

|                                                       |         |         |             |           |
|-------------------------------------------------------|---------|---------|-------------|-----------|
| Phyton (Austria)<br>Special issue:<br>"Global change" | Vol. 42 | Fasc. 3 | (105)-(119) | 1.10.2002 |
|-------------------------------------------------------|---------|---------|-------------|-----------|

## **"Free-Air" Ozone Canopy Fumigation in an Old-Growth Mixed Forest: Concept and Observations in Beech**

By

A. J. NUNN<sup>1)</sup>, I. M. REITER<sup>1)</sup>, K.-H. HÄBERLE<sup>1)</sup>, H. WERNER<sup>3)</sup>, C. LANGEBAEELS<sup>2)</sup>,  
H. SANDERMANN<sup>2)</sup>, C. HEERDT<sup>3)</sup>, P. FABIAN<sup>3)</sup> & R. MATYSSEK<sup>1)</sup>

**Key words:** Ozone, "free-air" fumigation, *Fagus sylvatica*, leaf injury, senescence, ACC (1-aminocyclopropane-1-carboxylic acid), polyamines.

### **S u m m a r y**

NUNN A. J., REITER I. M., HÄBERLE K.-H., WERNER H., LANGEBAEELS 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* (Horn, Austria) 42 (3) : (105)-(119).

A concept for the assessment and first observations on the O<sub>3</sub> sensitivity of adult beech trees (*Fagus sylvatica*) are reported. A novel "free-air" O<sub>3</sub> fumigation system was employed to the mixed canopy of a beech and spruce forest in Bavaria. In the unchanged, ambient air at the site, SUM0 reached 160 µl l<sup>-1</sup> h, and AOT40 more than 16 µl l<sup>-1</sup> h, while the vertical profile of O<sub>3</sub> levels in the stand hardly displayed gradients towards the forest floor. "Free-air" fumigation closely approached the targeted "2 x ambient" O<sub>3</sub> regime (with the O<sub>3</sub> levels confined to maximum 150 nl l<sup>-1</sup>) and increased AOT40 by a factor of 4, regarding trees in ambient air as "control". O<sub>3</sub> exposure promoted macroscopic symptoms (yellowish spots) indicating cell collapse in the mesophyll but differing from findings in controlled chamber studies. Autumnal senescence was accelerated in the sun and shade foliage of beech by up to 9 days under the experimentally enhanced O<sub>3</sub> regime. Concentrations of conjugated ACC (1-aminocyclopropane-1-carboxylic acid), a metabolic precursor of O<sub>3</sub>-induced ethylene formation, tended to be higher in shade leaves compared to sun leaves and indicated further increase under the "free-air" O<sub>3</sub> fumigation. Antagonistically to ACC levels, sun leaves had higher and shade leaves had lower levels of polyamines. After two growing seasons of "free-air" fumigation, the experiment is conceived to be continued for another three years and to relate whole-tree response patterns to ozone across a broad spectrum of structural, physiological and biochemical tree parameters to the actual O<sub>3</sub> flux into leaves.

<sup>1)</sup> Ecophysiology of Plants, TU München, Am Hochanger 13, D-85354 Freising, Germany.

<sup>2)</sup> GSF-Institute of Biochemical Plant Pathology, Ingolstädter Landstraße 1, D-85764 Oberschleißheim, Germany.

<sup>3)</sup> Bioclimatology, TU München, Am Hochanger 13, D-85354 Freising, Germany.

## Introduction

There is increasing awareness that juvenile trees may distinctly differ in their physiology, and thus in  $O_3$  response, from mature individuals (KELLY & al. 1995, KOLB & al. 1997, KOLB & MATYSSEK 2001), and that chamber conditions (altered micro-climate and water/nutrient availabilities, exclusion of competitors), under which young trees have typically been exposed to ozone, may strongly bias the sensitivity to stress (CHAPPELKA & CHEVONE 1992). During the nineties, the concept of 'Critical Levels for Ozone' was introduced by UN-ECE (FUHRER 1994, KÄRENLAMPI & SKÄRBY 1996) and defined as an AOT40 dose (i.e. the 'accumulated exposure to ozone above a threshold of  $40 \text{ nl } O_3 \text{ l}^{-1} \text{ air}$ ') which should indicate - when exceeded - the biomass production to decline by more than 10 % relative to plants under pre-industrial  $O_3$  regimes. The critical AOT40 dose for forest trees has been set to  $10 \mu\text{l } O_3 \text{ l}^{-1} \text{ h}$ , assuming  $O_3$  exposure below  $40 \text{ nl l}^{-1}$  and at night to be negligible. Such assumptions need to be regarded preliminary (FUHRER & al. 1997), given their derivation from few experiments with young trees grown in exposure chambers. Therefore, it is not surprising that the regional distribution of AOT40 is rather inconsistent with forest condition, being aware that this kind of threshold definition does probably not cover the different kinds of tree species, genotypes, forest types and site conditions (MATYSSEK & INNES 1999, VANDERHEYDEN & al. 2001). On the long run, AOT40 should be replaced with flux concepts of actual  $O_3$  uptake into leaves, providing a biologically meaningful  $O_3$  dose. In addition, assessments of  $O_3$  stress should reach beyond crown appearance and biomass production towards mechanistic measures of whole-tree performance (FUHRER & ACHERMANN 1999).

A major shortcoming is the analysis of the  $O_3$  sensitivity of adult forest trees. Branch cuvettes or bags (HAVRANEK & WIESER 1994, HOUPIS & al. 1991) and even large chambers for whole-tree exposure (HANSON & al. 1994) have been used to study  $O_3$  responses of adult trees. However, apart from the still occurring micro-climatic bias, also biochemical interactions between  $O_3$ -fumigated and non-fumigated branches (or branch sections) may be problematic (SANDERMANN & al. 1997, HENRIKSSON 2001). 'Free-air' fumigation systems enabling  $O_3$  exposure in the absence of branch or whole-plant enclosure appear to be the best choice (MUSSELMAN & HALE 1997, KARNOSKY & al. 2001), although most experiments of this kind with trees have been conducted on young individuals (MCLEOD & al. 1992, WULFF & al. 1992, OKSANEN 2001) or have applied gases other than ozone (HENDREY & al. 1992, LEWIN & al. 1994). One such approach was conducted in the canopy of tall sugar maple trees, although the 'free-air'  $O_3$  fumigation was restricted to clusters of sun and shade foliage (TJOELKER & al. 1995). It is obvious that the knowledge about  $O_3$  responses of adult trees under stand conditions is scarce, and that this deficit impedes the development of 'Critical Levels for Ozone' towards definitions of improved ecological relevance.

