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Ozone-Induced Long-Term Effects on Chromosomal Aberration Rates in Root-Tip Meristems of Spruce Trees do not Correspond to Changes in Tissue Antioxidant Status

By

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With 1 figure

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Summary

WONISCH A., TAUSZ M., MÜLLER M., SOJA G. & GRILL D. 2003. Ozone-induced long-term effects on chromosomal aberration rates in root-tip meristems of spruce trees do not correspond to changes in tissue antioxidant status. – *Phyton* (Horn, Austria) 43 (1): 147–160, with 1 figure. – English with German summary.

Ozone effects on 8-year-old clonal spruce trees [*Picea abies* (L.) KARST.] were investigated in a glasshouse fumigation system. Fumigated trees experienced ozone concentrations up to 80 nl l⁻¹ versus control plants exposed to ambient air background concentrations of maximum peaks below 40 nl l⁻¹. Antioxidants and pigments in one-year-old needles, antioxidants in fine roots, and chromosome damages and mitotic indices were determined at the end of the fumigation period and one year later after growing the trees under ambient conditions. Chromosomal aberration rates in root-tip meristems increased upon ozone treatment (7 to 8 % versus 4 to 5 % in controls) and remained at elevated levels for one year after the fumigation was

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terminated. However, these changes were not accompanied by alterations in the antioxidant systems in needles or roots. Irrespective of the treatment, roots contained less ascorbate and tocopherol than needles and the redox state of ascorbate and glutathione was more oxidized in the fine root tissues. Ozone treatment caused an increase in total glutathione concentrations in needles (860 vs 630 nmol g⁻¹ dry weight), but this effect vanished after the one year recovery period. It may be concluded that for long-lasting ozone effects chromosomes are more sensitive indicators than antioxidants in leaves or roots.

Zusammenfassung

WONISCH A., TAUSZ M., MÜLLER M., SOJA G. & GRILL D. 2003. Langzeiteffekte von Ozon auf das genetische Material im meristematischen Gewebe von Feinwurzeln und antioxidative Schutzsysteme von Fichten. – *Phyton* (Horn, Austria) 43 (1): 147–160, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Um mögliche Zusammenhänge zwischen der Entstehung von vermehrten Chromosomenaberrationen im meristematischen Gewebe und Änderungen im antioxidativen Schutzsystem der Feinwurzeln nach Ozoneinfluss zu untersuchen, wurden achtjährige Klonfichten [*Picea abies* (L.) KARST.] in den Glashäusern des Forschungszentrums Seibersdorf mit Ozon begast. Die behandelten Fichten erhielten Ozonkonzentrationen bis zu 80 nl l⁻¹ O₃, die Kontrolle wurde an Ozon-Außenkonzentrationen angepasst (bis zu 40 nl l⁻¹ O₃). Untersuchungen an einjährigen Nadeln (Antioxidantien und Pigmentausrüstung) sowie an Feinwurzeln (Antioxidantien, Mitoseindex und Chromosomenaberrationen) wurden sowohl sofort nach Beendigung des Glashausexperiments als auch ein Jahr später durchgeführt. Somit sollten auch mögliche Ozon-Langzeiteffekte im Experiment ermittelt werden. Die Ozonbehandlung führte zu erhöhten Chromosomenaberrationen (7 bis 8 % gegenüber 4 bis 5 %), welche auch nach einem Jahr Exposition der Fichten im botanischen Garten in Graz, existent waren. Das antioxidative Schutzsystem der einjährigen Nadeln und Feinwurzeln zeigte aber keine ozonbedingte Änderung. Unabhängig von der Behandlung wurden in den Feinwurzeln geringere Konzentrationen von Ascorbat und Tocopherol als in Nadeln festgestellt. Weiters konnten stärker oxidierte Redoxverhältnisse von Ascorbat und Glutathion gemessen werden. Die Ozonbehandlung führte auch zu erhöhten Gesamtkonzentrationen von Glutathion, allerdings konnte dieser Effekt ein Jahr später nicht mehr beobachtet werden.

