

New initiative

Response patterns in adult forest trees to chronic ozone stress: identification of variations and consistencies

Angela J. Nunn^{a,*}, Ilja M. Reiter^a, Karl-Heinz Häberle^a, Christian Langebartels^b,
Günther Bahnweg^b, Hans Pretzsch^c, Heinrich Sandermann^b, Rainer Matyssek^a

^a*Ecophysiology of Plants, TU München, Am Hochanger 13, D-85354 Freising, Germany*

^b*Institute of Biochemical Plant Pathology, GSF — National Research Centre for Environment and Health, D-85764 Oberschleißheim, Germany*

^c*Forest Yield Science, TU München, Am Hochanger 13, D-85354 Freising, Germany*

Received 27 December 2004; accepted 28 January 2005

*Responses of adult forest trees to chronic O₃ stress can be visualized in a survey table applying
a three-grade colour coding to each investigated parameter.*

Abstract

The responsiveness of adult beech and spruce trees to chronic O₃ stress was studied at a free-air O₃ exposure experiment in Freising/Germany. Over three growing seasons, gas exchange characteristics, biochemical parameters, macroscopic O₃ injury and the phenology of leaf organs were investigated, along with assessments of branch and stem growth as indications of tree performance. To assess response pattern to chronic O₃ stress in adult forest trees, we introduce a new evaluation approach, which provides a comprehensive, readily accomplishable overview across several tree-internal scaling levels, different canopy regions and growing seasons. This new approach, based on a three-grade colour coding, combines statistical analysis and the proficient ability of the “human eye” in pattern recognition.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: *Fagus sylvatica*; *Picea abies*; Tree-internal scaling levels; Colour coding; Long-term ozone exposure

1. Introduction

The responsiveness of adult trees as exposed, at their forest sites, to chronic O₃ stress is still uncertain, in particular, with respect to a process-oriented basis of cause-effect relationships (Kolb and Matyssek, 2001). Such a basis is needed for an ecologically meaningful risk assessment, in view of prognosticated increases of O₃ regimes and envisaged effects of O₃ on the carbon sink strength of trees and forests under continuing atmospheric CO₂ enrichment (Karnosky et al., 2003). Given the fact that available knowledge about the O₃ sensitivity

of trees predominantly derives from studies on juvenile individuals that were grown under controlled environments of cabinets or exposure chambers (Matyssek and Sandermann, 2003), new perspectives have been provided by free-air exposure techniques of trees to experimentally manipulated, gaseous regimes under otherwise unchanged field conditions (Karnosky et al., 2001). By such means, even whole-plant approaches of tall trees within joint stand canopies become feasible, with respect to process-based analyses of O₃ impact (Nunn et al., 2002; Werner and Fabian, 2002).

In contrast to controlled chamber studies, analyses of adult forest trees require integrative approaches while facing variable, multi-factorial site conditions besides the O₃ impact. Accounting, therefore, for potential scatter in O₃ responsiveness, the analysis of response patterns

* Corresponding author.

E-mail address: nunn@wzw.tum.de (A.J. Nunn).

(across the cell, organ and whole-tree level) is the preferential approach that, in addition, allows response scaling towards functional integration in tree performance (Matyssek and Sandermann, 2003). One analytical concept proposed by Schwela and Krause (1989) for evaluating response patterns was the ‘Chernoff–Flury Faces’ approach, which — while being mathematically complex — nevertheless may not be unaffected by subjective bias, i.e. the way of assigning tree parameters to facial features and weighing them. In fact, this latter approach has not become a common tool in air pollution research. In the following presentation, we introduce an evaluation approach of an ongoing free-air O₃ exposure experiment on adult beech and spruce forest trees, to provide a comprehensive, readily accomplishable overview integrating the O₃ responses across several tree-internal scaling levels and growing seasons.

2. Material and methods

2.1. Experimental approach

At a field site at ‘Kranzberger Forst’ (Freising, Germany, 48°25′ N, 11°39′ E) about 60-year-old beech (*Fagus sylvatica* L.) and spruce (*Picea abies* (L.) Karst.) trees were exposed to an experimentally enhanced O₃ regime using a free-air O₃ exposure system employed within the forest canopy (Nunn et al., 2002; Werner and Fabian, 2002). A twice-ambient O₃ regime (2×O₃) was applied throughout three growing seasons (2000–2002) to the entire crowns of five neighbouring beech and five neighbouring spruce trees. Comparisons were made with five trees each under unchanged ambient air (1×O₃=control). At 2×O₃, maximum O₃ levels were restricted to 150 nl O₃ l⁻¹ to prevent risk of acute O₃ injury. On-line O₃ analysis and 160 passive samplers distributed across the fumigated and non-fumigated canopy were employed for continuous O₃ monitoring. O₃ influx into leaves was assessed by parameterizing a model (Emberson et al., 2000) for the study trees using data collected on site (cf. Nunn et al., 2005). Scaffolding and a research crane provided access to sun and shade crowns. In each study tree, one branch in the sun crown and shade crown each was chosen for analysis to account for the structural and ecophysiological extremes within tree crowns. At regular intervals over three growing seasons, leaf organs were examined for phenology and macroscopic O₃ symptoms, gas exchange characteristics and biochemical parameters, along with assessments of branch and stem growth as indications of tree performance (Nunn et al., 2002; Nunn, 2005).

