Comparison of ozone uptake and sensitivity between a phytotron study with young beech and a field experiment with adult beech (*Fagus sylvatica*)

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Juvenile beech trees in phytotrons are more sensitive to ozone than adult forest trees due to lower defence capacity and growth conditions.

Abstract

Chamber experiments on juvenile trees have resulted in severe injury and accelerated loss of leaves along with reduced biomass production under chronically enhanced O3 levels. In contrast, the few studies conducted on adult forest trees in the field have reported low O3 sensitivity. In the present study, young beech in phytotrons was more sensitive to O3 than adult beech in the field, although employed O3 regimes were similar. The hypotheses tested were that: (1) differences in O3 uptake were caused by the ontogenetically higher stomatal conductance of young compared to adult trees, (2) the experimental settings in the phytotrons enhanced O3 uptake compared to field conditions, and (3) a low detoxification capacity contributes to the higher O3 sensitivity of the young trees. The higher O3 sensitivity of juvenile beech in the phytotrons is demonstrated to relate to both the experimental conditions and the physiological responsiveness inherent to tree age.

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1. Introduction

Amongst gaseous air pollutants, ozone (O3) as prevailing in the lower troposphere by chronically enhanced regimes is potentially most harmful to plants (Sandermann et al., 1997). In forest trees, O3 impact is indicated by reductions in photosynthesis, visible leaf injury, premature leaf loss and growth limitation (Reich, 1987; Pearson and Mansfield, 1994; Lippert et al., 1996; Vollenweider et al., 2003a; Matyssek and Sandermann, 2003). As a means of O3 risk assessment in plants, UNECE introduced, in the 1990s, the concept of “Critical Levels for Ozone”. An exposure-based AOT40 threshold
was proposed which, when exceeded, should indicate a reduction in biomass production by more than 10% relative to pre-industrial ozone regimes (Fuhrer, 1994; Skärby and Karlsson, 1996). AOT40 was set to 10 μl O₃ l⁻¹ h but has been recently revised to 5 μl O₃ l⁻¹ h for forest trees (LRTAP Mapping Manual, 2004), regarding exposure below 40 nl O₃ l⁻¹ and at night to be negligible. However, the regional exceedance of AOT40 turned out to be inconsistent with forest growth and symptom development in Europe (Matyssek and Innes, 1999). In addition, the AOT40 definition ignores variation in O₃ sensitivity among different genotypes and site conditions (VanderHeyden et al., 2001; Matyssek and Sandermann, 2003).

During recent years, efforts have been made to replace AOT40 with a flux concept of actual ozone uptake into leaves (Emborson et al., 2000; Karlsson et al., 2003, 2004; Tuovinen et al., 2004; Wieser and Emberson, 2004; Massman, 2004). Combined with measures of the detoxification capacity, the flux concept is postulated to evaluate the O₃ stress experienced by trees (Massman et al., 2000; Wieser et al., 2002; Massman, 2004; Matyssek et al., 2004). Until recently, debates on O₃ thresholds in trees have mainly been based on phytotron or open-top chamber studies with young trees (Skärby and Karlsson, 1996; Matyssek and Innes, 1999; Karlsson et al., 2004). However, a limited number of field studies tended to characterize adult trees as less sensitive to ozone than juvenile trees (Grunke and Miller, 1994; Fredericksen et al., 1996b; Wellburn et al., 1997; Kolb and Matyssek, 2001). In addition, growth conditions in chambers differed from field sites in microclimate and water/nutrient availability as well as exclusion of competitors so that the responsiveness to ozone may have been biased (Chappelka and Chevone, 1992).

This paper investigates the responses of young and adult beech, in a joint phytotron and field study, to experimentally enhanced O₃ levels (Nunn et al., 2002; Werner and Fabian, 2002; Grams et al., 2002; Kozovits et al., 2005). We used a correlational O₃ uptake model (Emborson et al., 2000) to relate O₃ flux to growth, photosynthesis and visible leaf injury. The model was parameterised for the respective phytotron and forest conditions. Model validation for a humid and a dry year in the field was undertaken.

While pursuing the question why young trees in growth chambers rather than adult trees in the field appear to be more O₃ sensitive, the following hypotheses were examined: (1) under high light conditions, stomatal conductance and therefore ozone uptake of young trees is higher than in adult trees (cf. Kolb and Matyssek, 2001). (2) Given the typically non-limiting growth conditions of juvenile trees in phytotron studies, e.g. with respect to water supply (Matyssek and Sandermann, 2003), ozone uptake is higher, on average, than in adult trees in the field. (3) Given lower photosynthetic rates of juvenile trees when typically growing underneath the stand canopy, lower leaf area-based levels of antioxidants in relation to O₃ flux promote O₃ injury as compared to adult trees (cf. Kolb and Matyssek, 2001; Wieser et al., 2003).

