

Phyton (Horn, Austria)	Vol. 34	Fasc. 2	321-335	29. 12. 1994
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The Effects of Interactions between Ozone and CO₂ on the Chromosomes of Norway Spruce Root Meristems

By

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With 2 Figures

Received May 21, 1994

Key words: *Picea abies*, chromosomal aberrations, ozone, CO₂, interactions.

Summary

MÜLLER M., GRILL D. & GUTTENBERGER H. 1994. The effects of interactions between ozone and CO₂ on the chromosomes of Norway spruce root meristems. – *Phyton* (Horn, Austria) 34 (2): 321–335, with 2 figures. – English with German summary.

A Norway spruce (*Picea abies* (L.) Karsten) test system was used to study the immediate and after effects of increased ozone or elevated CO₂ or both, on root tip chromosomes. Five-year-old potted spruce trees were exposed in environmental chambers to elevated concentrations of ozone (0.1 cm³m⁻³) for the study of an immediate effect and to elevated concentrations of carbon dioxide (750 cm³m⁻³) and ozone (0.08 cm³m⁻³) as single variables or in combination and then transferred to a field for the observation of an after effect.

Elevated ozone caused an increased number of chromosomal abnormalities directly after finishing the fumigation and also 21 months later. Elevated CO₂ more likely induced a decrease rather than an increase in the number of chromosomal aberrations. The most common abnormalities were chromosome stickiness, in the form of connections, clumped metaphases and amorphous chromatin masses. An increased number of chromosomal aberrations especially chromosome stickiness reflects highly toxic effects, usually of an irreversible type leading to cell death.

Zusammenfassung

MÜLLER M., GRILL D. & GUTTENBERGER H. 1994. Die Auswirkungen von Ozon und CO₂ auf die Chromosomen von Wurzelmeristemen der Fichte. – *Phyton* (Horn,

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Austria) 34 (2): 321–335, mit 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In dieser Studie wurde ein Testsystem mit der Fichte (*Picea abies* (L.) Karst.) angewendet, um die direkten Auswirkungen und die Langzeiteffekte von erhöhtem Ozon oder erhöhtem CO₂ bzw. beider Substanzen auf die Wurzelspitzen-Chromosomen zu untersuchen. Fünf Jahre alte getopfte Fichten wurden in Klimakammern exponiert und mit erhöhtem Ozon (0.1 cm³m⁻³) begast, um die Auswirkungen dieses Schadstoffes direkt nach Beendigung der Begasung auf das genetische Material zu beobachten. Um Langzeiteffekte im genetischen Material der Fichte zu beobachten, wurden die Jungfichten mit erhöhtem Kohlendioxid (750 cm³m⁻³) und Ozon (0.08 cm³m⁻³) als Einzelkomponenten bzw. in Kombination begast und im Anschluß daran normalen Umweltbedingungen ausgesetzt.

Die Auswirkungen von erhöhtem Ozon zeigten sich direkt nach Beendigung der Begasung, sowie auch 21 Monate später in Form einer Zunahme der Chromosomen-Anomalien. Erhöhtes CO₂ bewirkte eher eine Verminderung als eine Zunahme von Chromosomen-Aberrationen. Die häufigsten Anomalien waren Chromosomen-Verklebungen, in Form von Verbindungen, von verklumpten Metaphasen und amorphen Chromatinmassen. Eine erhöhte Anzahl von Chromosomen-Aberrationen, speziell der Chromosomen-Verklebungen, weisen auf stark erhöhte toxische Einflüsse hin, die gewöhnlich irreversibel sind und zum Zelltod führen.