2.2. Statistics

Annual courses of the investigated parameters were tested separately in each species using the repeated

measures module of the general linear model (GLM, SPSS 12.0, SPSS Inc., USA), employing a nested design for crown zone (sun/shade) and O₃ regime. Discrete sampling dates were tested for statistical significance of differences between O₃ regimes, using the univariate covariance module of the general linear model. Where appropriate, covariates like cross-sectional stem area and tree height were considered in the design of the statistical model. Statistical significance was accepted at the $p < 0.05$ level. Trends, which were marked with colours in Table 1, are reported at $p < 0.15$ (and $p < 0.25$ for visual injury assessment).

3. Results and discussion

Response patterns to chronic O₃ stress were reported to occur in juvenile trees across different tree-internal scaling levels and expected to exist also in adult individuals (Matyssek and Sandermann, 2003; Kolb and Matyssek, 2001). Also, Karnosky et al. (2003) reported consistency in responses at 1.5×O₃ in *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* from the leaf-towards the ecosystem-level. In our study, O₃ effects were found in adult beech and spruce at the cell (biochemical parameters) and leaf level (phenology and photosynthetic parameters). In branches, incipient reduction in leaf biomass was indicated, whereas no reduction in radial stem growth, as an integrative parameter of whole-tree performance, has been found at the current stage of the experiment (Table 1; data from Nunn, 2005). The question arose on how to identify, in a kind of surveying approach, response patterns and their consistency to chronic O₃ stress across tree-internal scaling levels and consecutive growing seasons. To summarize responses to 2×O₃ by tree species and foliage type, investigated tree parameters were - as a result of the statistical analysis - assigned to a three-grade colour coding (beginning at $p < 0.15$, see above): yellow, no difference between the two O₃ regimes; red, negative (i.e. reduction, decline), and green, positive influence of 2×O₃ (i.e. stimulation, enhancement) on the respective parameter. The outcome of this procedure was presented in a survey table (Table 1), where statistically significant effects were marked, in addition, with asterisks. The screening of O₃ responses for consistency is exemplified, in the following, through Table 1 by comparisons between growing seasons, foliage types and tree species:

3.1. Inter-annual variation

The presented responses to O₃ exposure were investigated across three growing seasons. In the shade foliage of beech, for example, responses of photosynthetic parameters varied among years and depended on

Table 1

Responses of structural, phenological, physiological and biochemical parameters on different tree-internal scaling levels (stem, branch, leaf, biochemical parameters) in beech and spruce in a field experiment at Kranzberger Forst, Germany

parameter year of treatment	beech sun crown				beech shade crown				spruce sun crown				spruce shade crown			
	zero	one	two	three	zero	one	two	three	zero	one	two	three	zero	one	two	three
	1999	2000	2001	2002	1999	2000	2001	2002	1999	2000	2001	2002	1999	2000	2001	2002
ozone uptake		*	*			*	*			*						*
tree																
radial stem increment																
phenology																
bud break		*								*		*				
autumnal senescence		*	*	*												
length of growing season		*														
branch																
increment of leaf biomass											*					
volume increment																
SLA																
<i>Apiognomon</i> (visual symptoms)																
phenology																
bud break		*								*	*			*	*	
autumnal senescence																
length of growing season																
leaf																
leaf injury																
intercostal stippling (visual)						none	none	none								
intercostal stippling (microscopic)								none								
necrosis			*													
chlorosis																
DNA content of <i>Apiognomon</i> (spring)																
DNA content of <i>Apiognomon</i> (autumn)																
herbivory: leaf hoppers						*	*	*								
herbivory: beech weavils							*	*								
chlorophyll fluorescence																
Yield (dusk and dawn)																
Yield (noon)																
gas exchange																
A2000			*			*		*							*	
A360						*		*							*	
rday								*								
CE								*								
pheff								*								
WUE								*								
gH ₂ O / A360		*				*	*									
gH ₂ O		*				*	*								*	
gH ₂ O night																
Vcmax						*		*							*	
Jmax						*		*							*	
cell (biochemical parameters)																
conjugated ACC																
putrescine										*	*			*	*	
spermidin																
spermin																
salicylic acid																
conjugated salicylic acid			*				*									
gentisic acid																
conjugated gentisic acid																