This paper presents the concept of a "free-air"  $O_3$  fumigation experiment which has been set into operation within the canopy of an old-growth, mixed forest of beech (*Fagus sylvatica* L.) and spruce (*Picea abies* (L.) Karst.) in southern Ger-

many. The methodology of the technical set-up has recently been published by WERNER & FABIAN 2002. Examples will be presented on the ambient and experimentally enhanced  $O_3$  regimes within the canopy and on first observations of the  $O_3$  responsiveness in the beech trees.

A control group of trees in ambient air ( $1 \times O_3$ ) serves as the reference for those trees which are exposed to an experimentally enhanced “2 x ambient”  $O_3$  regime ( $2 \times O_3$ ) as employed by the ‘free-air’ fumigation system (Fig. 1). In the enhanced  $O_3$  regime maximum peak  $O_3$  concentrations are restricted to  $150 \text{ nl } O_3 \text{ l}^{-1}$ . This reduces the risk of acute  $O_3$  injury. Thereby a new chronic  $O_3$  regime is experimentally established representing an enhanced but still site-relevant constraint on the trees. From tree responses to the experimentally enhanced  $O_3$  regime processes are concluded which may be affected or at risk in the control trees. Five beech and spruce individuals were chosen in  $2 \times O_3$  and another five trees of each species in the  $1 \times O_3$  canopy. In each tree individual, the analysis addresses a broad spectrum of structural, physiological and biochemical parameters (Fig. 1).

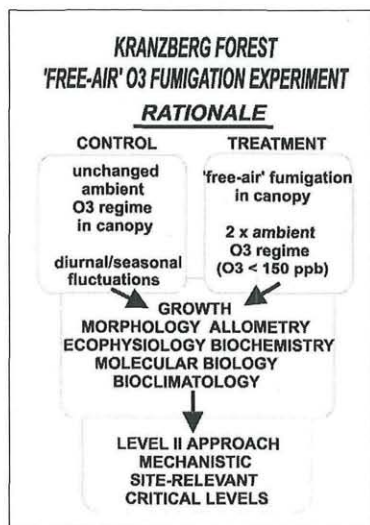


Fig. 1. Concept of the “free-air”  $O_3$  fumigation experiment at Kranzberg Forest: trees in unchanged ambient air serve as “control”, while another group of trees is experimentally exposed to a “2 x ambient”  $O_3$  regime (maximum  $150 \text{ nl } O_3 \text{ l}^{-1}$ ). A broad scope of structural and functional tree parameters are being assessed at several levels of biological organization in order to approach whole tree responses to ozone and a quantitative risk assessment.

Potential changes in the allometry of sun and shade crowns as well as stems are being assessed along with the carbon budget and demand for water and nutrients. Finally tree responses will be related to the  $O_3$  flux into leaves, and the spectrum of investigated parameters will allow to view AOT40 in a mechanistic context of whole-tree performance. Each tree is considered as an individual case study. The aim is to derive consistency patterns from synchronously occurring  $O_3$  responses at

(108)

the cell, organ and whole-tree level. The response patterns form the basis for comparisons between the two  $O_3$  regimes. A database is being created that will provide mechanistic, ecologically meaningful "Critical Levels for Ozone" and quantitative risk assessment of  $O_3$  impact on trees (FUHRER & ACHERMANN 1999).

## Material and Methods

The study is conducted in a mixed 55 to 60-year-old forest stand near Kranzberg (Germany/ 48°25'08"N, 11°39'41" E, elevation 485m) on about 27m high beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees which form a closed canopy. Resulting from a common planting practice in managed forests, beech forms dense groups of up to 50 individuals within the spruce stand. A scaffolding allows access to sun and shade crowns of about 30 neighboring beech and spruce trees (Fig. 2).

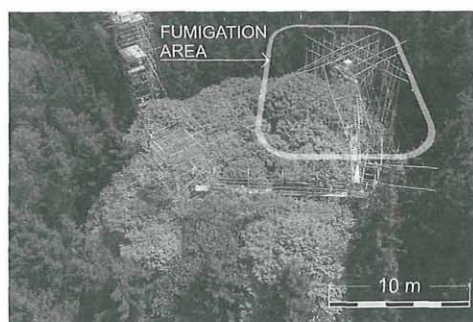


Fig. 2. Areal view, taken from the research crane, on the experimentation site of Kranzberg Forest near Freising, Germany. Frame outlines the canopy with "free-air" ozone fumigation. Canopy appearing in light-grey and dark-grey denote beech and spruce trees respectively.

Long-term climatic conditions at Kranzberg Forest are: annual mean air temperature 7.0-7.5 °C, mean air temperature during the growing season 14.5-15.0 °C, annual precipitation 730-790 mm, precipitation during the growing season 410-520 mm, length of the growing season 150-155 days (PRETZSCH & al. 1998). The growing season of 1999 prior to the onset of the "free-air"  $O_3$  canopy fumigation in 2000 serves as a reference. 1999 was slightly warmer and drier than the first two years of experimentation, as 2000 was rather wet and cool in July, and 2001 was characterized by low April temperatures and extended rainy periods during June and September (Table 1). Deposition of total-N was 9.8 kg ha<sup>-1</sup> a<sup>-1</sup>, and that of SO<sub>4</sub>-S amounted to 6.3 kg ha<sup>-1</sup> a<sup>-1</sup> in 1996, these levels being close to the averages across Bavarian beech forests (PRETZSCH & al. 1998). The "free-air"  $O_3$  fumigation was started in May 2000.

Ozone is generated from oxygen-enriched air and spread through a tubing system across the joint canopy (about 1500 m<sup>3</sup>) of the 10 trees in this treatment. The enhanced  $O_3$  levels are controlled on a half-hourly basis by a computerized feedback system based on the online  $O_3$  analysis at 6 sampling positions inside and outside the fumigation zone (WERNER & FABIAN 2002). Experimental maximum levels are restricted to 150 nl  $O_3$  l<sup>-1</sup>, by this preventing the risk of acute  $O_3$  injury. In addition, 120  $O_3$  passive-samplers (WERNER 1992) distributed across the canopy within the fumigated and non-fumigated zone record the  $O_3$  exposure by two-week intervals. AOT40 was

calculated according to FUHRER 1994 as the sum of 1-hourly mean  $O_3$  concentrations over 40 nl  $O_3$  l<sup>-1</sup> air during daylight hours, i.e., for global radiation > 50 W m<sup>-2</sup> per growing season.