Introduction

Air pollution, predicted shifts in climate (BOLIN & al. 1986) and expected increases in atmospheric CO₂ concentration (KEELING & al. 1989, CONWAY & al. 1988) are critical parts of the changing global environment. Atmospheric CO₂ concentrations are expected to approximately double during the next century (HARRINGTON 1987). Increased CO₂ concentrations will contribute to increases in "greenhouse gases" and this will result in some degree of climatic change (JOHNSON 1993). In plants, however, the increase in atmospheric CO₂ may also have beneficial effects, since CO₂ often is a limiting factor for photosynthesis. Tree species exposed to elevated CO₂ concentrations for one or two growth phases showed increases in photosynthesis, in biomass production, and improved water and nutrient use efficiency (KOZLOWSKI & al. 1991). In contrast, stressful environmental conditions, as caused by air pollutants such as ozone inhibit photosynthesis, alter carbon allocation (GARNER & al. 1989) and may reduce plant growth (SHELburne & al. 1993). Unfortunately the impact of ozone (as a non-accumulative compound) on plants is very difficult to detect. The influence on plants is mainly measured by various responses in plant metabolism (SEN GUPTA & al. 1991, POLLE & al. 1993), photosynthesis (SEN GUPTA & al. 1991), growth parameters (WISELOGEL & al. 1991), or winter hardiness (LARSEN & al. 1990). Most of the plant responses are considered to be caused by the oxidative properties of ozone (ELSTNER 1983). In general, two main mechanisms of ozone damage occur, the oxidation of polyunsaturated fatty acids to acid peroxides and the oxidation of sulfhydryl

groups of enzymes, proteins and peptides, making cellular membranes sensitive targets for ozone damage (GUDERIAN 1985, JÄGER & al. 1986). From studies on different test systems ozone was identified as an agent that is genotoxic to microorganisms (e. g. DUBEAU & CHUNG 1982), plants (e. g. MA & al. 1982), insects (e. g. ERDMAN & HERNANDEZ 1982) and cell cultures (e. g. THOMASSEN & al. 1991). Results from in vivo cytogenetic studies with laboratory animals after inhalation exposure are contradictory (e. g. RITHIDECH & al. 1990). To study the genotoxic effects of ozone in plants, various test systems have been used. In an early study, it was observed that root meristem cells of *Vicia faba* exposed to 4000 ppm ozone air, exhibited chromosome-type aberrations (FETNER 1958). The effects of ozone on *Vicia faba* meiotic chromosomes of buds exposed to 2 ppm were also chromosome-type aberrations (JANAKIRAMAN & HARNEY 1976). These test systems were carried out in the laboratory by exposing plants to high doses of ozone for a few minutes to a few hours.

The classical and commonly used test for environmental monitoring by chromosomal aberrations is the *Allium*-test, developed by LEVAN 1938. Based this and the basic study of a slovenian group (DRUSKOVIC 1988a), in 1989 we worked out a Norway spruce test system. This plant test system has been called "cytogenetic bioindication" and was first used at natural sites to assess the vitality of spruce plants (DRUSKOVIC 1988b, MÜLLER & al. 1991a). In addition to traditional methods of bioindication this cytogenetic method was supposed to provide information data about plant vitality. At high elevation sites, however, the correlation between the other bioindication methods and the cytogenetic method was poor (MÜLLER & al. 1991a, 1992). One explanation for this finding is that at higher elevations pollutants are present, like photooxidants including ozone, but that symptoms are difficult to identify by conventional methods in spruce trees.

The aim of this study is to use the classification of chromosomal aberrations as a test system for detection of air pollutants with spruce trees fumigated in environmental chambers. Thus it is possible to investigate if increased ozone causes an immediate effect and if increased ozone and/or CO₂ cause a long term after effect on the genetic material of spruce plants. After effects of ozone e. g. for secondary metabolites and antioxidants like catechin (LANGEBARTELS & al. 1990), and for pigments in spruce needles (LÜTZ 1992) are well known.

Material and Methods

Plants and soils.

Two different clones of three-year-old spruce trees (*Picea abies* (L.) Karst., clone 4611 for the investigation of the immediate effects and clone 773 for the investigation of the after effects, respectively) were potted in acidic, sandy forest soil and kept outdoors for 2 years prior to the experiment. The plants were irrigated regularly,

fertilized twice a year, and protected from pathogens as described elsewhere (POLLE & al. 1993).

Experimental conditions.

Climate chamber studies were undertaken in the phytotron at the GSF-Munich.

Immediate effects

The spruce trees were transferred to the environmental chambers and grown in independent growth chambers of a large phytotron, under completely controlled conditions. During a 42 days experimental period in 1993, the plants were exposed to a climatic programme simulating variations of temperature and day length, as observed at higher elevations in the Bavarian forest. The maximum irradiance was about $1200 \mu\text{molm}^{-2}\text{s}^{-1}$ PAR. The lack of higher light intensities was compensated by extended noon time illumination resulting in daily mean energy values comparable to field conditions. The relative humidity within the chambers varied in the following range: maximum rh 98–100%; mean maximum rh 78%; total mean rh 78%; mean minimum rh 63% (BLANK & al. 1990). Each experimental group consisted of eight potted 5-year-old trees. The pots (14-cm-diameter) were installed in temperature-controlled root boxes to provide root temperatures comparable to field conditions and connected to an automatic irrigation system to provide water at a soil water tension of 150 hPa.