Aufgrund der erhobenen Daten kann man daraus schließen, dass für Langzeiteffekte von Ozon die Ermittlung von Chromosomenaberrationen im meristematischen Gewebe der Feinwurzeln sensitivere Indikatoren darstellen als das antioxidative Schutzsystem der Nadeln und Feinwurzeln.

Introduction

Ozone is a major atmospheric trace gas which negatively affects forest trees (REICH 1987, MATYSSEK & al. 1995, SANDERMANN & al. 1997). Nowadays alpine forests in central Europe can experience O₃ episodes above 120 nl l⁻¹ (SMIDT & GABLER 1994). Such concentrations are known to impair the physiological performance of conifer seedlings in chamber experiments

(SANDERMANN & al. 1997). Under field conditions, reduced photosynthetic capacity has been reported for mature spruce and larch trees after more than 12 weeks of exposure to mean O_3 concentrations higher than 100 nl l^{-1} (WIESER 1999). However, it is still doubtful if the atmospheric ozone concentrations in the Alps exceed the critical levels for damage to Norway spruce under natural conditions.

Ozone is a strong oxidant, and probably acts through the formation of toxic active oxygen species (AOS). The antioxidative defence systems protect plant cells through removal of AOS. Important low molecular weight antioxidants are ascorbate, glutathione, and α -tocopherol. These molecules and protective pigments (xanthophylls, carotenes) are important markers of stress to plants. It has been shown that ascorbate and glutathione in conifer needles increased after O_3 exposure under controlled conditions (MEHLHORN & al. 1986, KRONFUB & al. 1998).

In previous experiments in open-top chambers (WONISCH & al. 1998) and in a glasshouse system (WONISCH & al. 1999), moderate ozone concentrations did not induce such changes in the antioxidative system in needles of young spruce trees. On the other hand, in both experiments long-lasting effects of ozone treatment on the root-tip meristems of the trees were observed. The number of chromosome damages in dividing cells increased upon ozone exposure and this increase persisted up to two years after the exposure was terminated (MÜLLER & al. 1996, WONISCH & al. 1998, 1999). Chromosome damages in dividing tissues have been found upon different pollutant exposures (MÜLLER & al. 2000d) and are regarded a sensitive stress indicator (MÜLLER & al. 1998). The direct consequence for the growth of the whole tree, however, is still unclear. Furthermore, the signalling pathway from the location of the pollution impact (the foliage) to the site of effects (root-tips) is still enigmatic. Several substances have been tested in feeding experiments with young spruce seedlings. Plant hormones such as jasmonates (MÜLLER & al. 2000c), and redox substances such as glutathione (MÜLLER & al. 2000b) were able to induce chromosome damages.

The present study tests the hypothesis that the development of chromosome damages in root-tips upon ozone exposure may be accompanied by changes in the redox system in the roots rather than the shoots. While it is unlikely that ozone directly induces oxidative stress in the roots since it is readily decomposed in the leaf tissues (LAISK & al. 1989), the idea that ubiquitous and easily transportable redox systems such as glutathione or ascorbate may be involved in stress sensing and signal transduction is intriguing (MAY & al. 1998, FOYER & NOCTOR 2001). The involvement of hydrogen peroxide (an ROS) in stress signalling has been shown recently (KARPINSKI & al. 1999), and an involvement of redox pools (such as ascorbate or glutathione) is also conceivable. For this

purpose, the determination of biochemical redox markers in fine roots of ozone exposed spruce seedlings was included in the study. At the same time, chromosome damages in root-tips were recorded.

Abbreviations: GSH – glutathione; GSSG – oxidized glutathione; asc – ascorbate; dw – dry weight.

Material and Methods

Plant material

Norway spruce trees (*Picea abies* [L.] Karst.) from the clone 884611 FBVA Vienna (provenance Ottenstein, Austria, 550–600 m a.s.l.) were potted in acidic (14 cm diameter pots), sandy forest soil and kept outdoors in the botanical garden in Graz prior to the experiment. During this time, plants were irrigated regularly, fertilized twice a year and protected from pathogens, which was necessary in only one case more than two years prior to the experiment (paraffin oil based pesticide, Paramaag Sommer, Austria). At the beginning of the experiment, trees were 8 years old.

