CHROMOSOMAL ABERRATIONS IN OZONE-IMPACTED SPRUCE AS A TEST OF CYTOLOGICAL DAMAGE IN FOREST TREES

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Received November 21, 1995; accepted May 7, 1996

ABSTRACT

A system for testing the cytological damage to trees from air pollution was developed by classification of chromosomal aberrations in root tip meristems of young spruce trees (*Picea abies* (L.) KARST.). The system was tested from 1989 to 1995. The objective was to develop a system for early detection of environmental influences on forest tree species. Results obtained from different natural sites and from ozone fumigation experiments lead us to recommend of this system as an easy and sensitive screening method for damage caused by mixed air pollutants on spruce and also damage from non-accumulating compounds such as ozone. Despite the widespread occurrence of ozone injury, mechanisms of the damaging process and plant defence systems against ozone attacks are still poorly understood. Considering the importance of our forest tree species, it is essential to characterize their short- and long-term responses to a variety of environmental and pollution impacts.

Key words: *Picea abies* (L.) KARST., chromosomal aberrations, environmental monitoring, O_3, natural conditions

INTRODUCTION

There is growing concern about the genotoxicity of complex air pollutant mixtures leading to dieback of our forest ecosystems. Several plant test systems have been developed to detect genotoxic effects of various agents. One of these systems is the classification of chromosomal aberrations; it is a sensitive method for the assessment of genotoxic effects caused by chemical treatments. The cytological studies are relatively simple to perform and give valuable information on pollutant effects on cell division and chromosome structure (LEVAN 1938, FISKESJÖ 1985). FISKESJÖ (1989) studied aluminium toxicity in root tips of spruce, beech and oak and found disturbances in dividing cells. He also found mitotic effects, growth restriction and browning of the roots. In another study, it was shown that spruce trees are mitotically active throughout the whole year and therefore also may be affected by pollutants during dormancy (MATSCHKE *et al.* 1994). SCHUBERT & RIEGER (1994) evaluated sister chromatid exchange frequencies and suggested this as a suitable and reliable criterion of genotoxic effects in spruce seedlings.

We believe that a new method or modification of one of the current test systems would be useful. In our search for an easy and reliable test assay, we classified chromosomal aberrations, with spruce as a bioindicator. Using the work of DRUŠKOVIČ (1988) on spruce as a starting point, in 1989 we developed a test system by classification of chromosomal aberrations with spruce as the bioindicator plant. In the present paper, the results from field studies and from ozone-fumigation experiments from 1989 to 1995 are summarized and discussed.

CHROMOSOMAL ANALYSIS

After preparation of the root tips, meristem cells are seen to be separated. Interphases and all mitotic stages, which are distinctly pink coloured, can be observed (MÜLLER & GRILL 1992). Due to the effect of the metaphase blocker (1-bromonaphtalene), metaphases are the most frequent mitosis stages. The normal metaphase consists of $2n = 24$ chromosomes (see, *e g.*, MIYAKE 1903, SAX & SAX 1933, PRICE 1989). The main type of chromosomal aberration in our work was chromosome stickiness, resulting in connections, clumped metaphases and amorphous chromatin masses. This type of aberration may result from improper folding of the chromosome fiber into single chromatids so that chromosomes become attached to each other by subchromatid bridges (MCGILL *et al.* 1974, KLÁŠTERSKÁ *et al.* 1976). STEPHEN 1979 also suggested that stickiness is a type of physical adhesion that involves mainly the proteinaceaous matrix of the chromatin material. Chromosome stickiness reflects highly toxic effects, usually of an irreversible type and probably leading to cell death (LIU *et al.* 1993). Additional but

less frequent defects found in this series of experiments are breaks, fragments and rings, which are genotoxic effects that may transfer the damage to subsequent generations of cells (FISKESJÖ 1994). Under conditions of constant ozone treatment, a significantly larger number of sticky chromosomes was observed. Under field conditions there was also an increased number of cases of chromosome stickiness, but in addition many more breaks and fragments occurred (MÜLLER *et al.* 1994, MÜLLER & BERMADINGER-STABENTHEINER in press).

It is difficult to decide whether or not certain influences or combinations lead to specific chromosomal abnormalities. DRUŠKOVIČ (1988) suggested that acute influences create abnormalities on single chromosomes, evidenced as breaks and fragments and long-lasting effects that cause stickiness.