The treatments were $0.02 \text{ cm}^3 \text{ ozone m}^{-3}$ (referred to as "control") and $0.1 \text{ cm}^3 \text{ ozone m}^{-3}$. Two chambers (each with four trees) were used for each treatment. Fumigation was provided for 24 hours per day for 42 days (February to April 1993). Background concentrations of about $0.008\text{--}0.010 \text{ cm}^3\text{m}^{-3}$ of SO_2 and NO_x were present in the chambers.

After effects

The spruce trees were transferred to the environmental chambers and grown in four independent growth chambers of a large phytotron, under completely controlled conditions. During the 6-month experimental period in 1989, the plants were exposed to a climatic programme simulating variations of temperature, and day length as observed at higher elevations in the Bavarian forest as previously described under immediate effects. Each experimental group consisted of 2 large pots (50 kg), each containing three 5-year-old plants. The pots were installed in temperature-controlled root-boxes and irrigated as previously described under immediate effects.

The experiment, planned and conducted by T. PFIRRMANN 1992, was designed to study the effects of enhanced ozone, enhanced CO_2 and potassium deficiency on biomass and physiology of the cloned trees.

The spruce trees were exposed to different experimental conditions (Table 1). Fumigation was provided for 24 weeks (April 1989 to October 1989) for 24 hours per day. Background concentrations of about $0.008\text{--}0.010 \text{ cm}^3\text{m}^{-3}$ of SO_2 and NO_x respectively, were present in all four chambers.

After the end of the fumigation period, plants were transferred in the fall of 1989 to a field near the institute and exposed to ambient air and weather conditions until final sampling was done after 21 months in summer 1991. To avoid a decline of

Table 1

Experimental conditions used to examine the after effects of enhanced O₃ and elevated CO₂ levels on chromosomal structures. Fumigation was provided for 24 hours/day for 24 weeks (April 1989 to October 1989). Background concentrations of about 0.008-0.010 cm³m⁻³ of SO₂ and NO_x respectively, were present in all four chambers.

O₃, CO₂ = in cm³m⁻³

experimental groups	O ₃	CO ₂
1	0.02	350
2	0.08	350
3	0.02	750
4	0.08	750

the potassium deficient trees, they were now fertilized with potassium and other nutrients in April 1990, just before the new flush developed.

Sample treatment.

Sampling of tissue was as described by MÜLLER & al. 1991b. The root tips from all trees sampled were prepared on the same day, treated with 1-bromonaphthalene and fixed in ethanol:glacial acetic acid (3:1, v/v). After fixation, the root tips were hydrolyzed in 3N HCl for 3 min at 63°C, stained in freshly prepared Schiff's reagent, and squashed in a few drops of carmine acetic acid.

Evaluation.

The cells in metaphase were classified in the following categories: metaphase, gap, break/fragment, ring, connection, clumping, amorphous chromatin masse. Gap and ring were scored and classified in one category as were connection, clumping, amorphous chromatin mass (= stickiness).

100 to 200 metaphases per replicate tree of the two experimental groups (for the immediate effect) and 100 to 200 metaphases from one pot with three plants (for the after effect), respectively, were examined for chromosomal aberrations. Each affected metaphase was counted as an aberration and the percentages of abnormalities in total metaphases were calculated. All slides were coded and examined blind.

Statistics.

Statistical analysis was carried out by means of the software package NCSS. Significance of differences between treatment and control (for the immediate effect) was calculated using MANN-WHITNEY-test (according to BORTZ & al. 1990). A non-parametric method was necessary because of small sample sizes, which amount to n = 8.

For the presentation of the results from the phytotron study (immediate effects), the form of notched box-plots was used. A box plot is divided into different fractiles (percentiles); the horizontal line in the center of the plot is the median. The horizontal line at the top of the box limits the 75 percentile, that at the bottom the 25 percentile. The small horizontal lines, which are the borders of the vertical lines,

show the 90 percentile (at the top) and the 10 percentile (at the bottom). The notches were calculated by the following equation: Median $\pm 1.57 \times (75 \text{ percentile} - 25 \text{ percentile})n^{1/2}$, n = number of replicates.