Experimental conditions

Fumigation was carried out in a glasshouse chamber system at the Austrian Research Centers, Seibersdorf. Exposure time was February 7 until May 6. Two variants [non-filtered air (control) and ozone (O_3)], in two chambers, were applied. For the O_3 variant, O_3 was added to non-filtered air (control) during the light period. Daily ozone concentrations in the fumigation chambers were 80 nl l^{-1} applied for 8 hours a day 7 days a week. Control chambers experienced maximum ozone peaks below 40 nl l^{-1} (ambient concentrations at Seibersdorf). Twenty-one randomly chosen trees were used per variant. The temperature in the chambers was by about 2°C above ambient, relative humidity and light intensity were similar to ambient conditions. Background concentrations of (maximum) 3 nl l^{-1} SO_2 , and 8 nl l^{-1} NO_x were present in the chambers. Plants were irrigated regularly and neither fertilization nor treatments with pesticides and herbicides were applied during the fumigation.

Sampling dates and sampling conditions

Eleven trees of each treatment were sampled at the end of the fumigation period (May 6). The remaining trees (10 per experimental group) were transferred to the botanical garden in Graz and grown under ambient conditions until September 24 of the following year. Biochemical analyses were performed on needle material of the youngest fully developed age class (1st needle age class) and on fine roots ($< 1 \text{ mm}$ diameter). Sampling was carried out after transferring trees for one day to dim light (less than $40 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in the PAR range) in the laboratory to standardize conditions with respect to short-term light-dependent changes. The material was immersed immediately (within seconds) in liquid nitrogen and lyophilised afterwards. Lyophilised material was ground in a dismembrator (Braun, Germany) and the dry powder was stored in air-tight plastic vials at -25°C until analysis.

Growth parameters

At the youngest shoots of the terminal and the lateral twigs in the first and second whorl shoot length and shoot fresh-weight was measured. Shoots developed in the fumigation chambers were already fully developed at the first sampling date (beginning of May), probably due to higher temperatures in the chambers compared to ambient conditions. At the second sampling date (September 24) shoots were fully developed corresponding to the season.

Biochemical analyses

Chloroplast pigments were determined using the HPLC gradient-method described by PFEIFHOFFER 1989. This method permits separation of all important chloroplast pigments in one step: Column Spherisorb S5 ODS2 250 × 4,6 mm with precolumn S5 ODS2 50 × 4,6 mm. Solvent A: acetonitrile : methanol : water = 100 : 10 : 5 (v/v/v), solvent B: acetone : ethylacetate = 2 : 1 (v/v), linear gradient from 10 % solvent B to 70 % solvent B in 18 min, run time 30 min, flow 1 ml min⁻¹, photometric detection at 440 nm. The needle dry powder was shaken in ice-cold acetone and centrifuged at 2 °C and 15000 × g. The supernatants of three re-extractions were combined and adjusted to a fixed final volume. Extracts were injected (20 µl) using a cooled autosampler.

α-Tocopherol was determined following a method by WILDI & LÜTZ 1996, which was slightly modified: Acetone extracts (see above) were subjected to a isocratic HPLC analysis (Column Spherisorb S5 ODS2 250 × 4,6 mm with precolumn S5 ODS2 50 × 4,6 mm) using methanol as solvent. Tocopherol was detected directly by fluorometry (excitation 295, emission 325 nm).

The determination of glutathione (oxidized and reduced) was conducted using a gradient HPLC-analysis (Column Spherisorb S5 ODS2 250 × 4,6 mm with precolumn S5 ODS2 50 × 4,6 mm) according to KRANNER & GRILL 1994. Lyophilised needle powder was extracted in 0.1 M HCl and incubated with 200 mM CHES-buffer (2-8-N-cyclohexylamino-ethanesulfonic acid) and DTT (dithiothreitol) to reduce thiol-groups. The SH-groups were labelled by monobromobimane. For the determination of the content of oxidized thiols SH-groups were blocked using 50 mM NEM (N-ethyl-maleimide). The excess of NEM was removed with toluene and derivatisation with monobromobimane was done as described above. Separation and determination of the derivatised thiols were completed according to the gradient-method described in KRANNER & GRILL 1994. Lyophilisation and storage conditions did not change the glutathione redox state compared to extracts of frozen material in young spruce trees cultivated at the Institute of Plant Physiology in Graz.

