the cell material activity which is only enhanced by 1.3 times.

It is important from the viewpoint of the sensitivity of this biochemical test that the short-term (2-day) ozone exposure had almost the same magnitude of effect on the extracellular peroxidase activity as the long-term exposure. In the 2-day experiment all plants were exposed to ozone in Geneva air, half of this group retained in filtered air returned to the ascorbic acid, peroxidase and SOD levels of the 30-day plants in filtered air after only 2 weeks plus 2 days.

However, only 2 days of ozone treatment was required to bring the levels of the three parameters back in the direction of the 30-day ozone treatment. Saplings pretreated with carbon-filtered air may be more sensitive to ozone than those maintained in ambient air with ozone concentrations ranging up to average daily peaks of 0.07 ppm. The 30-day exposure had a much greater effect on peroxidase activity of cell material than the short-term exposure. Increases of total peroxidase activity as a result of ozone or sulfur dioxide injury to woody plants has already been reported[15, 23].

These data confirm our previous results with *Sedum album* leaves[8], indicating that extracellular peroxidases are more sensitive to ozone than those from cell material. This increase of extracellular activity is believed to be the result of an activated secretion of peroxidases from cells to the periplasmic spaces, which seems to be dependent on calcium[8]. The secreted peroxidases are involved, among other metabolic processes, in defense mechanisms against various stresses[11].

Intercellular fluid of Norway spruce needles also displayed a superoxide dismutase-like activity (table 2). All control tests, including the inhibition of KCN and the absence of interference by ascorbic acid (possible scavenger of the superoxide anion) on NBT reduction, confirmed the presence of this enzyme in periplasmic spaces. Moreover, vacuolar, chloroplastic, and cytosolic enzyme

Table 1. Peroxidase activity ($\Delta A_{470} \cdot min^{-1} \cdot mg^{-1}$ protein) in the intercellular fluid and cell material from Norway spruce needles after a short- and long-term ozone exposure. The values are the average ± SD of 6 different saplings. Means for the 30-day experiment are separated using the least significant range (LSR)

Experiment		Intercellular fluid		Cell material	
		Current-year	1-year-old	Current-year	1-year-old
2 days	Filtered	43 ± 10	526 ± 134	32 ± 6	243 ± 42
	Ozone	126 ± 30	1169 ± 244	42 ± 8	306 ± 36
30 days	Filtered	58 ± 20a	512 ± 91a	32 ± 8a	216 ± 56a
	Ambient	126 ± 42a	1074 ± 292b	61 ± 19b	341 ± 84ab
	Outside	100 ± 40a	1208 ± 344b	57 ± 18b	448 ± 110bc
	Ozone	196 ± 79b	1679 ± 264b	80 ± 14b	539 ± 160c
LSR		87.77	429.7	25.3	177.53

Table 2. Superoxide dismutase activity (units $\cdot mg^{-1}$ protein) in the intercellular fluid and cell material from current-year and 1-year-old needles after a short- and long-term ozone exposure. The values are the average ± SD of 6 different saplings. Means for the 30-day experiment are separated using the least significant range (LSR)

Experiment		Intercellular fluid		Cell material	
		Current-year	1-year-old	Current-year	1-year-old
2 days	Filtered	712 ± 4	105 ± 14	326 ± 66	457 ± 4
	Ozone	801 ± 111	187 ± 40	386 ± 70	541 ± 128
30 days	Filtered	975 ± 145a	85 ± 17a	328 ± 51a	442 ± 75a
	Ambient	1081 ± 165a	101 ± 15a	518 ± 51b	644 ± 111ab
	Outside	1153 ± 181a	203 ± 28b	606 ± 139bc	795 ± 172b
	Ozone	1555 ± 223b	235 ± 35b	708 ± 113c	925 ± 172b
LSR		282.23	37.76	148.06	216.04