2. Material and methods

2.1. Experimental design

In the field experiment at “Kranzberger Forst” (near Freising, Germany, 48°25’08” N, 11°39’41” E, elevation 485 m a.s.l.) exposure to above-ambient O₃ concentrations was realised through a free-air O₃ exposure approach employed within the forest canopy (Nunn et al., 2002; Werner and Fabian, 2002). The entire crowns of five adult beech trees (57–60 years old) were exposed throughout three growing seasons (2000–2002) to a twice-ambient O₃ regime (2 × O₃) and compared to five individuals in unchanged ambient air (1 × O₃) which served as controls. At 2 × O₃, maximum O₃ concentrations were restricted to 150 nl O₃ l⁻¹ to prevent risk of acute O₃ injury (Nunn et al., 2002; Matyssek et al., 2004). In each study tree, one branch in the sun crown and one in the shade crown was chosen for analysis to account for the structural and ecophysiological extremes within tree crowns. In phytotrons (GSF, National Research Centre for Health & Environment, Munich, Germany; Payer et al., 1993; Thiel et al., 1996), monocultures of 20 beech saplings (3–4 years old) per container (70 l; filled with forest soil) were exposed to 1 × O₃ or 2 × O₃ regimes (two containers each) throughout two growing seasons (Grams et al., 2002; Kozovits et al., 2005). The microclimate and O₃ levels of the preceding year of the field experiment were reproduced on an hourly basis throughout the growing seasons in the phytotrons, where maximum irradiance reached 950 μmol photons m⁻² s⁻¹. In 1998, the year before the phytotron study, containers had been kept in a greenhouse under charcoal-filtered air. During the winters of 1998/1999 and 1999/2000 (i.e., the time period between the growing seasons in the phytotrons) containers were kept under ambient air conditions. The containers were regularly watered and fertilized to prevent drought and nutrient deficiency. Leaves of juvenile and adult beech reflected non-limiting N supply (1.8 ± 0.2% and 2.4 ± 0.1%, respectively; leaf dry weight related, means ± SE; Le Tacon, 1981; Schütt et al., 1992).

2.2. Ozone exposure

In the field, ozone concentrations were measured by 10-min intervals (type 8811 UV analysers, Monitor Labs/USA) at two positions each in the O₃-fumigated.
and non-fumigated canopy. In the phytotrons, measurements were taken by 30-min intervals for each treatment using UV analysers (Payer et al., 1993). In both experiments, AOT40 was calculated according to Fuhrer (1994) as the sum of the differences between hourly mean O₃ concentrations above a threshold of 40 nl O₃ l⁻¹ and that threshold during daylight hours (at global radiation > 50 W m²). SUM0 is the sum of all hourly O₃ concentrations. Calculations of SUM0, AOT40 and O₃ uptake were restricted to the actual length of the growing seasons (i.e., the presence of foliage on the plants) in each O₃ regime. In the phytotrons, under 2 × O₃, SUM0 (Table 1) was about twice as high as under 1 × O₃ (SUM0 = 97 μl l⁻¹ h) across the two years of observation. In the field experiment, SUM0 was increased by a factor of 1.8 in the 2 × O₃ regime compared to 1 × O₃ (140 μl l⁻¹ h). AOT40 (Table 1) was 6–7 times higher under 2 × O₃ than in the 1 × O₃ regime (11 μl⁻¹) in the phytotrons and 4.4 times higher in the 2 × O₃ regime compared to 1 × O₃ (16 μl l⁻¹ h) under field conditions. In the field experiment, SUM0 was about 21% higher than in the phytotrons, as in these latter systems the growing season was reduced by 2–4 weeks in the first experimental year. In the second experimental year, plants were harvested on 31 August. AOT40 reached, under 2 × O₃, similar levels in the phytotrons and in the field study, given the low O₃ concentrations at the field site during September and October. In the first year, SUM0 in September accounted for 11–13% of total SUM0, and in parallel, AOT40 for 1–7% of total AOT40. Under 1 × O₃, AOT40 was about 30% lower in the phytotrons.

The ozone distribution in the canopy of the fumigated and non-fumigated beech trees in the field was assessed by two-week intervals using passive samplers for O₃ detection (Werner and Fabian, 2002). For each investigated branch, the ratio was determined between recordings of passive samplers next to the branch and close to the O₃ analysers. The ratios which ranged between 0.72 and 1.08 were used for adapting CU to the O₃ distribution inside the canopy.