Concentrations of ascorbic acid and dehydroascorbic acid were measured simultaneously according to TAUSZ & al. 1996 using a derivatisation procedure with o-phenylenediamine. Extracts of lyophilised needle powder in metaphosphoric acid (1.5 %) were treated with o-phenylenediamine and separation was carried out on an isocratic HPLC system (Column Spherisorb S5 ODS2 250 × 4.6 mm with precolumn S5 ODS2 50 × 4.6 mm) using hexadecylammoniumbromide as ion pairing agent in aqueous methanol (30 % vol) as solvent. Detection was performed at 348 and at 248 nm simultaneously. Lyophilisation and storage conditions did not change the

ascorbate redox state compared to samples of fresh material in spruce needles (TAUSZ & al. 1996).

Chromosomal analyses

Three to 5 root tips per plant were cut and fixed in 5 ml ethanol:glacial acetic acid (3:1, v/v). After fixation the root tips were hydrolysed in 3 M HCl for 3 min at 63 °C, stained in freshly prepared Schiff's reagent and squashed in a few drops of 45 % acetic acid (treatment modified according to MÜLLER & al. 1991). The cells in metaphase and anaphase were classified in the following categories (according to FISKEŠJÖ 1994, for the *Allium*-test): normal metaphase, normal anaphase, vagrant chromosomes, chromosome stickiness (= bridges and sticky chromosomes), and fragments. One hundred to 200 meta- and anaphases (from cells with intact cell walls) per replicate tree were examined for chromosomal aberrations. The percentages of abnormalities in total meta- and anaphases were calculated. For the determination of the mitotic index (MI) a minimum of 500 cells per replicate sample was analysed and the percentages calculated.

Statistics

Differences between treatments and harvest dates were verified using two-way analysis of variance (ANOVA). If ANOVA F-test was significant cross comparisons among groups were computed using least squared difference (LSD) method. Normal distribution of the data could not be tested, since $n=10$ is too small to allow this. Small violations of normality assumption will usually not adversely bias ANOVA. If the assumption of homogeneity of variances was violated (checked by Levene's test) logarithmic transformation of raw data was used. If means and variances were strongly correlated or homogeneity of variances not met by data transformation, ANOVA was skipped and differences between treatments within the same harvest date were evaluated using Mann-Whitney's U-test. The same was done when less than 10 valid replicates per group were present. $P < 0.05$ was regarded significant. All computing was performed on Statistica (StatSoft, Tulsa, USA) software package.

Results

While there were no ozone-dependent differences in the weight or length of young shoots directly at the end of the fumigation period (data not shown), the new flush that developed the following season under ambient conditions showed small differences in growth between formerly ozone-treated and control trees. While the average weight of terminal shoots was equal without regard of the ozone treatment, the average shoot length of controls was 110 ± 33 mm (mean $\pm$ SD) versus 90 ± 29 at formerly ozone treated trees.

Pigment and antioxidant concentrations were significantly different between the two harvesting dates: Chlorophyll concentrations increased and the ratios of carotenoids over chlorophyll decreased, with the exception of β -carotene contents, which did not change (Table 1). Ascorbate and

Table 1.

Photosynthetic pigments in current season's needles of ozone exposed spruce trees. Means and standard deviations of n = 11 (harvest 1996) and n = 10 (harvest 1997) trees per group. Asterisks indicate significant differences between control and ozone variant within the same harvest.

	Harvest 1996 (fumigated)		Harvest 1997 (post-fumigated)		ANOVA Effect	
	Control	Ozone	Control	Ozone	Harvest	Treatment
Total chl [$\text{mg g}^{-1}\text{dw}$]	2.44 ± 0.55	2.71 ± 0.68	3.06 ± 0.74	3.01 ± 0.54	0.024	ns
<i>Carotenoids</i> [$\mu\text{g mg}^{-1}\text{chl}$]						
V+A+Z	26 ± 6	28 ± 9	47 ± 7	44 ± 4	<0.001	ns
Neoxanthin	68 ± 6	67 ± 22	48 ± 3	47 ± 4	<0.001	ns
Lutein	176 ± 18	185 ± 51	118 ± 5	124 ± 11	<0.001	ns
α -Carotene	77 ± 9	81 ± 20	67 ± 8	65 ± 7	0.002	ns
β -Carotene	53 ± 10	58 ± 11	54 ± 6	53 ± 4	ns	ns
Epoxidation state	96 ± 2	94 ± 2	98 ± 1	98 ± 1		ns