Analysis: Macroscopic leaf symptoms were identified according to HARTMANN & al. 1995, INNES & al. 2001 and the "Ozone Validation Center" (WSL/FSL Birmensdorf/Switzerland). Autumnal leaf senescence was estimated as the discoloured and shed leaf area in proportion to total branch foliage area (using nets for assessing the shed leaves). Bud break was classified based on BRÜGGER 1998. The date of 50 % leaf senescence on branches and the date of bud break were calculated using the sigmoidal Boltzmann fit (ORIGIN 6.0, Microcal Inc., USA). Free and conjugated ACC was analysed from beech leaf samples (n=5) from one shade and one sun branch of each experimental tree on 20 June and 1 August 2000 according to and LIZADA & YANG 1979. Free polyamines (putrescine, spermidine and spermine) were determined from the same samples using the method described in LANGEBARTELS & al. 1991. The statistical analysis was performed using GraphPad Prism 3.0 (GraphPad, Inc.).

Table 1. Air temperature and precipitation of the years 1999-2001 as measured at about 6 km east of Kranzberg Forest (Weihenstephan, LANDTECHNIK 2002).

| Weihenstephan | Mean Air Temperature [°C] |      |      | Average Rainfall [mm] |       |       |
|---------------|---------------------------|------|------|-----------------------|-------|-------|
| Year/month    | 1999                      | 2000 | 2001 | 1999                  | 2000  | 2001  |
| April         | 8.7                       | 9.9  | 6.9  | 73.1                  | 68.0  | 53.4  |
| May           | 14.5                      | 14.7 | 15.6 | 110.6                 | 96.9  | 51.6  |
| June          | 15.5                      | 17.5 | 14.1 | 79.0                  | 69.6  | 112.1 |
| July          | 18.8                      | 15.1 | 17.9 | 76.0                  | 129.3 | 52.5  |
| August        | 17.3                      | 18.3 | 18.6 | 60.6                  | 68.6  | 103.3 |
| September     | 16.2                      | 13.5 | 10.9 | 65.1                  | 97.6  | 139.4 |
| October       | 8.8                       | 9.8  | 12.1 | 41.1                  | 84.8  | 46.8  |
| Annual mean   | 9.1                       | 9.3  | 8.6  | 736.2                 | 884.9 | 892.7 |
| April-October | 14.3                      | 14.1 | 13.8 | 505.5                 | 614.8 | 559.1 |

## Results

The  $O_3$  regime of the ambient air at Kranzberg Forest was similar during the three investigated growing seasons.  $O_3$  levels repeatedly exceeded 40 nl l<sup>-1</sup> from April through September (Fig. 3).  $O_3$  levels were more evenly distributed in 1999, whereas highest levels of 80 to 100 nl l<sup>-1</sup> occurred in 2000 and 2001. SUM0 ranged between 150 and 160  $\mu$ l l<sup>-1</sup> h, and AOT40 between 15.6 and 16.3  $\mu$ l l<sup>-1</sup> h during the three growing seasons (Tab. 2). Hence, AOT40 annually exceeded the "Critical Level" of 10  $\mu$ l l<sup>-1</sup> h. "Free-air" fumigation enhanced SUM0 during 2000 and 2001 in the sun-exposed canopy up to 267  $\mu$ l l<sup>-1</sup> h, whereas AOT40 reached about 72  $\mu$ l l<sup>-1</sup> h. In the shaded canopy, the fumigation effect was less pronounced (Tab. 2). Between bud break and senescence of beech, from May to October, the ratio of

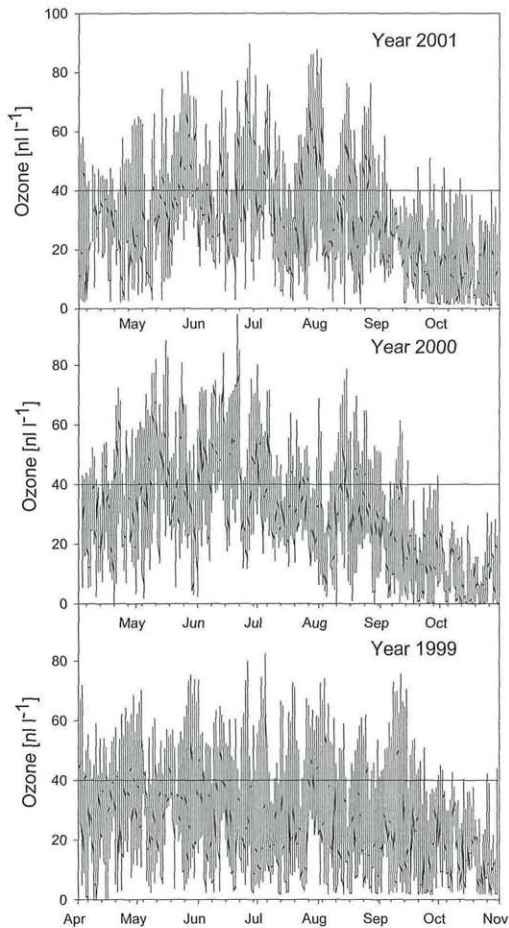


Fig. 3. Seasonal courses of the O<sub>3</sub> levels in the unchanged ambient air (1xO<sub>3</sub>) at Kranzberg Forest measured at the upper edge of the canopy during the years 1999, 2000, 2001. Horizontal line denotes the 40 nl O<sub>3</sub> l<sup>-1</sup> threshold level of the AOT40 definition.

SUM0<sub>fumigation</sub>/SUM0<sub>reference</sub> was – in the sun crown – 1.61 in 2000, and 1.93 in 2001, closely approaching the target ratio of 2 as proposed by the experimental concept (Tab. 2). The ratio of AOT40<sub>fumigation</sub>/AOT40<sub>reference</sub> was enhanced by a factor of about 4. In the shade crown, the corresponding ratios of SUM0 and AOT40 reached 1.5 and 4.1, respectively (Tab. 2). AOT40 mainly increased between May and September (as exemplified for 2000 in Fig. 4), exceeding 10 µl l<sup>-1</sup> h

in the unchanged, ambient air in July. Under “free-air” fumigation the “Critical Level” was already crossed during May, and the increase of AOT40 was only slightly delayed in the shade relative to the sun crown (Fig. 4). The vertical gradient of O<sub>3</sub> across the canopy towards the forest floor was rather small during the growing season (Fig. 5). Apparently, the open, branchless space between the trunks enabled the lateral airflow to advect O<sub>3</sub> from the forest edge towards the study site. Fig. 6 illustrates the sun canopy and the distribution of the “O<sub>3</sub> cloud” released from the “free-air” fumigation, which stayed well within the canopy of the 2xO<sub>3</sub> experimental trees.

