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

By

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K e y w o r d s : Ozone, "free-air" fumigation, *Fagus sylvatica*, leaf injury, senescence, ACC (1-aminocyclopropane-1-carboxylic acid), polyamines.

S u m m a r y

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A concept for the assessment and first observations on the O_3 sensitivity of adult beech trees (*Fagus sylvatica*) are reported. A novel "free-air" O_3 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 $\mu l\ l^{-1}$ h, and AOT40 more than 16 $\mu l\ l^{-1}$ h, while the vertical profile of O_3 levels in the stand hardly displayed gradients towards the forest floor. "Free-air" fumigation closely approached the targeted "2 x ambient" O_3 regime (with the O_3 levels confined to maximum 150 $nl\ l^{-1}$) and increased AOT40 by a factor of 4, regarding trees in ambient air as "control". O_3 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_3 regime. Concentrations of conjugated ACC (1-aminocyclopropane-1-carboxylic acid), a metabolic precursor of O_3-induced ethylene formation, tended to be higher in shade leaves compared to sun leaves and indicated further increase under the "free-air" O_3 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_3 flux into leaves.

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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 nl O_3 l^{-1} 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 µl O_3 l^{-1} h, assuming O_3 exposure below 40 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 ($1xO_3$) serves as the reference for those trees which are exposed to an experimentally enhanced "2 x ambient" O_3 regime ($2xO_3$) 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 nl O_3 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 $2xO_3$ and another five trees of each species in the $1xO_3$ canopy. In each tree individual, the analysis addresses a broad spectrum of structural, physiological and biochemical parameters (Fig. 1).

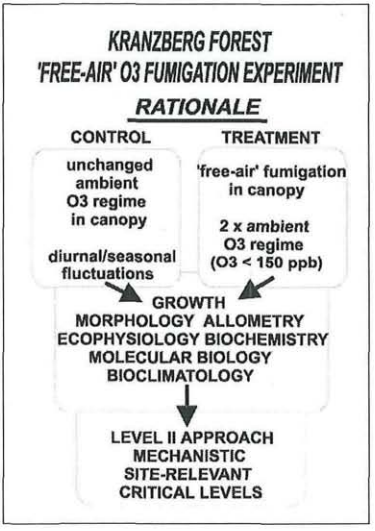

Fig. 1. Concept of the "free-air" O_3 fumigation experiment at Kranzberg Forest: trees in unchanged ambient air serve as "control", while another group of trees is experimentally exposed to a "2 x ambient" O_3 regime (maximum 150 nl O_3 l^{-1}). A broad scope of structural and functional tree parameters 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

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).

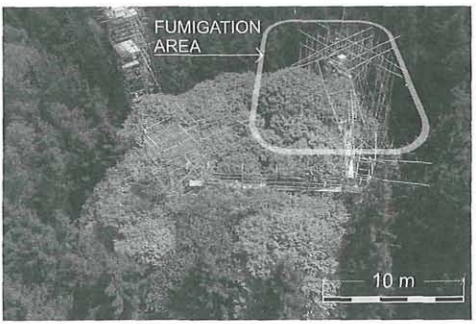

Fig. 2. Areal view, taken from the research crane, on the experimentation site of Kranzberg Forest near Freising, Germany. Frame outlines the canopy with "free-air" ozone fumigation. Canopy appearing in light-grey and dark-grey denote beech and spruce trees respectively.

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^{-1} a^{-1}, and that of SO_4-S amounted to 6.3 kg ha^{-1} a^{-1} 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^3) 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^{-1}, 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^{-1} air during daylight hours, i.e., for global radiation > 50 W m^{-2} 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 sigmiodal 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^{-1} from April through September (Fig. 3). O_3 levels were more evenly distributed in 1999, whereas highest levels of 80 to 100 nl l^{-1} occurred in 2000 and 2001. SUM0 ranged between 150 and 160 µl l^{-1} h, and AOT40 between 15.6 and 16.3 µl l^{-1} h during the three growing seasons (Tab. 2). Hence, AOT40 annually exceeded the "Critical Level" of 10 µl l^{-1} h. "Free-air" fumigation enhanced SUM0 during 2000 and 2001 in the sun-exposed canopy up to 267 µl l^{-1} h, whereas AOT40 reached about 72 µl l^{-1} 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