2.3. Phenology, growth and physiological measurements

In the field study on adult trees, autumnal leaf senescence was assessed as the discoloured and shed leaf area (shed leaves collected with nets wrapped around branches) in proportion to the total branch foliage area. Bud break was classified according to Meier (1997). Macroscopic leaf symptoms were identified according to Hartmann et al. (1995) and Innes et al. (2001) and validated for ozone effects by the “Ozone Validation Centre” (WSL/FSL Birmensdorf/Switzerland). To monitor seasonal variability in injury development, 20 leaves per experimental branch were classified during 2000–2002 by two-week intervals for determining the symptomatic leaf area. In the phytotrons, leaf abscission was assessed only in 1999 through counting the attached leaves by one to two-week intervals, beginning at the end of July (when all of the formed leaves were still attached to the tree). O₃-induced injury established in beech as chlorotic and necrotic dots or small necrotic areas which spread across the whole leaf lamina. Plants were classified by monthly intervals according to the percentage of symptomatic foliage area.

Radial stem growth was assessed on an annual basis in both experiments by calculation of the cross-sectional area from diameter measurements at the stem base in the phytotrons (calipers) and at breast height in the field experiment (dendrometer bonds).

In the phytotrons, rates of net CO₂ uptake (Uₐ) along with stomatal conductance for water vapour (gₛ) were assessed under saturating light conditions (> 400 μmol photons m⁻² s⁻¹, 22–30 °C, 360 μl CO₂ l⁻¹; > 50% relative air humidity) by IRGA with an open flow porometer system (CQP130, Walz, Effeltrich, Germany). In the field study, gₛ for modelling was measured using an open flow porometer (Li-6400; Li-Cor, Inc., Nebraska, USA) at seven times during the growing seasons of 2000–2002, on sunny days each between 8 a.m. and 3 p.m. (ambient light conditions, 25 °C air temperature, 40 ± 10% relative air humidity depending on transpiration.

<table>
<thead>
<tr>
<th>Year</th>
<th>Phytotron study</th>
<th>Field study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 × O₃</td>
<td>2 × O₃</td>
</tr>
<tr>
<td>SUM0 (μl l⁻¹ h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>97.0</td>
<td>195.0</td>
</tr>
<tr>
<td>Second</td>
<td>91.9</td>
<td>175.2</td>
</tr>
<tr>
<td>Third</td>
<td>126.4 ± 0.3</td>
<td>234.0 ± 0.7</td>
</tr>
<tr>
<td>AOT40 (μl l⁻¹ h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>11.1</td>
<td>70.6</td>
</tr>
<tr>
<td>Second</td>
<td>9.5</td>
<td>64.0</td>
</tr>
<tr>
<td>Third</td>
<td>16.3 ± 0.1</td>
<td>67.2 ± 0.1</td>
</tr>
</tbody>
</table>

In the latter study, data refer to individual branches: mean values ± 5E (n = 20 per treatment, crown zone and year). Phytotron study: experimental time 2 May–26 September (31 August second year); field study: growing season from 1 May to 15 October.
rate, 360 μl CO₂ l⁻¹). Additionally, maximum \( J_{\text{CO}_2} \) and maximum \( g_s \) were assessed under saturating light conditions (1500 μmol photons m⁻² s⁻¹). Both porometer systems were calibrated against the same standards and cross-compared for consistency in data assessment. Measurements in the dark were conducted four times during 2002 between 9 p.m. and 11 p.m. All measurements were performed in the proximity of the 20 experimental branches. Rates of \( J_{\text{CO}_2} \) and \( g_s \) were related to projected leaf area.

Maximum \( g_s \) vs. maximum \( J_{\text{CO}_2} \) provides the potential of \( \text{O}_3 \) uptake (mitigated by \( \text{O}_3 \) fluctuations and boundary layers) in relation to detoxification capacity (Kolb and Matyssek, 2001). Ascorbate, glutathione and tocopherol levels in leaves have been regarded as indicators of the anti-oxidative capacity in trees (Luwe and Heber, 1995; Luwe, 1996; Wellburn and Wellburn, 1996; Polle et al., 2000; Tausz et al., 2002; Wieser et al., 2002, 2003). Ascorbate and tocopherol were determined from liquid-nitrogen frozen leaf samples according to Wildi and Lütz (1996). Analysis of glutathione was performed according to Schupp and Rennenberg (1994).