Data in this line did not meet assumptions for ANOVA. Comparisons between control and ozone treated groups were calculated separately according to the harvest date using Mann-Whitney test.

tocopherol increased whereas glutathione concentrations decreased in the needles upon exposure to ambient conditions after the fumigation.

Fine roots contained only low amounts of ascorbate and tocopherol compared to the needle tissues. Total ascorbate amounted to about 15 % of the needle concentrations, and tocopherol to less than 10 %. Glutathione concentrations were of comparable magnitude to those of needles. With the exception of ascorbate which was not measured in samples of the 1996 harvest, antioxidant concentrations in roots decreased upon exposure of trees to ambient conditions. The redox states of glutathione and ascorbate were more oxidized in roots than in needles.

Ozone treatment had only very few significant effects on the antioxidant system: Needles of ozone treated trees contained by about one third more total glutathione than control needles directly after the ozone exposure (Table 2). Fine roots of ozone treated trees contained less α -tocopherol compared to control trees after recovery under ambient conditions (Table 3).

The mitotic indices in the root meristems of spruce trees did not vary significantly between ozone treated and control trees. Eight to 10 % of dividing cells in total meristem cells were counted in all experimental groups (data not shown).

The chromosomal aberration rates increased significantly upon ozone treatment (Fig. 1). The negative effect of ozone on the root meristems persisted for one year after the trees were transferred to outdoor conditions. In control roots harvested directly after the fumigation chromosomal aberrations were equally represented by vagrant chromosomes, fragments, and chromosome stickiness, all types accounting for about one third of the

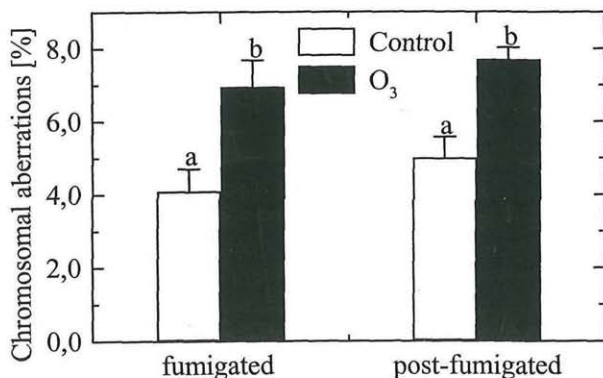


Fig. 1. Chromosomal aberration rates in root-tip meristems of young spruce trees. Differences between O₃ fumigated variants and controls were evaluated using Mann-Whitney's U-test. Means and SD of n=6 replicates.

Table 2.
Antioxidants in current season's needles of ozone exposed spruce trees. Means and standard deviations of n = 11 (harvest 1996) and n = 10 (harvest 1997) trees per group. Asterisks indicate significant differences between control and ozone variant within the same harvest.

	Harvest 1996 (fumigated)		Harvest 1997 (post-fumigated)		ANOVA Effect	
	Control	Ozone	Control	Ozone	Harvest	Treatment
Total asc [$\text{mg g}^{-1}\text{dw}$]	1.82 ± 0.31	2.02 ± 0.66	2.41 ± 0.39	2.34 ± 0.30	0.002	ns
Oxidised asc [% of total]	13 ± 3	10 ± 3	14 ± 10	11 ± 1		
α -Tocopherol [$\mu\text{g g}^{-1}\text{dw}$]	166 ± 21	191 ± 41	231 ± 37	221 ± 29		
GSH [$\text{nmol g}^{-1}\text{dw}$]	632 ± 91	$861 \pm 301^*$	504 ± 51	537 ± 74	<0.001	ns
%GSSG	7 ± 2	9 ± 1	11 ± 2	9 ± 2		

Data in these lines did not meet assumptions for ANOVA. Comparisons between control and ozone treated groups were calculated separately according to the harvest date using Mann-Whitney test.