Shown are three growing seasons with free-air O₃ fumigation (2000–2002), and the year 1999 prior to the onset of the experiment. Explanation of colours: yellow, no difference between the 1×O₃ (unchanged ambient air, control) and the experimentally enhanced 2×O₃ regime; red, negative influence of 2×O₃ on tree parameter; green, positive influence of 2×O₃ on tree parameter. Coloured trends are marked for $p < 0.15$ (and $p < 0.25$, in the case of phenology). Statistically significant differences of $p < 0.05$ are marked with asterisks. Dashed lines indicate the parameter was not determined. A2000, max. rate of photosynthesis at 2000 ppm CO₂; A360, max. rate of photosynthesis at 360 ppm CO₂; rday, respiration during daytime; CE, carboxylation efficiency; pheff, photosynthetic efficiency; WUE, water use efficiency; gH₂O, stomatal conductance to water vapour; gH₂O night, night-time stomatal conductance; Vcmax, max. rate of Rubisco activity; Jmax, potential rate of electron transport; conjugated ACC, L-aminocyclopropane-1-carboxylate.

the seasonal stage (Table 1). In June 1999, the year prior to the onset of the free-air O₃ fumigation, Vcmax (maximum rate of Rubisco activity) was slightly lower in the future 2×O₃ trees compared to the control. In August 2000, no difference existed between the two O₃ regimes, whereas in June 2001 Vcmax was significantly enhanced under 2×O₃. After three growing seasons, in August 2002, Vcmax was significantly reduced under 2×O₃. The length of growing season was significantly reduced in the shade crown of beech under 2×O₃ in fall of 2000, the first year of O₃ fumigation. Such a reduction was not detectable in 2001, given the rather cool and humid weather conditions in September (cf. Nunn et al., 2002), but was indicated again in 2002. However, when viewing the tree canopy as a whole, autumnal leaf shedding was consistently accelerated under 2×O₃ throughout all three years (Table 1).

3.2. Sun versus shade foliage

In beech and spruce, gas exchange responded more sensitively to O₃ impact in shade rather than sun foliage. However, O₃-induced reductions in the annual increments of leaf biomass and branch volume were more distinct in the sun crowns, although biochemical parameters in general showed similar responses to 2×O₃, irrespective of foliage type (Table 1).

3.3. Beech versus spruce

The shade foliage of both species displayed significant reductions in Vcmax, A2000 (max. rate of photosynthesis at 2000 μl l⁻¹ CO₂) and A360 (max. rate of photosynthesis at 360 μl l⁻¹ CO₂) under 2×O₃ (Table 1). Beech exhibited slightly enhanced 1-aminocyclopropane-1-carboxylate levels (ACC, ethylene precursor, injury induction) in 2000 and 2001, but no response in counteracting putrescine among the biochemical parameters under 2×O₃. In contrast, spruce shade foliage developed slightly enhanced ACC in 2001 but significantly lowered putrescine levels (Table 1).

3.4. Conclusions

Overall, the visualized effects of 2×O₃ were largely inconsistent in the adult beech and spruce trees across the internal scaling levels, foliage types and growing seasons. Nevertheless, even in the first year of O₃ fumigation responses to 2×O₃ were present at different scaling levels in beech: enhanced ACC (cell level), O₃-induced injury (leaf level) and accelerated foliar senescence in fall (branch level). These responses are linked to enhanced ethylene production, which was shown, in the same trees, to be the driving agent in the oxidative cell death cycle (Nunn, 2005).

Different approaches to graphically communicate multivariate studies have been suggested in the literature. Schwela and Krause (1989) used Chernoff–Flury faces in a multivariate cluster analysis, evaluating effects of dry and wet deposition on *Picea abies*. In their approach, however, the assignment of variables to face features and their relation to changes of parameters in absolute terms is arbitrary to some extent, although complex mathematical treatment forms an underlying platform. Karnosky et al. (2003) described the influence of 1.5×O₃ on *Populus tremuloides*, using upwards (enhancement) and downwards directed arrows (decrease) in a table for each investigated parameter. This latter approach does not require complex mathematical analysis, although the arrow arrangement restricts the potential in the visualization of response patterns across different scaling levels.