Results and Discussion

Chromosomal analysis

Interphase nuclei and all mitotic stages were observed (distinctly pink coloured – MÜLLER & GRILL 1992) in the root meristem cells of spruce trees. Due to the effect of 1-bromonaphthalene metaphases are the most frequent stages. These induced metaphases are most suitable for the observation of chromosomal abnormalities, because the splitting of chromosomes into daughter chromosomes is delayed and the observation of the chromosomal aberrations is facilitated.

The normal metaphase of Norway spruce consists of $2n = 24$ chromosomes (cp. MIYAKE 1903, SAX & SAX 1933, PRICE 1989). The most common abnormalities observed were chromosome stickiness and breaks or fragments. Chromosome stickiness manifested itself in the form of connections, clumped metaphases and amorphous chromatin masses. Chromosome stickiness may result from the entanglement of chromatin fibres (MCGILL & al. 1974) or from breakage and exchange among chromatin fibres on the surface of adjoining chromosomes or chromatids (KLASTERSKA & al. 1976). STEPHEN 1979 suggested that stickiness is a type of physical adhesion that involves mainly the proteinaceous matrix of the chromatin material. DRUSKOVIC 1988a distinguished between unspecific aberrations (e. g. chromosome stickiness) caused by long-lasting influences or chemicals and specific aberrations (e. g. breaks and fragments) caused by acute influences or radiation. Effects of different chemicals on chromosomes have also been studied in various other plant species (e.g. EL-SADEK & ASHOUR 1978, GRANT 1982, MA 1982, BRIAND & KAPOOR 1989, GEORGE & GEETHAMMA 1990, KUMAR & al. 1991). Such structural reconstructions of the chromosome lead to a decrease or an increase of the number of genes thereby resulting in a change in plant vitality.

Phytotron study (immediate effects)

Observations of visible damage were done on a qualitative basis at the end of the ozone treatment. No visible evidence of injuries due to the effect of ozone was observed in any of the plants at the end of fumigation. The typical chlorotic banding pattern on needles, characteristic of ozone (KRESS & al. 1982, WILLIAMS 1986) could not be observed.

Differences in the results between the trees of the replicated chambers could not be observed. The differences in the number of chromosomal aberrations between the two experimental variants are shown in Figure 1. The control variant responded with an aberration rate of $2.1\% \pm 0.3$

(= mean \pm standard deviation) which is comparable to the aberration rate observed in plants grown in charcoal-filtered air in open-tops (unpublished). The treatment with $0.1 \text{ cm}^3 \text{ ozone m}^{-3}$ resulted in an $4.4\% \pm 0.8$ increase in chromosomal abnormalities. The finding that fumigation with $0.1 \text{ cm}^3 \text{ ozone m}^{-3}$ caused a two-fold increase in chromosomal aberrations in the root meristems by the end of the fumigation period is surprising,

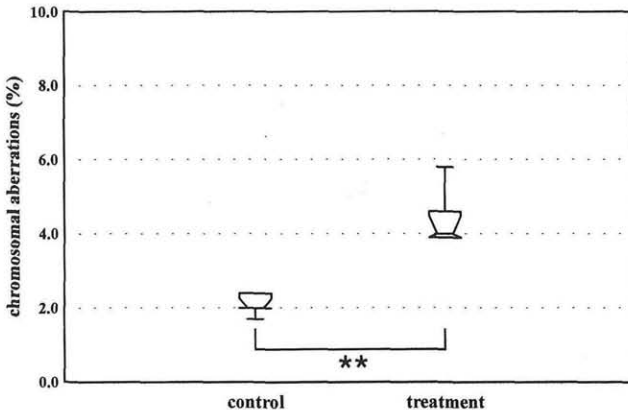


Figure 1.: Immediate effects of increased O_3 on the root tip chromosomes of young spruce plants.