Table 3.

Antioxidants in fine roots of ozone exposed spruce trees. Means and SD of n = 11 (harvest 1996) and n = 10 (harvest 1997) trees per group. Asterisks indicate significant differences between control and ozone variant within the same harvest.

	Harvest 1996 (fumigated)		Harvest 1997 (post-fumigated)		ANOVA Effect	
	Control	Ozone	Control	Ozone	Harvest	Treatment
Total asc [$\text{mg g}^{-1}\text{dw}$]	nd	nd	$0,38 \pm 0,05$	$0,40 \pm 0,04$		
Oxidised asc [% of total]	nd	nd	30 ± 9	24 ± 7		
α -Tocopherol [$\mu\text{g g}^{-1}\text{dw}$]	19 ± 7	16 ± 6	17 ± 8	$9 \pm 5^*$		
GSH [$\text{nmol g}^{-1}\text{dw}$]	627 ± 284	538 ± 147	238 ± 190	210 ± 76	<0.001	ns
%GSSG	16 ± 3	14 ± 2	35 ± 9	36 ± 9		

Data in these lines did not meet assumptions for ANOVA. Comparisons between control and ozone treated groups were calculated separately according to the harvest date using Mann-Whitney test.

total aberrations. In root meristems of all other experimental groups, vagrant chromosomes accounted for more than two thirds of the total aberrations (data not shown).

Discussion

The shoot length of the young spruce trees seemed to be slightly impaired in the season following the fumigation. On the other hand, only marginal changes in the antioxidative system of the spruce needles were induced by exposure to double ambient ozone concentrations. An increase of total glutathione concentrations by about 30 % is in agreement with some previous studies (MEHLHORN & al. 1986). However, as in other studies under comparable conditions (WONISCH & al. 1998, 1999) ascorbate, tocopherol, and chloroplast pigments did not change upon the applied ozone treatment. Some changes in these variables can be induced by transferring the trees from the chambers to ambient conditions (WONISCH & al. 1999), which explains significant differences between the harvest times.

The redox systems in the fine roots of spruce seedlings were hardly affected by ozone treatment, although upon one year exposure to ambient conditions after the fumigation experiment, the fine roots of formerly ozone treated trees contained less α -tocopherol than those of non-treated controls. However, since α -tocopherol is mainly present in thylakoid membranes (FRYER 1992), root tissue contains very low amounts compared to needles. Since major antioxidants in root tissues were glutathione and ascorbate, the concentrations of which did not change upon ozone exposure, the physiological importance of the tocopherol changes remains doubtful.

Despite the only marginal ozone effects on the antioxidative protection systems in fine-root and needle tissues, chromosomal aberration rates were persistently increased in root-tips meristems of ozone treated trees. This effect is in good agreement with results from previous studies on different clones of spruce trees (MÜLLER & al. 1996, WONISCH & al. 1998, 1999), and it is the more noteworthy, since the root-tips have developed under ambient ozone conditions after the fumigation has been terminated. The rate of cell divisions in the meristems is represented by the mitotic index, which remained unaffected by ozone treatments.

From the present study there is no evidence that chromosome damages are accompanied by changes in the redox systems. But some experimental limitations of the present investigations must be taken into account: biochemical antioxidant determination results in average tissue concentrations of fine roots, consisting mainly of fully developed root tissue. Root-tip meristems are only a small part of the fine roots sampled. Some recent data showed that intercellular (SANCHEZ-FERNANDEZ & al. 1997) and intracellular (MÜLLER & al. 2000a) distribution of the glutathione redox

system is not uniform. Some results still suggest a possible impact of changes in the glutathione redox system on the cell cycle and on chromosome damages (MÜLLER & al. 2000c). Recent experiments with transgenic plants suggest that elevated glutathione biosynthetic capacity in chloroplasts is accompanied by greatly enhanced sensitivity to oxidative stress (CREISSEN & al. 1999). However, a reliable method to measure specific antioxidant localization in fine roots of spruce is not available yet.

In summary, it can be concluded from the present results that for some long-lasting ozone effects chromosomal aberrations may be a more sensitive marker than changes in the tissue antioxidant status.