FIELD STUDIES

Between 1989 and 1995, we applied our plant test system to natural spruce stands in Austria. The results showed that the classification of chromosomal aberrations is a valuable tool in environmental monitoring under natural conditions. Our method works with young spruce trees and results obtained from young trees can be correlated with results from older trees. Therefore it is possible to use the system to determine the vitality of spruce stands (MÜLLER *et al.* 1991, 1992). The data suggested that an environmental effect (*i.e.* effects of air pollutants) was more important than an effect of the soil or of the provenance of the tree tested (MÜLLER *et al.* 1992, 1994). The test results provide valuable information about the condition of the trees, especially of spruce stands at upper-elevation sites (MÜLLER & BERMADINGER–STABENTHEINER in press). Plants on higher sites are affected by lower temperatures, higher radiation levels and higher concentrations of naturally-occurring photooxidants, especially ozone, as compared with plants growing on lower sites (TRANQUILLINI 1979, BOLHAR–NORDENKAMPF & LECHNER 1989). When trees are growing on natural sites, it is difficult to determine which factors have the greatest impact. In experiments, each different environmental factor may be varied while other factors are kept constant, thus enabling the researcher to test the plant's reaction to the particular factor (FANGMEIER *et al.* 1992).

OZONE FUMIGATION EXPERIMENTS

Two representative ozone fumigation experiments are described here. They were undertaken in climate chambers (GSF Munich) and in greenhouses (Research Centre Seibersdorf). In all experiments, observations of visible damage were made on a qualitative basis (1) during the fumigation, (2) at the end of the fumigation, and (3) during a post-fumigation period under natural air and weather conditions for observation of long-term effects. No visible evidence of injuries or of stunted growth due to increased ozone was observed in any of the plants at the end of the fumigation experiments or up to two years later (MÜLLER & GRILL 1994, 1995, MÜLLER *et al.* 1995, in press). The data showed that the spruce clones used in our experiments were not sensitive to ozone treatments. The well-known symptoms of ozone injury, including bleaching, chlorotic mottling, changes in pigmentation and necrosis (KRESS *et al.* 1982, WILLIAMS 1986, POLLE *et al.* 1993) could not be

Table 1 Average hourly ozone concentration (nl $\cdot l^{-1}$) calculated using all 24 hours per day for the entire exposure. 24-hour dose (ppm * h). Effects on Norway spruce root cells treated with ozone for 42 days (climate chambers) and for 50 days (greenhouses), respectively. The chromosomal aberrations were scored in metaphase. For the variants 8 to 10 trees were used, and if possible 100 to 200 cells per tree were examined for chromosomal abnormalities. Numbers in brackets are the standard deviation. The symbols indicate significant differences between the two variants of each experiment for immediate effect and long-term after-fumigation effect (*p<0.05)**

Climate chamber studies	Direct effect		Long-term after effect	
	Control	Ozone	Control	Ozone
24-hour average 24-hour dose (42 days) chromosomal abberations (%)	20 20.16 2.1 (0.2)***	100 100.80 3.9 (0.7)***	4.3 (0.6) ***	5.6 (0.6) ***
Greenhouse studies	Control	Ozone	Control	Ozone
24-hour average 24-hour dose (50 days) chromosomal abberations (%)	23.30 27.76 3.3 (0.3) ***	56.17 67.40 7.5 (0.4) ***	4.2 (0.8) ***	8.1 (1.3) ***

observed. Visible damage had not been found in other experiments in which concentrations of about 50–70 nl ozone l^{-1} were used (ALSCHER *et al.* 1989, WALLIN *et al.* 1990). It has been suggested that acute short-term exposure to high ozone concentrations (> 200 $nl \cdot l^{-1}$) generally results in visible damage, whereas long-term, chronic exposure to lower ozone concentrations generally leads to reduced growth without foliar damage (HEAGLE 1989).

The results of all investigations under defined conditions of enhanced level of ozone (80 to 100 $nl \cdot l^{-1}$) showed a strong influence of ozone on the genetic material in the root tips of spruce. The amount of chromosomal defects increased with increasing ozone dose (Table 1), indicating that the genetic material of the root tips of spruce trees is a sensitive measure for oxidative stress, although the root tips did not come in direct contact with ozone.

In other studies with different test systems evaluating genetic defects, ozone was identified as an agent that is genotoxic to plants (*e.g.* MA *et al.* 1982). Also, ozone was found to be genotoxic to microorganisms (DUBEAU & CHUNG 1982), insects (ERDMAN & HERNANDEZ 1982) and to cell cultures in vitro (*e.g.* THOMASSEN et al. 1991). Results from *in vivo* cytogenetic studies with laboratory animals after inhalation exposure are contradictory (RITHIDECH *et al.* 1990). The first investigations on genotoxicity of ozone to higher plants were carried out with high ozone concentrations. In an early study (FETNER 1958), *Vicia faba* was exposed to 4000 $\mu l \cdot l^{-1}$ ozone for 15, 30, and 60 minutes; this treatment induced chromosome aberrations in the root meristem cells. The effects of ozone on *Vicia* were also studied by JANAKIRAMAN & HARNEY (1976) by investigations of the meiotic chromosomes of the buds. Exposures to concentrations of 2 $\mu l \cdot l^{-1}$ for 4 or 8 hours also caused chromosome aberrations. Further studies of the genotoxic effects of volatile air pollutants in the laboratory and in field monitoring were carried out with *Tradescantia*. Somatic mutations in stamen hairs (SCHAIRER *et al.* 1979) and micronucleus formation in the meiotic pollen mother cells were used as criteria (MA *et al.* 1982). Ozone exposure for 6 hours at 5 $\mu l \cdot l^{-1}$ caused mutations in stamen hairs, whereas the test for micronucleus formation (5.5 hours at 5 $\mu l \cdot l^{-1}$) was negative. In many of these investigations, high ozone concentrations were supplied and the direct effects were then determined after a certain time.