Difference is statistically significant between the treatment and the control variant (Mann-Whitney-test ** $p < 0.05$).

because analyses of experiments with extremely high concentrations (800 ppb ozone for 36 days, for 7 hours/day) showed negligible differences in the pigment contents of the needles (LÜTZ 1992). However, it is well known, that ozone not only limits the growth of the whole plant, but also reduces the growth of the roots more than that of surface organs (MATYSSEK & al. 1990). A possible explanation for this fact might be the comparatively well-characterized ozone effects on the photosynthetic apparatus (e. g. REICH & al. 1987) in combination with the logical assumption that primary impact occurs at the site of phloem loading in the leaf. Thus, the results of the classification of chromosomal aberrations clearly demonstrate that the meristematic cells of the root tips of spruce plants provide a valuable tool for studying the effects of ozone. At a time where no visible evidence of the effect of ozone had been observed in any of the plants the number of chromosomal abnormalities in the treatments with $0.1 \text{ cm}^3 \text{ ozone m}^{-3}$ ozone was increased. Thus, the classification of chromosomes gave clear evidence of important ozone-affected alteration of the spruce trees not evident to visual inspection.

Phytotron/Field study (after effects)

As mentioned previously the five-year-old trees in the climate chambers were treated in different ways and then transferred to a field near the institute and exposed to ambient air and weather conditions (cf. material and methods). The clonal spruce trees used in this experiment appeared to be relatively ozone-sensitive, because pigment reduction and chlorotic spots were observed in the previous year's needles (1988) after exposure to $0.08 \text{ cm}^3 \text{ ozone m}^{-3}$ for one growth phase in 1989 (POLLE & al. 1993). Ozone induced pigment reduction in the previous year's needles was also reported by WALLIN & al. 1990. Visible damage was not found in other experiments in which average ozone concentrations similar to those of the present investigation were used (ALSCHER & al. 1989, WALLIN & al. 1990). It is possible that the constant concentration of $0.08 \text{ cm}^3 \text{ ozone m}^{-3}$ supplied in the present study for one growing period imposed higher stress on the spruce trees than exposure to fluctuating ozone concentrations in other experiments (ALSCHER & al. 1989, PAYER & al. 1990, WALLIN & al. 1990, POLLE & al. 1993).

Ozone effects

In the present investigation elevated ozone caused as an immediate effect a decreased production of above ground biomass (POLLE & al. 1993). This response has frequently been found in different coniferous species (e. g. SHAFER & al. 1987, ADAMS & al. 1988). After 21 months under normal air and weather conditions the trees from the former experimental groups with increased ozone ($0.08 \text{ cm}^3 \text{ m}^{-3}$) showed a reduction in photosynthetic capacity and a development of yellow mottling of the 1989 spruce needles during growth in the field 1990 (LIPPERT 1992). The trees of this experimental variant responded with an increased number of chromosomal abnormalities (9.2%) compared to the control variant (3.8% – Fig. 2), indicating a long-term effect of ozone on the genetic material of young spruce trees. Thus, the observed chromosomal aberrations are not only a consequence of direct impact of ozone on the genetic material, but also of secondary reactions. If there were no after effects on the genetic material, the experimental groups would have to show the similar number of chromosomal aberrations, because from our studies at natural sites, we can suggest that the genetic material responds quickly to changes of environmental influences with an increase or a decrease of chromosomal aberrations (MÜLLER & al. 1992). This explains the elevation of the aberration rate from the experimental group from the control chamber under field conditions (Fig. 2. – 3.8%) compared to the results from the studies for the immediate effects (Fig. 1 – 2.1 ± 0.3). After effects of ozone are known for various biochemical parameters. LANGEBARTELS & al. 1990 report such effects concerning secondary metabolites and antioxidants like catechin.

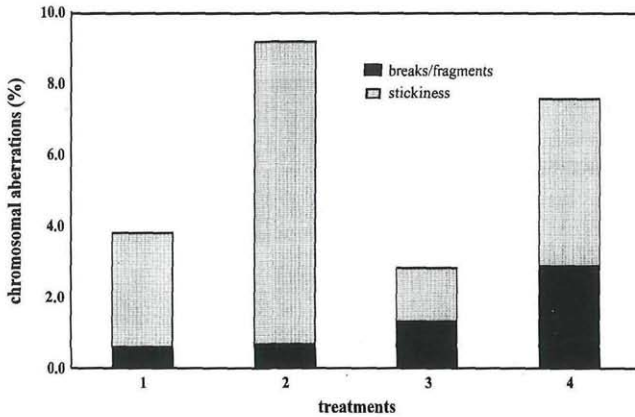


Figure 2. After effects of enhanced O_3 and elevated CO_2 on the root tip chromosomes of spruce plants 21 months after fumigation had ceased. Abbreviations 1–4: represent the experimental groups from Table 1.