The advantage of the visualization approach proposed here is the combination of colours with an arrangement of the investigated parameters along tree-internal scaling levels, which provides a more objective (in relation to Schwela and Krause, 1989) but nevertheless statistically founded overview of chronic O₃ stress on adult forest trees (in the absence of complex mathematical treatment). The synergism derived from statistical analysis and the proficient ability of the ‘human eye’ in pattern recognition, therefore, offers a link between the complex graphical Chernoff–Flury faces and the arrow table approach by Karnosky et al. (2003), which lacks the graphical dimension.

Acknowledgements

We are grateful to Dr H. Werner, C. Heerdt, M. Leuchner, Professor Dr P. Fabian (Bioclimatology, TUM) and G. Fröhlich (Bayerische Landesanstalt für Landwirtschaft) for providing climatic background data and performing the canopy fumigation and O₃ analysis. Dr R. Grote and P. Wipfler are acknowledged for supplying data of the radial stem increment. We gratefully acknowledge the skilful technical assistance of Dipl. Ing. T. Feuerbach, N. Hofman, A. Knötig, P. Kuba, J. Lebherz, I. Süß, R. Ludwig and L. Göbl. The investigations were funded by Bayerisches Staatsministerium für Umwelt, Gesundheit und Verbraucherschutz; and by Deutsche Forschungsgemeinschaft (DFG) through SFB 607 ‘Growth and Parasite defence — Competition for Resources in Economic Plants from Agronomy and Forestry’.

References

- Emberson, L.D., Ashmore, M.R., Cambridge, H.M., Simpson, D., Tuovinen, J.P., 2000. Modelling stomatal ozone flux across Europe. *Environmental Pollution* 109, 403–413.

- Karnosky, D.F., Gielen, B., Ceulemans, R., Schlesinger, W.H., Norby, R.J., Oksanen, E., Matyssek, R., Hendry, G.R., 2001. FACE systems for studying the impact of greenhouse gases on forest ecosystems. In: Karnosky, D.F., Ceulemans, R., Scarascia-Mugnozza, G.S., Innes, J.L. (Eds.), *The Impact of Carbon dioxide and Other Greenhouse Gases on Forest Ecosystems IUFRO Task Force on Environmental Change Reports*, vol. 3. CABI Publishing, pp. 297–324.
- Karnosky, D.F., Zak, D., Pregitzer, K., Awmack, C., Bockheim, J., Dickson, R., Hendrey, G., Host, G., King, J., Kopper, B., Kruger, E., Kubiske, M., Lindroth, R., Mattson, W., McDonald, E., Noormets, A., Oksanen, E., Parsons, W., Percy, K., Podila, G., Riemenschneider, D., Sharma, P., Thakur, R., Sober, A., Sober, J., Jones, W., Anttonen, S., Vapaavuori, E., Manskovska, B., Heilman, W., Isebrands, J., 2003. Tropospheric O₃ moderates responses of temperate hardwood forests to elevated CO₂: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* 17, 289–304.
- Kolb, T.E., Matyssek, R., 2001. Limitations and perspectives about scaling ozone impacts in trees. *Environmental Pollution* 115, 373–392.
- Matyssek, R., Sandermann, H., 2003. Impact of ozone on trees: an ecophysiological perspective. *Progress in Botany* 64, 350–404.
- Nunn, A.J., 2005. Risiko-Einschätzung der chronisch erhöhten Ozonbelastung mittels 'Free-Air'-Begasung von Buchen (*Fagus sylvatica*) und Fichten (*Picea abies*) eines forstlich begründeten Mischbestandes. Dissertation. Ecophysiology of Plants, Department of Ecology, Life Science Center Weihenstephan. TUM, Freising, pp. 218.
- Nunn, A.J., Reiter, I., Häberle, K.H., Werner, H., Langebartels, C., Sandermann, H., Heerdt, C., Fabian, P., Matyssek, R., 2002. 'Free-air' ozone canopy fumigation in an old-growth mixed forest: concept and observations in beech. *Phyton (Austria)* 42, 105–119.
- Nunn, A.J., Kozovits, A.R., Reiter, I.M., Heerdt, C., Leuchner, M., Lütz, C., Liu, X., Grams, T.E.E., Häberle, K.H., Werner, H., Matyssek, R., 2005. Comparison of ozone uptake and sensitivity between a phytotron study with young beech and a field experiment with adult beech (*Fagus sylvatica*). *Environmental Pollution*, in press. doi: 10.1016/j.envpol.2005.01.036.
- Schwela, D.H., Krause, G.H.M., 1989. Chernoff–Flury Faces: A statistical means for representing multivariate response parameters of air pollution induced injury on plants. *Environmental Pollution* 61, 59–75.
- Werner, H., Fabian, P., 2002. Free-air fumigation of mature trees. *Environmental Science and Pollution Research* 9, 117–121.