2.4. \( \text{O}_3 \) flux model and parameterisation

\( \text{O}_3 \) flux (\( F_{\text{O}_3} \); Eq. (1)) and cumulative uptake (CU; Eq. (2)) were calculated from stomatal conductance for ozone (\( g_{\text{O}_3} \)) on a diurnal basis throughout the growing season (SGS = start of growing season; EGS = end of growing season), assuming that the intercellular ozone concentration is close to zero (Laisk et al., 1989).

\[
F_{\text{O}_3} = g_{\text{O}_3}[\text{O}_3]
\]

(1)

\[
\text{CU} = \sum_{\text{EGS}}^{\text{SGS}} F_{\text{O}_3}
\]

(2)

\( g_{\text{O}_3} \) was calculated through a correlative stomatal conductance model (Eq. (3); based on Emberson et al. (2000),

\[
g_{\text{O}_3} = \begin{cases} \text{PPFD} = 0: & g_{\text{night}} g_{\text{O}_3,\text{max}} \\ \text{PPFD} > 0: & \max \left( f_{\text{phen}} g_{\text{min}} g_{\text{O}_3,\text{max}} \right) \end{cases}
\]

(3)

with \( g_{\text{O}_3} \) = stomatal conductance to ozone at given climatic conditions, \( g_{\text{O}_3,\text{max}} \) = maximal stomatal conductance to ozone at non-limiting climatic conditions, \( g_{\text{night}} \) = night-time stomatal conductance as percentage of \( g_{\text{O}_3,\text{max}} \), \( g_{\text{min}} \) = minimum stomatal conductance during daylight hours as percentage of \( g_{\text{O}_3,\text{max}} \). The functions \( f_{\text{phen}}, f_{\text{night}}, f_{\text{temp}}, f_{\text{VPD}} \) and \( f_{\text{soil}} \) represent fractional modifications of \( g_{\text{O}_3,\text{max}} \) depending on pheno-

logical, irradiance, air temperature, vapour pressure deficit of the air (VPD) and soil water potential, respectively, and are expressed as values between 0 and 1. Maximum stomatal conductance for water vapour (\( g_s \)) was derived from porometry for each study branch at several positions along the main axis. \( g_{\text{O}_3,\text{max}} \) was calculated from maximum \( g_s \) by multiplication with 0.613, i.e., the ratio of the diffusivities of water vapour vs. ozone (Laisk et al., 1989). Minimum stomatal conductance for ozone during daytime (\( g_{\text{min}} \)) was set to 13% of \( g_{\text{O}_3,\text{max}} \), according to Emberson et al. (2000).

In the field experiment, the analysis was performed for 10 sun and 10 shade branches (Nunn et al., 2002). \( F_{\text{O}_3} \) was calculated for four sections along each branch axis, based on diurnal measurements of photosynthetic photon flux density (PPFD) in each section (Reitmayer et al., 2002). Measurements taken at the field site during 2002 revealed night-time conductance (\( g_{\text{night}} \)) to be close to zero at the beginning of the growing season and gradually reaching 13% of \( g_{\text{O}_3,\text{max}} \) towards the end of the growing season. Fraction \( f_{\text{night}} \) was modelled as a linear increase across the growing season (Eq. (4); Table 2).

\[
f_{\text{night}} = (m_{\text{night}} \text{doy}) + tf_{\text{night}}
\]

(4)

with \( f_{\text{night}} \) = function for calculating \( g_{\text{night}} \) as fraction of \( g_{\text{O}_3,\text{max}} \), doy = day of year; \( m_{\text{night}} \) = slope and \( tf_{\text{night}} \) = intercept of linear regression between doy and \( f_{\text{night}} \). However, in the exceptionally dry summer of 2003 (Staatsforstverwaltung, 2004), stomatal opening at night stayed below 13% of \( g_{\text{O}_3,\text{max}} \). Therefore, \( g_{\text{night}} \) was set to a constant mean nocturnal \( g_s \) of 2.5 mmol O₃ m⁻² s⁻¹ (cf. Matyssek et al., 2004).

The model parameterisation of \( f_{\text{soil}} \) in the field experiment was derived from diurnal gas exchange measurements (Walz, Effeltrich, Germany; Götz, 1996) during the summer of 2003. To incorporate soil drought, the empirical coefficients for \( f_{\text{soil}} \) calculation (SWP_max and SWP_min; SWP = soil water potential, Table 2) were adapted by minimizing the difference between modelled and measured levels of \( g_s \) as well as
Table 2
Standard parameters of the ozone-flux model (Emerson et al., 2000) compared to new parameterisation obtained from porometry of sun and shade foliage at the field site "Kranzberger Forst"