Table 3. Ascorbic acid content ($\mu g \cdot g^{-1}$ f. wt) in the intercellular fluid and cell material from current-year and 1-year-old needles after a short- and long-term ozone exposure. The values are the average ± SD of 6 different saplings. Means for the 30-day experiment are separated using the least significant range (LSR)

Experiment	Intercellular fluid		Cell material	
	Current-year	1-year-old	Current-year	1-year-old
2 days Filtered	2.4 ± 0.5	2.8 ± 0.4	521 ± 55	550 ± 43
Ozone	1.1 ± 0.1	1.7 ± 0.4	452 ± 50	491 ± 65
30 days Filtered	2.6 ± 0.4a	2.4 ± 0.3a	496 ± 72a	526 ± 85a
Ambient	1.8 ± 0.3b	1.9 ± 0.4ab	429 ± 58a	439 ± 70a
Outside	1.7 ± 0.5bc	1.8 ± 0.6ab	449 ± 58a	438 ± 46a
Ozone	1.2 ± 0.2c	1.4 ± 0.3b	323 ± 49b	306 ± 52b
LSR	0.652	0.73	97.01	101.02

markers were never detected in the intercellular fluid, indicating the absence of contamination by other cell compartments. Extracellular SOD accounts for about 1% of the total activity in the needles. Specific activity is higher in the intercellular fluid of current-year needles because of its lower protein content. As shown before for peroxidase activity, a general stimulation of SOD was observed after ozone exposure. Saplings exposed to ozone for 2 days responded by an enhancement of extracellular activity (+80%) in 1-year-old needles. SOD from cell material was only activated by 20%. No significant differences in extracellular SOD activity from current-year needles were observed between filtered, ambient, or outside treatments after a 30-day fumigation experiment. Extracellular SOD from 1-year-old needles increased three times after ozone exposure. The 30-day fumigation increased the SOD activity in cell material two-fold more than the 2-day fumigation.

This stimulation of SOD activity has also been observed in bleached spruce needles[9] and after ozone fumigation of beans[17]. Ozone-induced oxy- and peroxyl-radicals can react with plasma membrane components, induce lipid peroxidation, and modify the membrane permeability[14]. Extracellular SOD activity would contribute to the protection of the plasma membrane against ozone or oxy-radical reaction products[18]. Increasing levels of SOD could be accompanied by an excellent ozone protection as previously shown for other plant material[17].

Periplasmic space accounted for about 0.5% of the total content of ascorbic acid in Norway spruce needles (table 3). When saplings are fumigated with ozone, extracellular ascorbic acid is reduced by one-half, whatever the length of the exposure. A decrease in the ascorbic acid content is also observed in the cell material. This decrease is more important after a long-term (−40%) than a short-term exposure (−12%). Levels of ascorbic acid from ambient air and outside saplings were always intermediate between those from filtered air and ozone fumigated saplings. The decrease of ascorbic acid content in Norway spruce needles was observed after SO_2 exposure[13]. Barnes found that exposure of pine needles to 50 ppb ozone induced an accumulation of ascorbic acid, whereas 150 ppb ozone had no effect[2]. Ascorbic acid can play a protective effect by scavenging toxic free radicals[5], and in this way maintain the stability of plant cell membranes. The decrease of extracellular ascorbic acid could be explained by the activation of an ascorbate-specific peroxidase which uses ascorbate as an electron donor. This type of response was observed in *Sedum album* leaves after ozone exposure[7]. The decrease of ascorbic acid in cell material suggested that saplings cannot generate a continuous supply of ascorbic acid to meet stress level requirements as long as tissue damage continues.