Table 2. SUM0 and AOT40 for the growing seasons of the years 1999 through 2001 at Kranzberg Forest (ozone exposure given as [μl l<sup>-1</sup> h]; ratios without units).

| Year                                                  | 1999 | 2000 | 2001 |
|-------------------------------------------------------|------|------|------|
| <hr/>                                                 |      |      |      |
| “control” (1xO <sub>3</sub> )                         |      |      |      |
| Apr. 1 – Oct. 31                                      |      |      |      |
| SUM 0                                                 | 151  | 160  | 152  |
| AOT 40                                                | 16.1 | 15.6 | 16.3 |
| “free-air” fumigation sun crown (2xO <sub>3</sub> )   |      |      |      |
| Apr. 1 – Oct. 31                                      |      |      |      |
| SUM 0                                                 | 148  | 243  | 267  |
| AOT 40                                                | 15.7 | 62.4 | 71.8 |
| “free-air” fumigation shade crown (2xO <sub>3</sub> ) |      |      |      |
| Apr. 1 – Oct. 31                                      |      |      |      |
| SUM 0                                                 | 144  | 231  | 210  |
| AOT 40                                                | 15.7 | 61.9 | 49.4 |
| Sun crown                                             |      |      |      |
| $SUM0_{fumigation}/SUM0_{reference}$                  | 0.98 | 1.61 | 1.93 |
| May 10 – Oct. 31                                      |      |      |      |
| Sun crown                                             |      |      |      |
| $AOT40_{fumigation}/AOT40_{reference}$                | 0.98 | 4.12 | 4.88 |
| May 10 – Oct. 31                                      |      |      |      |
| Shade crown                                           |      |      |      |
| $SUM0_{fumigation}/SUM0_{reference}$                  | 0.95 | 1.53 | 1.47 |
| May 10 – Oct. 31                                      |      |      |      |
| Shade crown $AOT40_{fumigation}/AOT40_{reference}$    | 0.98 | 4.12 | 3.31 |
| May 10 – Oct. 31                                      |      |      |      |
| <hr/>                                                 |      |      |      |

Macroscopic leaf symptoms were observed in 2000 and 2001 in the sun crown of one free-air fumigated beech individual. They consisted of yellowish-brown discrete spots, filling small intercostal fields (4<sup>th</sup> order veins), and were irregularly spread across the leaf blade (Fig. 7). The occurrence of such symptoms was increased in 2001 under the 2xO<sub>3</sub> treatment. The symptoms were comparable with those previously observed in the sun crowns of beech growing in the Bavarian Forest (BAUMGARTEN 1999), but differed from injury found in phytotron O<sub>3</sub> exposure studies (GRAMS & al. 1999, GÜNTHARDT-GOERG & al. 2000), i.e. the bounda-

ries around spots were more discrete in the field. However, collapse of palisade tissue, reflecting “programmed cell death” (RAO & al. 2000), was also detected in the field, preceded by wrinkling of cell walls and coagulation of cell contents (Günthardt-Goerg pers. comm., Ozone Validation Center, Birmensdorf/Zurich). This structural decline is consistent with observations from controlled O<sub>3</sub> chamber studies (MATYSSEK & al. 1991, GÜNTHARDT-GOERG & al. 1993). Same leaf injury was found to a minor extend (about 5 % of the sun foliage) in beech trees under the ambient ozone regime. Spruce did not display visual needle injury in any needle age class.

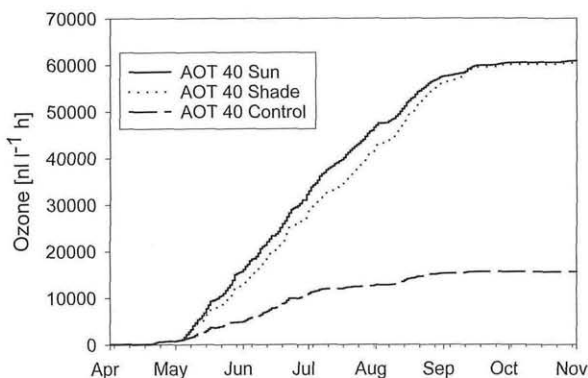


Fig. 4. Seasonal course of AOT40 during 2000 (the first year of “free-air” fumigation) at Kranzberg Forest in the sun and shade crown of the fumigated canopy (2xO<sub>3</sub>) and the upper edge of the canopy of the control trees (1xO<sub>3</sub>).

Before the beginning of ozone fumigation (reference year 1999) all investigated beech trees showed similar progress in leaf senescence in sun and shade crowns (Fig. 8). In 2000, accelerated autumnal leaf senescence was observed in the sun and shade crowns in 2xO<sub>3</sub>, preceding the control by about 7 to 9 days (Fig. 8). In 2001, the second season of O<sub>3</sub> fumigation accelerated senescence of sun crowns by 4 days relative to the control, whereas no response was observed in the shade crowns. Bud break of beech in both O<sub>3</sub> treatments as well as sun and shade crown occurred within two days at the end of April from 1999 through 2001 and was not affected by ozone fumigation.

Fresh-weight related, conjugated ACC was up to twice as high in shade compared to sun leaves. Although no differences for sun leaves between the treatments were found, up to two times higher conjugated ACC levels were indicated for shade leaves in 2xO<sub>3</sub> (Table 3). Free ACC was not detectable. 1.5-Fold higher levels of free polyamines per unit of fresh-weight were determined in sun leaves. In June, the 2xO<sub>3</sub> treatment showed a significantly higher level of putrescine and a slightly elevated concentration spermidine in the shade crown, whereas in August

the shade leaves under  $1xO_3$  had about 20 % higher levels of putrescine and spermidine. In the sun crown, only putrescine was enhanced through ozone in June. Spermidine did not significantly differ between the two  $O_3$  regimes in the beech leaves.