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Recensio

FÜHRER Erwin & NOPP Ursula 2001. Ursachen, Vorbeugung und Sanierung von Waldschäden. – Lex. 8°, X + 514 Seiten; kart. – Facultas Verlags- und Buchhandels AG Wien. – € 44,-. – ISBN 3-85076-528-8.

Dieser umfangreiche Band faßt Ergebnisse von fast 20 Jahren Waldschadensforschung in fichtenreichen Wäldern in Österreich zusammen. „Nachdem nun über 1000 Publikationen, Dissertationen, Diplomarbeiten und Kongreßbeiträge als wissenschaftliche Mosaiksteinchen zu ausgewählten Fragen der Waldschadensproblematik von Forschern der „Forschungsinitiative gegen das Waldsterben“ (FIW) veröffentlicht wurden, war es endlich Zeit, die für die forstliche Praxis relevanten Ergebnisse dieser Forschungstätigkeit in verwertbarer Form darzulegen“ (Vorwort p. VI). Das Buch richtet sich an alle, die in irgendeiner Weise mit Waldschadensproblemen konfrontiert sind, also in erster Linie an Förster, Leiter von Forstbetrieben, Berater und Konsulenten in der Forst- und Holzwirtschaft etc., aber auch an Ökologen, Naturschützer und Studenten.

Nach der Einleitung mit einem „Orientierungsleitfaden“ für die behandelten Themen folgt Abschnitt II: Zielsetzung und Zieldefinition als Grundlage der Feststellung von Sanierungsbedarf und Sanierungserfolg (p. 15–39). In III. Aspekte der Differentialdiagnose wird eine tabellarische Übersicht mit Abbildungen für die möglichen Ursachen von Nadelverlusten und Vergilbungserscheinungen gebracht (p. 41–47). Im Abschnitt IV über den Kronenzustand werden u. a. waldwachstumskundliche Vitalitätsindizes diskutiert (p. 49–65). Abschnitt V enthält eine Übersicht über die wichtigsten ökologischen Ansprüche und Eigenschaften von 25 heimischen Baumarten (5 Nadel-, 15 Laubgehölze; p. 67–85), da falsche Baumartenwahl eine wichtige Ursache für spätere Waldschäden ist. In „Bodendegradation–Ernährungsstörungen–Walddüngung und andere Gegenmaßnahmen“ (VI, p. 87–112) wird neben der Diagnose u.a. auch auf Alternativen zur Düngung eingegangen. 62 Seiten (VII, p. 123–184) umfaßt der Abschnitt „Habitat–Waldschaden–disposition–Waldschaden“. Im umfangreichsten Abschnitt [VIII „Prädispositionsabschätzung zur Gefährdungserkennung und Schadensvorbeugung (p. 185–424)] werden Immissions-, Sturm-, Schnee- und Schälsschäden sowie drei Schadinsekten behandelt und Prädispositionsschlüssel erarbeitet. Abschnitt IX gilt der biochemischen und strukturellen Indikation von Streß bei Fichten (p. 425–431). Auf p. 428 wird als Methode der Früherkennung von Waldschäden das „cytogenetische“ (richtiger chromosomenmorphologische) Verfahren erwähnt, dem der Rezensent besonders skeptisch gegenübersteht, weil seiner Erfahrung nach viele „Aberrationen“ präparativ bedingt sind und kaum von vorher entstandenen zu unterscheiden sind. Schließlich werden Fragen der Datenqualität von Erhebung über Schulung bis Reproduzierbarkeit diskutiert (X, p. 433–448). Im Sinne des Zieles der ganzen Forschungen, der Umsetzung der Ergebnisse in der forstlichen Praxis, wird auch auf dabei auftretende Hürden und die Möglichkeiten, sie zu überwinden, eingegangen [XI Akzeptanz von Vorschlägen zur Waldsanierung (p. 449–472)]. Es folgen Anhänge bezüglich Nährstoffmangelsymptomen, ausgewählter Nadelspiegelwerte von wichtigen Ionen, Bodenkennwerte, Düngungsbedarf, Meliorationsmaßnahmen, Durchforstungsstrategien und Waldinventuren und Waldschäden sowie ein ausführliches Sachregister.

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