In our experiments, we also worked with ambient concentrations under so-called "natural conditions", necessitating consideration of the distance from the area of ozone entry (the top of the tree) to the root meristems. Ozone enters the mesophyll via stomata, where it can be dissolved in water and converted into reactive oxygen forms such as superoxide anions, hydroxyl radicals, and hydrogen peroxide (GRIMES *et al.* 1983, MEHLHORN *et al.* 1990, KANOFSKY & SIMA 1991). The active oxygen forms are probably responsible for the plasma membrane lipid peroxidation that has been demonstrated to occur after ozone treatment (PAULS & THOMPSON 1980, HEATH 1987, CHEVRIER *et al.* 1990). There is also increasing evidence that aldehydes generated endogenously during the process of lipid peroxidation are causally involved in some of the physiological effects associated with oxidative stress in cells and tissues (ESTERBAUER *et al.* 1991). The role of aldehydes in chromosomal damage in plants is still unknown. In trees, the long-lived aldehydes must be translocated in the phloem to the root tips and the toxic peroxidation products may damage the phloem. Phloem damage interrupts translocation of assimilates, which may correlate with reductions in root growth. Biomass measurements of pine trees exposed to ozone showed no differences in root biomass compared to control trees, but the fumigation period was short compared with the total life span of a tree (SPENCE *et al.* 1990). ALSCHER *et al.* (1989) observed no effects of ozone on root biomass or carbohydrate content in red spruce seedlings in the first field season. However, in the second year starch content of the roots decreased and sugar content and its rate of accumulation increased with increasing ozone dose, but root-biomass was not affected (AMUNDSON *et al.* 1991). Various studies have noted accumulations of soluble sugars and starch in shoots that were explained by reduced or delayed phloem translocation (TINGEY *et al.* 1976, VOGELS *et al.* 1986, GORRISSEN &VAN VEEN 1988, SPENCE *et al.* 1990, WILLENBRINK & SCHATTEN 1993). If ozone reduces only the phloem loading, the long-term after effect may not be evident for years. From our observations, we suggest that the effects in the root tips are the results of secundary reactions, caused either by the translocation of toxic peroxidation products as aldehydes to the root tips or by hormones.

Results of our investigation of a long-term after-fumigation effect under normal air and weather conditions showed that there was an increased number of chromosomal abnormalities (Table 1), i. e. a long-term effect of ozone on the genetic material of spruce plants. From our results obtained at natural sites, we suggest that the chromosomes of spruce trees respond to changing environment with an increase or a decrease in the number of aberrations (MÜLLER *et al.* 1992). This can explain why the proportion of fumigated variants (trees with chromosomal abberations), as compared to the control variants, was higher under field conditions than in climate chambers and greenhouses. Approximately 4–5 % of spruce trees grown in natural areas have been found to have chromosomal defects in meristems (MÜLLER *et al.* 1991, 1992). If no long-term after-fumigation

effects were visible in preparations from fumigated variants, all series of our experiments would show the same frequency of chromosomal defects under field conditions. In our experiments, the ozone-fumigated variants growing under normal air and weather conditions had 5.6–8.1 % chromosomal abnormalities, while the control variants had 4.2–4.3 % (Table 1). Post-fumigation long-term effects of ozone are well-known. Effects on plant growth and vitality in loblolly pine were reported by SPENCE *et al.* (1990), on secondary metabolites and antioxidants such as catechin by LANGEBARTELS *et al.* (1990) and on pigments in spruce needles by LÜTZ (1992).

ACKNOWLEDGEMENTS

We are grateful to GERHARD SOJA (Research Centre Seibersdorf) and to CORNELIUS LÜTZ (GSF Munich) for the fumigation experiments. Many thanks to BÄRBEL KÖHLER and to ANITA GEISZINGER for technical assistance, to MICHAEL TAUSZ for the statistical examination and to Helmut Guttenberger for his help and for valuable comments. This work is supported by the 'Fonds of the Austrian Nationalbank No. 5419' (1993–1997).

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