Table 3. Levels of ACC and polyamines on June 20th and August 1st 2000, given as means and two-sided standard deviation ( $n=5$ ). \*\* show a significant difference of means according to the Mann-Whitney U-Test (two-tailed,  $p<0.01$ ).

| Treatment<br>( $n=5$ ) | Free-ACC<br>[nmol/gFW] | conj.-ACC<br>[nmol/gFW] | Putrescine<br>[nmol/gFW] | Spermidine<br>[nmol/gFW] | Spermine<br>[nmol/gFW] |
|------------------------|------------------------|-------------------------|--------------------------|--------------------------|------------------------|
| $1xO_3$ sun            | n.d.                   | 16                      | 147                      | 205                      | 138                    |
| June                   |                        | +/- 6                   | +/- 52                   | +/- 52                   | +/- 22                 |
| $2xO_3$ sun            | n.d.                   | 15                      | 168                      | 187                      | 130                    |
| June                   |                        | +/- 5                   | +/- 66                   | +/- 32                   | +/- 27                 |
| $1xO_3$ shade          | 0                      | 23                      | 35**                     | 58                       | 91                     |
| June                   | +/- 0                  | +/- 8                   | +/- 4                    | +/- 14                   | +/- 9                  |
| $2xO_3$ shade          | n.d.                   | 33                      | 53**                     | 71                       | 94                     |
| June                   |                        | +/- 17                  | +/- 13                   | +/- 9                    | +/- 8                  |
| $1xO_3$ sun            | 0                      | 9                       | 208                      | 215                      | 116                    |
| August                 | +/- 0                  | +/- 7                   | +/- 80                   | +/- 35                   | +/- 19                 |
| $2xO_3$ sun            | n.d.                   | 7                       | 180                      | 219                      | 115                    |
| August                 |                        | +/- 1                   | +/- 123                  | +/- 43                   | +/- 18                 |
| $1xO_3$ shade          | n.d.                   | 9                       | 65                       | 126                      | 79                     |
| August                 |                        | +/- 3                   | +/- 22                   | +/- 22                   | +/- 14                 |
| $2xO_3$ shade          | n.d.                   | 19                      | 55                       | 103                      | 84                     |
| August                 |                        | +/- 15                  | +/- 15                   | +/- 19                   | +/- 14                 |

## Discussion

The “free-air” fumigation proved the experimental  $O_3$  enrichment to closely reach the targeted  $O_3$  regime within the group of the  $2xO_3$  trees (cf. WERNER & FABIAN 2002). AOT40 distinctly exceeded the currently defined “Critical Level for Ozone” of  $10 \mu l/l \cdot h$  in the unchanged, ambient air at the study site, throughout the three investigated growing seasons. The approximate experimental doubling of the  $O_3$  exposure (i.e. SUM0) raised AOT40 by a factor of about 4, this effect being somewhat larger in the sun than shade crown. In the unchanged ambient air, the vertical  $O_3$  gradient across the stand was rather small, underlining the importance of the stand structure for  $O_3$  deposition and, by this,  $O_3$  degradation. At Kranzberg Forest, the branchless space between the trunks up to a height of 17 m allowed advection of air from the forest edge, thereby levelling out potential  $O_3$  gradients. However, also in the large forested area of the Bavarian Forest, the vertical  $O_3$  gradient was much less pronounced (decline by 20 % across the canopy, and 33 % towards the forest floor (BAUMGARTEN & al. 2000), as compared with a spruce stand (Ebersberg Forest near Munich), where the dense foliation led to distinct diurnal courses in  $O_3$  concentration underneath the canopy (MATYSSEK & al. 1997). PLEIJEL & al. 1996 even found an increase in  $O_3$  concentration at night in a spruce stand, when the wind velocity was low.

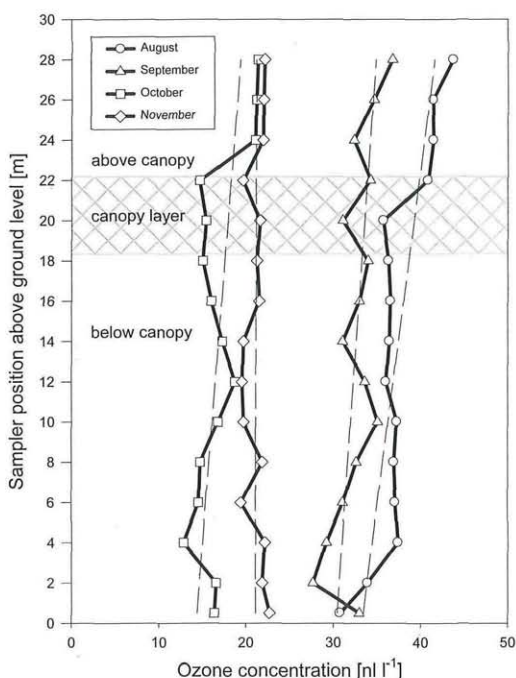


Fig. 5. Vertical profile of the ozone concentrations across a group of beech trees within the stand. Monthly means for four months during 1997. Linear regressions (broken lines) show vertical gradient.

The advantages of the “free-air” fumigation approach chosen in this study are (1) that  $O_3$  experimentation is feasible with adult trees under stand conditions, (2) whole-tree responses can be analysed in the absence of micro-climatic bias, (3) an experimental  $O_3$  regime can be created which throughout several growing seasons has relevance for evaluating chronic  $O_3$  impact on trees, and (4) a “control” of trees in unchanged, ambient air can be defined at the site (KARNOSKY & al. 2001). Already during the two initial years of experimentation, the “free-air” fumigation promoted the occurrence of macroscopic leaf injury and accelerated leaf senescence in fall. It appears that macroscopic leaf symptoms can vary depending on growth conditions, tree age and genetic constitution (Günthardt-Goerg, pers. comm.). The microscopic decline of the beech leaves at Kranzberg Forest was consistent with observations from controlled  $O_3$  chamber studies (GÜNTHARDT-GOERG & al. 1993, MATYSSEK & al. 1991). Although  $O_3$  injury cannot be uncritically viewed, to the full extent, as an expression of accelerated, natural senescence (GRAMS & al. 1999, LANGEBAEELS & al. 1997, LU & ZHANG 1998, MATYSSEK &

al. 1995, THOMAS & STODDART 1980), the elevated O<sub>3</sub> regime did promote autumnal discolouration and leaf loss. Remarkably, senescence was also accelerated in the experimentally O<sub>3</sub>-exposed shade leaves, although the latter had not developed macroscopic O<sub>3</sub> injury. This might indeed reflect particular O<sub>3</sub> sensitivity in light-limited leaves (KOLB 2001, TJOELKER & al. 1995).