LÜTZ 1992 observed long-term effects on pigment contents in spruce needles. Furthermore, LAISK & al. 1989, did not observe a direct ozone influence on the chloroplast and they too described their ozone effects as secondary reactions caused by ozone.

CO_2 -effects

In the present experiment, exposure of spruce trees to enhanced CO_2 for 6 months had no significant immediate effect on pigment content (POLLE & al. 1993), whereas photosynthesis was about two-fold higher in spruce plants grown at elevated CO_2 than in trees grown at ambient CO_2 concentration (PFIRRMANN 1992) and the production of above-ground biomass was increased (POLLE & al. 1993). Not only CO_2 is a major greenhouse gas, i. e. radiatively active, but it is essential to plant life and also stimulates growth (KRAMER 1981, DAHLMAN & al. 1985, WARRICK 1988).

21 months after the fumigation had ceased in this study the ambient CO_2 -treatment ($350 \text{ cm}^3 \text{ m}^{-3}$) resulted in a higher number (3.8%) of chromosomal aberrations than the experimental variant with $750 \text{ cm}^3 \text{ CO}_2 \text{ m}^{-3}$ (2.8% - Fig. 2). The trees in the ambient CO_2 -treatment with $0.02 \text{ cm}^3 \text{ ozone m}^{-3}$ showed similar chromosomal abnormalities to trees from so-called natural "clean air" areas, which show up to 4% of aberrations (MÜLLER & al. 1992). The higher number of chromosomal abnormalities (especially of chromosomal stickiness) in the ambient treatment ($350 \text{ cm}^3 \text{ m}^{-3}$) reflects effects on the genetic material of root tips. Thus, the results indicate that elevated CO_2 for one growth phase caused an after effect, but it caused an improvement rather than a deterioration in the amount of

chromosomal aberrations and hence in the vitality of a plant. Data dealing with effects of CO₂ concentration on belowground plant responses exist, but they are few and far between (TOGNONI & al. 1967, DEL CASTILLO & al. 1989, CHAUDHURI & al. 1990, ROGERS & al. 1992). Results from studies with e.g. *Glycine max* showed a significantly increased volume of the root system (taproot and all laterals). Root/shoot ratio and root weight ratio were boosted. Root diameter was 27% greater in the root hair zone. Light microscopy revealed a 23% increase in stele diameter and a 28% increase in cortex width (ROGERS & al. 1992).

Ozone and CO₂-effects

The spruce trees in the present study grown at elevated concentrations of both CO₂ and ozone, responded immediately after the fumigation period with mostly independent and additive effects. Ozone-induced growth reduction was prevented, although the needles contained the lowest pigment content and showed visible injuries (POLLE & al. 1993).

The treatments with elevated CO₂ in the presence of increased ozone showed after 21 months under normal air and weather conditions 7.8% chromosomal aberrations, the treatment with ambient CO₂ (350 cm³m⁻³), which resulted in 9.2% chromosomal abnormalities (Fig. 2). Thus indicating that elevated CO₂ during ozone fumigation caused more likely an improvement than a deterioration in the amount of chromosomal aberrations, too. However, the greater part of the current research on the physiological effects of elevated CO₂ levels and air pollutants is traditionally focussed on rapidly growing herbaceous plants, because its relatively short generation time enabled it to be grown from seedling to maturity in elevated CO₂ within a realistic time period (BARNES & PFIRRMANN 1992). One well known model is the radish plant, because its response to ozone is well characterized (ATKINSON & al. 1988, HELD & al. 1991). Elevated CO₂ reduced in this plant model the detrimental effects of ozone on plant growth. Growth of the plants exposed to a combination of the gases was not significantly different from that of plants treated with elevated CO₂ alone, and was markedly greater than that for plants exposed to O₃ at ambient CO₂ concentration (BARNES & PFIRRMANN 1992).