Table 4. Ethylene production ($nl \cdot g^{-1}$ f. $wt \cdot h^{-1}$) by current-year and 1-year-old needles incubated in the presence of 1 mm ACC. The cumulated ethylene concentration was estimated after 24 h

Experiment	Current-year	1-year-old
2 days Filtered	16.2 ± 2.5	5 ± 1.8
Ozone	16.8 ± 1.8	6.9 ± 0.6
30 days Filtered	18.1 ± 4.3a	6.9 ± 0.2a
Ambient	19.4 ± 2.5a	7.5 ± 0.6a
Outside	21.3 ± 2.5a	7.5 ± 1.8a
Ozone	18.8 ± 3.1a	15 ± 1.8b

Ethylene production was also used to monitor the effect of ozone on trees. Since ozone can modify the level of ACC[20] (the natural precursor of ethylene), Norway spruce stems were incubated with exogenous ACC, in order to determine the maximum capacity for ethylene production. In general, current-year needles produced 2.5 times more ethylene than 1-year-old needles, whatever the previous treatment had been (table 4). Fumigation with ozone did not affect the ethylene production of current-year needles, whereas 1-year-old needles produced more ethylene after short- (+40%) and long-term (+120%) exposures. No significant differences in ethylene production were observed between filtered, ambient, or outside treatments.

Increase of ethylene production has also been observed in Norway spruce after SO_2 exposure[4] and in other species after different stresses[16]. The ozone-induced ethylene production can have dangerous consequences because ethylene can react with ozone to form reactive components[9], such as formaldehyde and hydroperoxides, which can damage plant membrane integrity.

In summary, our results confirm the need for simultaneous screening of several biochemical parameters as a way of overcoming the lack of specificity of any single parameter for identifying a particular stress, e.g., ozone. Additional sensitivity, and perhaps specificity, may be obtained by measuring intercellular fluid in addition to cell material. Regardless of which plant response was measured in our study, the intercellular fluid is more sensitive to ozone exposure than cell material. This may allow a quantification of the physiological activation of these parameters in response to any stress[11]. An additional advantage of measuring extracellular enzyme acti-

vities is that of the calculation of the ratio extracellular/total activity, an internal parameter, that may be an improved indicator of stress, as proposed elsewhere[6].

Finally, the possibility of a practical application of these biochemical assays for detecting ozone stress to Norway spruce is encouraging. Saplings placed in the chamber fumigated with ambient air and trees maintained outside exhibited biochemical values which were close to those of saplings fumigated with ozone; i.e., the levels of pollutants in ambient air were high enough to induce a change in those biochemical parameters. More work is needed with large trees, in situ, to separate the possible effects of season, and several intrinsic tree variables, from changes induced by ozone and other stresses.

Abbreviations used: ACC, 1,aminocyclopropane-1-carboxylic acid; EDTA, ethylenediaminetetraacetic acid; NBT, nitrobluetetrazolium; SOD, superoxide dismutase; G6PDH, glucose-6-P-dehydrogenase; HPLC, high performance liquid chromatography.

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* Present address: Department of Botany and Plant Sciences, University of California, Riverside, California 92521, USA.