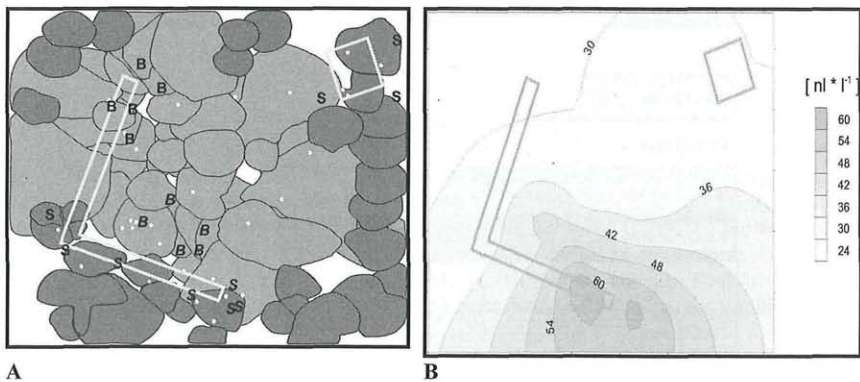


Fig. 6. Experimental site at Kranzberg Forest. A: crown projection of the experimental plot (light-grey = beech; dark-grey = spruce); B: Extension of the experimental O<sub>3</sub> regime in the sun crown as released from the “free-air” fumigation. Frames indicate scaffolding; dots denote the positions of the passive-samplers; B (beech) and S (spruce) indicate the stem positions of the experimental trees.

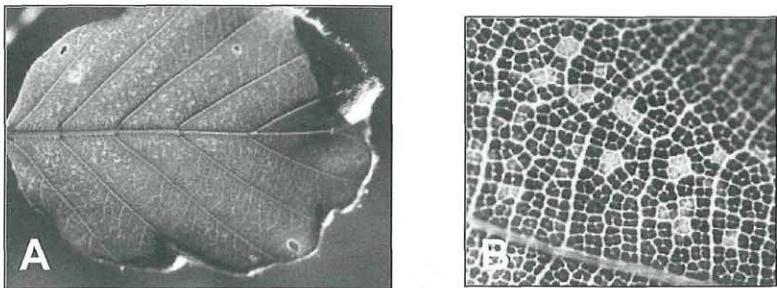


Fig. 7. Macroscopic leaf symptoms relating to O<sub>3</sub> impact. A: entire leaf; B: leaf section in detail. Light-grey areas denote yellowish discoloration reflecting incipient O<sub>3</sub> injury.

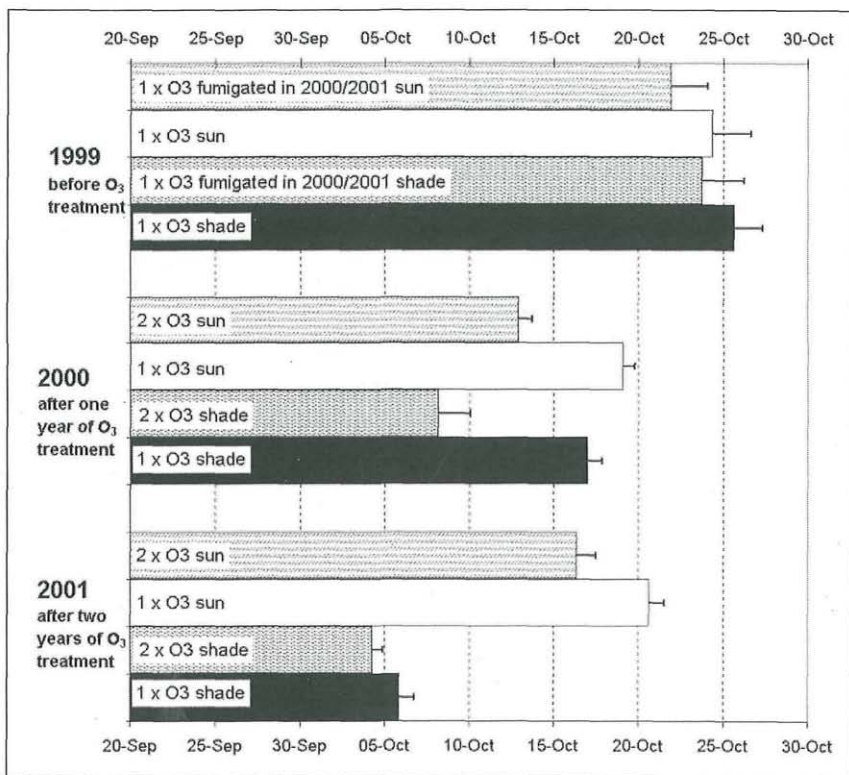


Fig. 8. Date during 1999, 2000, 2001 indicating mid autumnal leaf senescence as reflected by half of the leaves being discolored or lost. Bars represent means across branches of different trees ( $n=5$ ) each per foliage type and ozone treatment, expressing 50 % of leaf senescence in a branch. The error bars indicate the standard error of the Boltzmann fit applied.

Ethylene and polyamines are indicators of ozone sensitivity: A rapid surge in the foliar production of the gaseous hormone ethylene is a typical response of sensitive plant species to ozone (TINGEY & al. 1976). Ethylene is known to promote autumnal senescence, and its role in the propagation of ozone-induced cell death has recently been described (OVERMYER & al. 2000). Ethylene and polyamines share the same precursor, S-adenosyl-methionine, and mutually inhibit their biosynthesis. Consequently, a metabolic switch towards ethylene or polyamine biosynthesis has been postulated as a major factor in ozone sensitivity of herbaceous plants (LANGEBARTELS & al. 1991). When regarding the levels of conjugated ACC, the ethylene precursor, and polyamines in sun and shade leaves of beech at the Kranzberg site, they clearly show a parallel behaviour with high polyamine levels in sun leaves and elevated ACC levels in shade leaves. Levels of conjugated ACC were elevated under  $2xO_3$  in the shade crown where autumnal senescence was accelerated. The diamine putrescine showed increased levels in the

shade crown in June, while the values were low in August when conjugated ACC levels were twice as high as in the control leaves. It will be necessary to follow the annual time course more closely to define the role of these endogenous compounds as amplifying or ameliorating factors in ozone-induced injury and senescence of trees, respectively. The experiment is conceived to be continued for another three years and to relate whole-tree response patterns to ozone across a broad spectrum of structural, physiological and biochemical tree parameters to the actual O<sub>3</sub> flux into leaves.