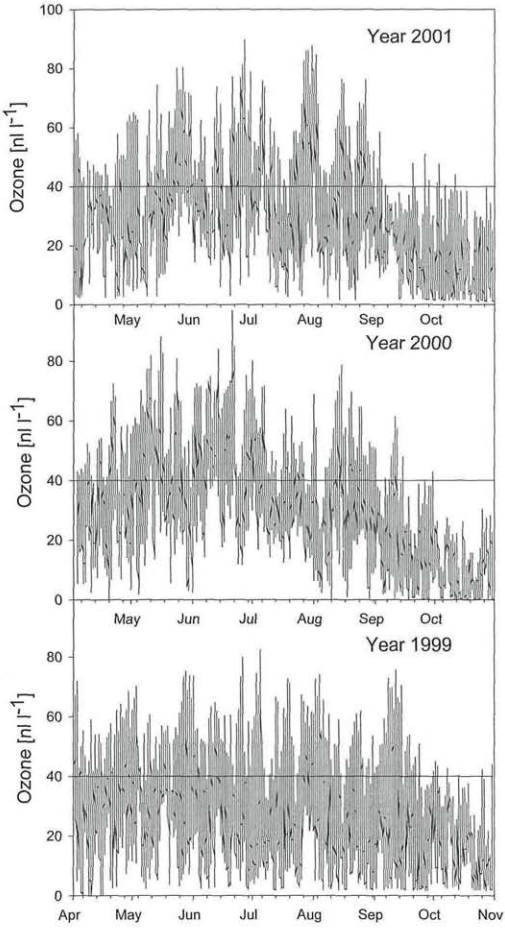

Fig. 3. Seasonal courses of the O_3 levels in the unchanged ambient air ($1xO_3$) at Kranzberg Forest measured at the upper edge of the canopy during the years 1999, 2000, 2001. Horizontal line denotes the 40 nl O_3 l^{-1} threshold level of the AOT40 definition.

$SUM0_{fumigation}/SUM0_{reference}$ 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 $AOT40_{fumigation}/AOT40_{reference}$ 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^{-1} 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_3 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_3 from the forest edge towards the study site. Fig. 6 illustrates the sun canopy and the distribution of the "O_3 cloud" released from the "free-air" fumigation, which stayed well within the canopy of the $2xO_3$ 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^{-1} h]; ratios without units).

Year	1999	2000	2001
"control" ($1xO_3$)			
Apr. 1 – Oct. 31			
SUM 0	151	160	152
AOT 40	16.1	15.6	16.3
"free-air" fumigation sun crown ($2xO_3$)			
Apr. 1 – Oct. 31			
SUM 0	148	243	267
AOT 40	15.7	62.4	71.8
"free-air" fumigation shade crown ($2xO_3$)			
Apr. 1 – Oct. 31			
SUM 0	144	231	210
AOT 40	15.7	61.9	49.4
Sun crown $SUM0_{fumigation}/SUM0_{reference}$ May 10 – Oct. 31	0.98	1.61	1.93
Sun crown $AOT40_{fumigation}/AOT40_{reference}$ May 10 – Oct. 31	0.98	4.12	4.88
Shade crown $SUM0_{fumigation}/SUM0_{reference}$ May 10 – Oct. 31	0.95	1.53	1.47
Shade crown $AOT40_{fumigation}/AOT40_{reference}$ May 10 – Oct. 31	0.98	4.12	3.31

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^{th} order veins), and were irregularly spread across the leaf blade (Fig. 7). The occurrence of such symptoms was increased in 2001 under the $2xO_3$ 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_3 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_3 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.

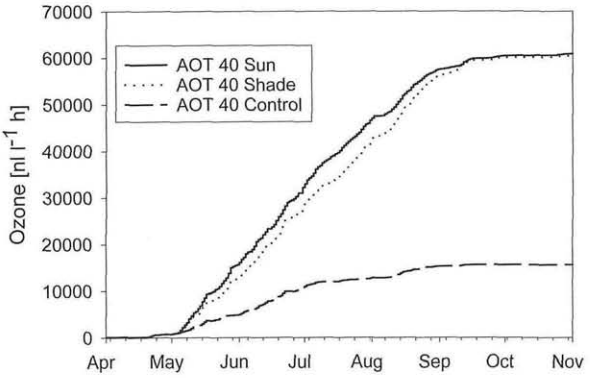

Fig. 4. Seasonal course of AOT40 during 2000 (the first year of "free-air" fumigation) at Kranzberg Forest in the sun and shade crown of the fumigated canopy ($2xO_3$) and the upper edge of the canopy of the control trees ($1xO_3$).