1 On sabbatical leave at the University of Geneva.
2 Barnes, R.L., Effects of chronic exposure to ozone on soluble sugar and ascorbic acid contents of pine seedlings. Can. J. Bot. *50* (1972) 215–219.
3 Beauchamp, C., and Fridovich, I., Superoxide dismutase: improved assays and an assay applicable to acrylamide gel. Analyt. Biochem. *44* (1974) 276–287.
4 Bucher, J.B., SO_2-induced ethylene evolution of forest tree foliage and its potential use as stress-indicator. Eur. J. For. Path. *11* (1981) 369–373.
5 Calabrese, E.J., The role of ascorbic acid in aquatic detoxification, in: Vitamin C, Nutrition and Environmental Health, vol. 1, p. 272. John Wiley, New York 1980.
6 Castillo, F.J., Extracellular peroxidases as markers of stress?, in: Molecular and Physiological Aspects of Plant Peroxidases. Eds H. Greppin, Cl. Penel and Th. Gaspar. Centre de Botanique, University of Genève, in press.
7 Castillo, F.J., and Greppin, H., Balance between anionic and cationic extracellular peroxidase activites in *Sedum album* leaves after ozone exposure. Analysis by high-performance liquid chromatography. Physiologia Pl. *68* (1986) 201–208.
8 Castillo, F.J., Penel, Cl., and Greppin, H., Peroxidase release induced by ozone in *Sedum album* leaves. Involvement of Ca^{2+}. Pl. Physiol. *74* (1984) 846–851.
9 Elstner, E.F., Osswald, W., and Youngman, R.J., Basic mechanisms of pigment bleaching and loss of structural resistance in spruce *(Picea abies)* needles: advances in phytochemical diagnosis. Experientia *41* (1985) 591–597.
10 Fridovich, I., The biology of oxygen radicals. Science *201* (1978) 875–880.
11 Gaspar, T., Penel, Cl., Castillo, F.J., and Greppin, H., A two-step control of basic and acidic peroxidases and its significance for growth and development. Physiologia Pl. *64* (1985) 418–423.
12 Gaspar, T., Penel, Cl., Thorpe, T., and Greppin, H., Peroxidases 1970–1980. A survey of their biochemical and physiological roles in higher plants. University of Geneve, Switzerland, 1982.
13 Grill, D., Esterbauer, H., and Welt, R., Einfluss von SO_2 auf das Ascorbinsäuresystem der Fichtennadeln. Phytopath. Z. *96* (1979) 361–368.
14 Heath, R.L., Initial events in injury to plants by air pollutants. A. Rev. Pl. Physiol. *31* (1980) 395–431.
15 Keller, Th., The influence of SO_2 on CO_2 uptake and peroxidase activity. Eur. J. For. Path. *14* (1984) 354–359.
16 Kimmerer, T.W., and Kozlowski, T.T., Ethylene, ethane, acetaldehyde, and ethanol production by plants under stress. Pl. Physiol. *69* (1982) 840–847.
17 Lee, E.H., and Bennett, J.H., Superoxide dismutase. A possible molecular enzyme against ozone injury in snap beans (*Phaseolus vulgaris* L.) Pl. Physiol. *69* (1982) 1444–1449.
18 Olszyk, D.M., and Tingey, D.T., Phytotoxicity of air pollutants. Evidence for the photodetoxification of SO_2 but not O_3. Pl. Physiol. *74* (1984) 999–1005.
19 Prinz, B., Krause, G.H.N., and Stratman, H., Waldschäden in der Bundesrepublik Deutschland. Landesanstalt für Immissionsschutz des Landes Nordrhein-Westfalen, Bericht No. 28 (1982).
20 Rodecap, K.D., and Tingey, D.T., The influence of light on ozone-induced 1-aminocyclopropane-1-carboxylic acid and ethylene production from intact plants. Z. Pfl. Physiol. *110* (1983) 419–427.
21 Schütt, P., and Cowling, E.B., Waldsterben – A general decline of forest in Central Europe: symptoms, development, and possible causes of a beginning breakdown of forest ecosystems. Pl. Disease *69* (1985) 548–558.
22 Sokal, R.R., and Rohlf, F.J., Biometry. W.H. Freeman and Co., San Francisco 1969.
23 Smidt, S., Begasungsversuche mit SO_2 und Ozon an jungen Fichten. Eur. J. For. Path. *14* (1984) 241–248.

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Trialkyllead. Occurrence, biological interactions, and possible impact on forest decline

by H. Faulstich, C. Stournaras* and K. P. Endres

Abteilung Physiologie, Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, D-6900 Heidelberg (Federal Republic of Germany)

Summary. Biochemical studies and experiments with cells have shown that trialkyllead (R_3Pb^+), a degradation product of tetraalkyllead (R_4Pb) antiknock agents, is highly cytotoxic. Trialkyllead may occur in rain and fog in concentrations much higher than those reported so far. From these data it has been proposed that trialkyllead may represent one of the factors responsible for the present forest decline. This article reviews the chemistry of trialkyllead, its molecular interactions, its toxic effects on cells and on plant cells in particular, and its occurrence in the environment. Evidence for and against the involvement of trialkyllead in forest decline will be discussed.
Key words. Organolead; phytotoxicity; rain water; acid rain; glutathione.