### A c k n o w l e d g e m e n t s

The study is supported by the "Bayerisches Staatsministerium für Landesentwicklung und Umweltfragen" and the "Deutsche Forschungsgemeinschaft" through SFB 607 "Growth and Parasite Defence – Competition for Resources in Economic Plants from Agronomy and Forestry". The skilful technical assistance by Th. FEUERBACH, L. GÖBL, R. LUDWIG, A. JUNGERMANN, T. HÄGELE, B. BAUMEISTER, I. SÜB, J. LEBHERZ, P. KUBA, N. HOFMANN and A. KNÖTIG is gratefully acknowledged. The expertise of Dr. M. S. GÜNTHARDT-GOERG (Ozone Validation Centre, Birmensdorf/Zurich) is highly appreciated.

### R e f e r e n c e s

- BAUMGARTEN M. 1999. Charakterisierung des physiologischen Zustands von Altbuchen in zwei Höhenlagen des Bayerischen Waldes unter Berücksichtigung der Standortfaktoren und der Ozonbelastung. - Doctoral thesis, Ludwig-Maximilians-Universität.
- , WERNER H., HÄBERLE K.-H., EMBERSON L. D., FABIAN P. & MATYSSEK R. 2000. Seasonal ozone response of mature beech trees (*Fagus sylvatica*) at high altitude in the Bavarian forest (Germany) in comparison with young beech grown in the field and in phytotrons. - *Environ. Pollut.* 109: 431 - 442.
- BRÜGGER R. 1998. Die phänologische Entwicklung von Buche und Fichte. Beobachtung, Variabilität, Darstellung und deren Nachvollzug in einem Modell. - Verlag des Geographischen Instituts der Universität Bern, Bern.
- CHAPPELKA A. & CHEVONE B. 1992. Tree response to ozone.- In: LEFOHN A. (Eds.), Surface level ozone exposure and their effects on vegetation, pp. 271 - 324. - Lewis, MI. Chelsea.
- FUHRER J. 1994. The critical level for ozone to protect agricultural crops - an assesment of data from European open-top chamber experiments.- In: FUHRER J. & ACHERMANN B. (Eds.), Critical levels for ozone - an UN-ECE Workshop Report, vol. 16, pp. 42 - 57. - Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene, Bern, Switzerland.
- & ACHERMANN B. 1999. Critical levels for ozone - level II, vol. Environmental documentation 115. - Swiss Agency for the Environment, Forests and Landscape, Berne, Switzerland.
- , SKÄRBY L. & ASHMORE M. R. 1997. Critical levels for ozone effects on vegetation in Europe. - *Environ. Pollut.* 97: 91 - 106.
- GRAMS T. E. E., ANEGG S., HÄBERLE K.-H., LANGEBAEDEL C. & MATYSSEK R. 1999. Interactions of chronic exposure to elevated CO<sub>2</sub> and O<sub>3</sub> levels in the photosynthetic light and dark reactions of European beech (*Fagus sylvatica*). - *New Phytol.* 144: 95 - 107.
- GÜNTHARDT-GOERG M. S., MATYSSEK R., SCHNEIDEGGER C. & KELLER T. 1993. Differentiation and structural decline in the leaves and bark of birch (*Betula pendula*) under low ozone concentration. - *Trees* 7: 104 - 114.

- , MCQUATTIE C.J., MAURER S. & FREY B. 2000. Visible and microscopic injury in leaves of five deciduous tree species related to current critical ozone levels.- *Environ. Pollut.* 109: 489 - 500.
- HANSON P., SAMUELSON L., WULLSCHLEGER S., TABBERER T. & EDWARDS G. 1994. Seasonal patterns of light-saturated photosynthesis and leaf conductance for mature and seedling *Quercus rubra* L. foliage: differential sensitivity to ozone exposure. - *Tree Physiol.* 14: 1351 - 1366.
- HARTMANN G., NIENHAUS F. & BUTIN H. 1995. Farbatlas Waldschäden: Diagnose von Baumkrankheiten. - 2. Auflage edition. - Eugen Ulmer Verlag, Stuttgart.
- HAVRANEK W. & WIESER G. 1994. Design and testing of twig chambers for ozone fumigation and gas exchange measurements in mature trees. - *Proc. Roy. Soc. Edinburgh Sect. B* 102: 541 - 546.
- HENDREY G., LEWIN K., KOLBER Z. & EVANS L. 1992. Controlled enrichment system for experimental fumigation of plants in the field with sulphur dioxide. - *J. Air Waste Manage Assoc.* 42: 1324 - 1327.
- HENRIKSSON J. 2001. Differential shading of branches or whole trees: survival, growth, and reproduction. - *Oecol.* 126: 482 - 486.
- HOUPIS J., COSTELLA M. & COWLES S. 1991. A branch exposure chamber for fumigating ponderosa pine to atmospheric pollution. - *J. Environ. Qual.* 20: 467 - 474.
- INNES J. L., SKELLY J. M. & SCHAUB M. 2001. Ozone and broadleaved species - a guide to the identification of ozone-induced foliar injury. - Haupt, Bern.
- KÄRENLAMP L. & SKÄRBY L. 1996. Critical levels for ozone in Europe: testing and finalizing the concepts. UN-ECE workshop report. - In: KÄRENLAMP L. & SKÄRBY L. (Eds.), Critical levels for ozone in europe: testing and finalizing the concepts. - UN-ECE workshop report, University of Kuopio, Kuopio.
- 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 report No. 3 of the IUFRO task force on environmental change, pp. 297 - 324.- CABI Publishing.
- KELLY J. M., SAMUELSON L., EDWARDS G., HANSON P., KELTING D., MAYS A. & WULLSCHLÄGER S. 1995. Are seedlings reasonable surrogates for trees? An analysis of ozone impacts on *Quercus rubra*. - *Water Air Soil Pollut.* 85: 1317 - 1324.
- KOLB T. 2001. Ageing as an influence on tree response to ozone: theory and observations. - In: HUTTUNEN S., BUCHER J., SUNDBERG B., JARVIS P. & MATYSSEK R. (Eds.), Trends in European forest tree physiology research, pp. 127 - 155.- Kluwer, Dordrecht, The Netherlands.
- & MATYSSEK R. 2001. Limitations and perspectives about scaling ozone impact in trees. - *Environ. Pollut.* 115: 373 - 393.
- , FREDERICKSEN T., STEINER K. & SKELLY J. 1997. Issues in scaling tree size and age responses to ozone: a review. - *Environ. Pollut.* 98: 195 - 208.
- LANDTECHNIK W. 2002. Landtechnik Weihenstephan - Agrarmeteorologische Meßstation im Schaugarten. - Landtechnik Weihenstephan: <http://dec2.tec.agrar.tu-muenchen.de/wetter/>.
- LANGEBARTELS C., ERNST D., HELLER W., LÜTZ C., PAYER H. D. & SANDERMANN H. 1997. Ozone responses of trees: results from controlled chamber exposures at the GSF phytotron. - In: SANDERMANN H., WELLBURN A. R. & HEATH R. L. (Eds.), Forest decline and ozone: a comparison of controlled chamber and field experiments. Ecological studies, pp. 163 - 200. - Springer, Berlin.
- , KERNER K., LEONARDI S., SCHRAUDNER M., TROST M., HELLER W. & SANDERMANN H. 1991. Biochemical plant responses to ozone. I. Differential induction of polyamine and ethylene biosynthesis in tobacco. - *Plant Physiol.* 95: 882 - 889.
- LEWIN K., HENDREY G., NAGY J. & MCMORTE R. 1994. Design and application of a free-air carbon dioxide enrichment facility. - *Agric. Meteorol.* 70: 15 - 29.