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 $2xO_3$, preceding the control by about 7 to 9 days (Fig. 8). In 2001, the second season of O_3 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_3 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 $2xO_3$ (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 $2xO_3$ 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 (n=5)	Free-ACC [nmol/gFW]	conj.-ACC [nmol/gFW]	Putrescine [nmol/gFW]	Spermidine [nmol/gFW]	Spermine [nmol/gFW]
$1xO_3$ sun June	n.d.	16 +/- 6	147 +/- 52	205 +/- 52	138 +/- 22
$2xO_3$ sun June	n.d.	15 +/- 5	168 +/- 66	187 +/- 32	130 +/- 27
$1xO_3$ shade June	0 +/- 0	23 +/- 8	35** +/- 4	58 +/- 14	91 +/- 9
$2xO_3$ shade June	n.d.	33 +/- 17	53** +/- 13	71 +/- 9	94 +/- 8
$1xO_3$ sun August	0 +/- 0	9 +/- 7	208 +/- 80	215 +/- 35	116 +/- 19
$2xO_3$ sun August	n.d.	7 +/- 1	180 +/- 123	219 +/- 43	115 +/- 18
$1xO_3$ shade August	n.d.	9 +/- 3	65 +/- 22	126 +/- 22	79 +/- 14
$2xO_3$ shade August	n.d.	19 +/- 15	55 +/- 15	103 +/- 19	84 +/- 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 µl/l*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.

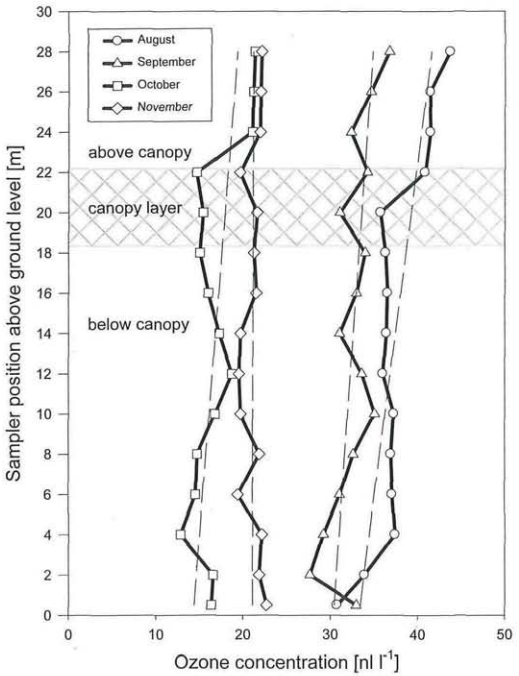

Fig. 5. Vertical profile of the ozone concentrations across a group of beech trees within the stand. Monthly means for four months during 1997. Linear regressions (broken lines) show vertical gradient.

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, LANGEBARTELS & al. 1997, LU & ZHANG 1998, MATYSSEK &

al. 1995, THOMAS & STODDART 1980), the elevated O_3 regime did promote autumnal discolouration and leaf loss. Remarkably, senescence was also accelerated in the experimentally O_3-exposed shade leaves, although the latter had not developed macroscopic O_3 injury. This might indeed reflect particular O_3 sensitivity in light-limited leaves (KOLB 2001, TJOELKER & al. 1995).