Conclusions

Ozone causes effects on the root tip meristems of spruce plants in form of an increased number of chromosomal aberrations. Enhanced ozone affects the genetic material not only directly after finishing the fumigation, but also causes an intensified "negative" memory effect created probably by secondary reactions. Elevated CO₂ shows a "positive" long term effect manifest as in a decreased number of damaged meristem cells. The impact of these environmental factors may be quite significant for forests and tree

vegetations. However, there has been a lack of experimental evidence for forest trees (SHUGART & al. 1986, KRAUSE 1988, JARVIS 1989). For this reason research on the effects of elevated CO₂ levels and air pollutants on tree physiology needs to be intensified.

Acknowledgements

We are grateful to Prof. Cornelius LÜTZ for the fumigation experiment, to Bärbel KÖHLER for technical assistance and to Michael TAUSZ for the statistic examination.

This project was supported by the "Bund-Bundesländerkooperation auf dem Gebiet der Rohstoff-, Energie- und Umweltforschung der Steiermärkischen Landesregierung und der Bundesministerien für Wissenschaft und Forschung, sowie für Land- und Forstwirtschaft" and the "Jubiläumsfonds der Österreichischen Nationalbank (project number 4479). We thank the GSF Forschungszentrum-Epoka (Germany) for the fumigation experiment and the Forstliche Bundesversuchsanstalt Vienna for plant material.

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Recensio

HARTL Helmut, KNIELY Gerhard, LEUTE Gerfried H., NIKLFELD Harald & PERKO Michael 1992. Verbreitungsatlas der Farn- und Blütenpflanzen Kärntens. Unter Mitwirkung zahlreicher Botaniker Österreichs und seiner Nachbarländer. Mit einem landeskundlichen Beitrag von Martin SEGER. – 4°, 451 Seiten, 7 Abb. (Karten), 180 Farbphotos, 2457 Rasterkarten; Ln. – Verlag des Naturwiss. Vereins für Kärnten, Klagenfurt. – AT\$ 360.–. – ISBN 3-85328-000-5.

Als erstes überrascht gleich der ungemein günstige, niedrige Preis für das schöne, sehr sorgfältig gestaltete und sehr gut ausgestattete Werk; das ist durch finanzielle Förderung durch das Land Kärnten möglich geworden.

Der Band mit der Dokumentation des gegenwärtigen Standes der Kartierung der Flora Kärntens ist in einen Einleitungsteil, einen Kartenteil und einen Anhangsteil gegliedert. Der Einleitungsteil (p. 11–59) enthält einen Abschnitt über die naturräumliche Gliederung Kärntens, eine Übersicht über die Vegetation Kärntens mit farbiger Karte der aktuellen Vegetation (Farbtafel 1, 14 Einheiten unterschieden), 15 Farbtafeln mit 180 Photos von Vegetation und Flora, eine Erforschungsgeschichte der Kärntner Flora (p. 45–49; ab der Ankunft WULFENS in Kärnten im Jahre 1764), einen Beitrag über die floristische Kartierung in den letzten 15 Jahren und den gegenwärtigen Stand des Projektes (p. 50–57) sowie ein Verzeichnis der Mitarbeiter (p. 58–59). Im Kartenteil (p. 63–372) sind die Signaturen auf Quadrantenbasis eingetragen und nach 3 Zeitstufen (Beobachtung vor 1900, 1900–1944, seit 1945) differenziert. Archäophyten sind nicht eigens ausgewiesen, Neophyten und unbeständige Adventive sind gekennzeichnet. Es sind nur Funde in Kärnten berücksichtigt. Auch im Falle von Quadranten, die teils in Kärnten, teils in einem der Nachbarländer liegen, ist nur dann eine Signatur eingetragen, wenn ein Vorkommen im Kärntner Anteil liegt. Auf pflanzengeographisch interessante Vorkommen knapp außerhalb der Landesgrenzen wird im Anhangsteil hingewiesen, doch ist hier etwas uneinheitlich vorgegangen worden; so sind im Bereich der Koralpe solche Vorkommen

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Zeitschrift/Journal: [Phyton, Annales Rei Botanicae, Horn](#)

Jahr/Year: 1994

Band/Volume: [34_2](#)

Autor(en)/Author(s): Müller Maria, Grill Dieter, Guttenberger Helmut

Artikel/Article: [The Effects of Interactions between Ozone and CO2 on the Chromosomes of Norway Spruce Root Meristems. 321-335](#)