- LIZADA M. C. C. & YANG S. F. 1979. A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. - *Anal. Biochem.* 100: 140 - 145.
- LU C. & ZHANG J. 1998. Changes in photosystem II function during senescence of wheat leaves. - *Physiol. Plant.* 104: 239 - 247.
- MATYSSEK R. & INNES J. L. 1999. Ozone - a risk factor for trees and forests in Europe? - *Water Air Soil Pollut.* 116: 199 - 226.
- , GÜNTHARDT-GOERG M. S., KELLER T. & SCHNEIDEGGER C. 1991. Impairment of gas exchange and structure in birch leaves (*Betula pendula*) caused by low ozone concentrations. - *Trees* 5: 5 - 13.
- , REICH P., OREN R. & WINNER W. E. 1995. Response mechanisms of conifers to air pollutants. - In: SMITH W. K. & HINCKLEY T. M. (Eds.), *Physiological ecology of coniferous forests. Physiological ecology series*, pp. 255 - 308. - Academic Press, New York.
- , MAURER S., FABIAN P. & PRETZSCH H. 1997. Scaling von Ozonwirkungen in Holzpflanzen bis zur Bestandsebene: Ausgangsbasis, Erfordernisse und Perspektiven. - *EcoSys. Kiel* 20: 49 - 58.
- MCLEOD A., SHAW P. & HOLLAND M. 1992. The Liphook fumigation project: studies of sulphur dioxide and ozone effects on coniferous trees. - *Forest Ecol. Manag.* 51: 121 - 127.
- MUSSELMAN R. C. & HALE B. 1997. Methods for controlled and field ozone exposures of forest tree species in North America. - In: SANDERMANN H., WELLBURN A. R. & HEATH R. (Eds.), *Forest decline and ozone: a comparison of controlled chamber and field experiments*, pp. 227 - 315. - Springer-Verlag, Berlin.
- OKSANEN E. 2001. Increasing tropospheric ozone level reduced birch (*Betula pendula*) dry mass within a five year period. - *Water Air Soil Pollut.* 130: 947 - 952.
- OVERMYER K., TUOMINEN H., KETTUNEN C., BETZ C., LANGEBAEELS C., SANDERMANN H. & KANGASJÄRVI J. 2000. The ozone-sensitive *Arabidopsis rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. - *Plant Cell* 12: 1849 - 1862.
- PLEIJEL H., WALLIN G., KARLSSON P. & SKÄRBY L. 1996. Ozone gradients in a spruce forest stand in relation to wind speed and time of the day. - *Atmos. Environ.* 30: 4077 - 4084.
- PRETZSCH H., KAHN M. & GROTE R. 1998. Die Fichten-Buchen-Mischbestände des Sonderforschungsbereiches "Wachstum oder Parasitenabwehr?" im Kranzberger Forst. - *Forstw. Cbl.* 117: 241 - 257.
- RAO M. V., KOCH J. R. & DAVIS K. R. 2000. Ozone: a tool for probing programmed cell death in plants. - *Plant Mol. Biol.* 44: 345 - 358.
- SANDERMANN H., WELLBURN A. R. & HEATH R. 1997. Forest decline and ozone: synopsis. - In: SANDERMANN H., WELLBURN A. R. & HEATH R. (Eds.), *Forest decline and ozone: a comparison of controlled chamber and field experiments. Ecological studies*, pp. 369 - 377. - Springer-Verlag, Berlin.
- THOMAS H. & STODDART J. 1980. Leaf senescence. - *Annu. Rev. Plant Physiol.* 31: 83 - 111.
- TINGEY D., STANDLEY T. & FIELD R. 1976. Stress ethylene evolution: a measure of ozone effects on plants. - *Atmos. Environ.* 10: 969 - 974.
- TJOELKER M., VOLIN J., OLEKSYN J. & REICH P. 1995. Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment. - *Plant Cell Environ.* 18: 896 - 905.
- VANDERHEYDEN D., SKELLY J., INNES J., HUG C., ZHANG J., LANDOLT W. & BEULER P. 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. - *Environ. Pollut.* 111: 321 - 331.
- WERNER H. 1992. Das Indigopapier, sensitives Element zum Aufbau von Passivsammlern zur Messung von Ozonimmissionen. - In: *Forstl. Forschungsberichte*, vol. 122, München.
- WERNER H. & FABIAN P. 2002. Free-air fumigation of mature trees. - *Environ. Sci. Pollut. Res.* 9: 117 - 121.
- WULFF A., HÄNNINEN O., TUOMAINEN A. & KÄRENLAMPI L. 1992. A method for open-air exposure of plants to ozone. - *Ann. Bot. Fennici* 29: 253 - 262.

# ZOBODAT - [www.zobodat.at](http://www.zobodat.at)

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

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

Jahr/Year: 2002

Band/Volume: [42\\_3](#)

Autor(en)/Author(s): Nunn A.:J., Reiter I. M., Häberle K.- H., Werner H., Langebartels C., Sandermann H., Heerdt C., Fabian P., Matyssek Rainer

Artikel/Article: ["Free Air" Ozone Canopy Fumigation in an Old- Growth Mixed Forest: Concept and Observations in Beech. 105-119](#)