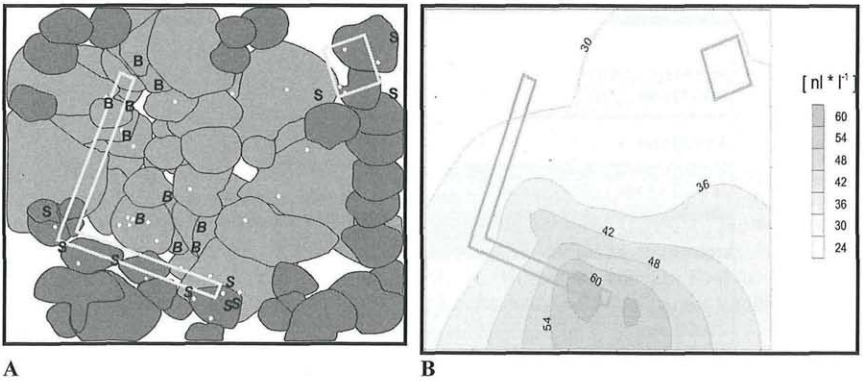

Fig. 6. Experimental site at Kranzberg Forest. A: crown projection of the experimental plot (light-grey = beech; dark-grey = spruce); B: Extension of the experimental O_3 regime in the sun crown as released from the "free-air" fumigation. Frames indicate scaffolding; dots denote the positions of the passive-samplers; B (beech) and S (spruce) indicate the stem positions of the experimental trees.

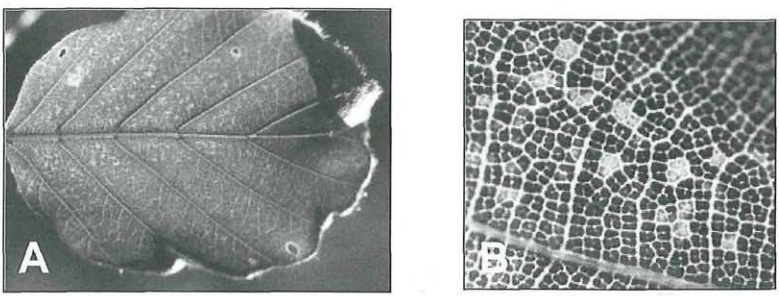

Fig. 7. Macroscopic leaf symptoms relating to O_3 impact. A: entire leaf; B: leaf section in detail. Light-gray areas denote yellowish discoloration reflecting incipient O_3 injury.

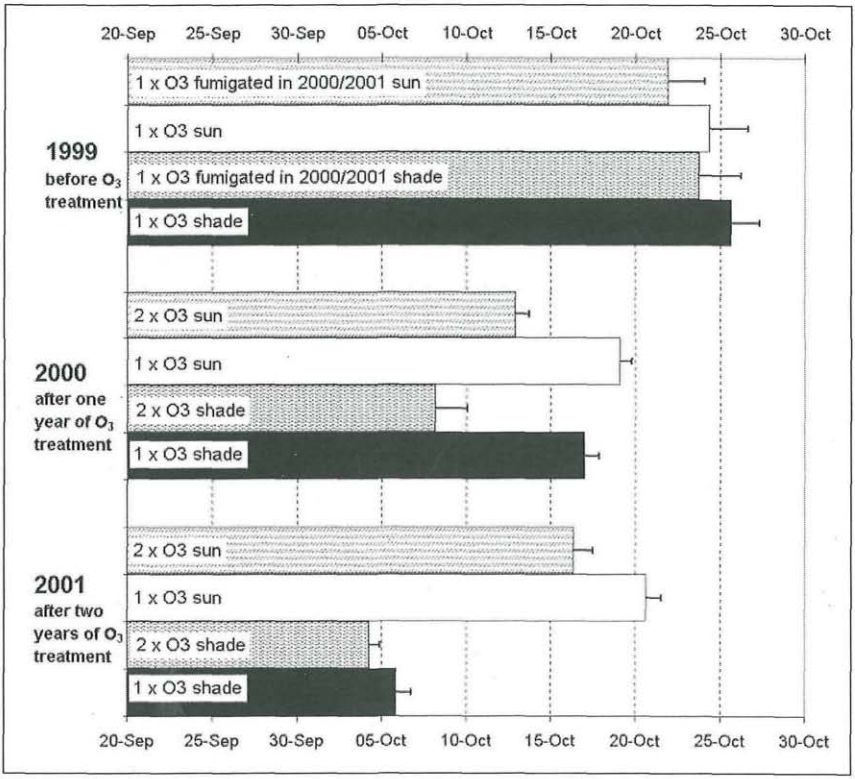

Fig. 8. Date during 1999, 2000, 2001 indicating mid autumnal leaf senescence as reflected by half of the leaves being discolored or lost. Bars represent means across branches of different trees (n=5) each per foliage type and ozone treatment, expressing 50 % of leaf senescence in a branch. The error 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_3 flux into leaves.

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