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Emberson et al. (2000)</th>
<th>Sun crown (this study)</th>
<th>Shade crown (this study)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( g_{O_3,max} ) (mmol O(_3) m(^{-2}) s(^{-1})) based on total leaf area</td>
<td>specific for each branch ((n = 10))</td>
<td>specific for each branch ((n = 10))</td>
<td></td>
</tr>
<tr>
<td>( g_{\text{min}} ) ((% g_{O_3,max}))</td>
<td>66</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>SGS (day of year)</td>
<td>calculated</td>
<td>branch and year as dependent on phenology data recorded in the field</td>
<td></td>
</tr>
<tr>
<td>EGS (day of year)</td>
<td>calculated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( f_{\text{phen}_a} )</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>( f_{\text{phen}_b} ) (days)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>( f_{\text{phen}_c} ) (days)</td>
<td>50</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>( f_{\text{light}_a} )</td>
<td>−0.006</td>
<td>−0.006</td>
<td>−0.06</td>
</tr>
<tr>
<td>( T_{\text{max}} ) ((^\circ C))</td>
<td>34</td>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td>( T_{\text{min}} ) ((^\circ C))</td>
<td>13</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>( T_{\text{opt}} ) ((^\circ C))</td>
<td>24</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>VPD(_{\text{max}}) ((\text{kPa}))</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>VPD(_{\text{min}}) ((\text{kPa}))</td>
<td>3.1</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>SWP(_{\text{max}}) ((\text{MPa}))</td>
<td>−1.0</td>
<td>−0.05</td>
<td>−0.05</td>
</tr>
<tr>
<td>SWP(_{\text{min}}) ((\text{MPa}))</td>
<td>−1.9</td>
<td>−1.25</td>
<td>−1.25</td>
</tr>
<tr>
<td>( g_{\text{night}} ) ((% g_{O_3,max}))</td>
<td>not implemented</td>
<td>1 ( \times O_3 ): 1.347 E − 03</td>
<td>1 ( \times O_3 ): 0.534 E − 03</td>
</tr>
<tr>
<td>( m_{\text{night}} )</td>
<td>in model</td>
<td>2 ( \times O_3 ): 1.396 E − 03</td>
<td>2 ( \times O_3 ): 0.501 E − 03</td>
</tr>
<tr>
<td>( f_{\text{night}} )</td>
<td></td>
<td>1 ( \times O_3 ): −207.8 E − 03</td>
<td>1 ( \times O_3 ): −63.7 E − 03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 ( \times O_3 ): −236.8 E − 03</td>
<td>2 ( \times O_3 ): −58.0 E − 03</td>
</tr>
</tbody>
</table>

\( g_{\text{O}_3,\text{max}} \): maximum stomatal conductance for ozone; \( g_{\text{min}} \): minimum stomatal conductance for ozone; SGS: start of growing season; EGS: end of growing season; \( f_{\text{phen}_a} \) to \( f_{\text{phen}_c} \): parameters for describing phenological response of \( g_s \); \( f_{\text{phen}_a} \): starting point for \( f_{\text{phen}_c} \)’s proportion between 0 and 1, \( f_{\text{phen}_c} \): days after SGS but prior to full stomatal capacity; \( f_{\text{light}_a} \): factor describing exponential light response of stomatal conductance; \( T_{\text{max}} \), \( T_{\text{min}} \), \( T_{\text{opt}} \): maximum, minimum and optimum temperature of \( g_s \) response to air temperature; VPD\(_{\text{max}}\), VPD\(_{\text{min}}\): maximum, minimum, minimum vapour pressure deficit determining \( g_s \); SWP\(_{\text{max}}\), SWP\(_{\text{min}}\): maximum, minimum soil water potential for stomatal conductance; \( g_{\text{night}} \): night-time stomatal conductance to ozone described by a linear response function during the growing season; \( m_{\text{night}} \): slope of linear regression, \( f_{\text{night}} \): intercept of linear regression. The latter slope and intercept as well as \( f_{\text{light}_a} \) are parameters of the model structure that are required in terms of the resolution given below.

Fig. 1. Soil water potential and function for response of stomatal conductance to soil moisture \( f_{\text{soil}} \) during the exceptionally dry year of 2003 at “Kranzberger Forst” at 5 cm soil depth as calculated according to Staatsforstverwaltung (2004), Karlsson et al. (2004) and own data. The broken line depicts the soil water potential measured during 2003 at “Kranzberger Forst”. The solid line represents \( f_{\text{soil}} \) calculated by the ozone-flux model through Table 2.

Accounting for a maximum reduction in the potential transpiration rate by about 50% in August and September (Fig. 1; Staatsforstverwaltung, 2004), SWP\(_{\text{max}}\) was set to −0.05 MPa (cf. Karlsson et al., 2004), and SWP\(_{\text{min}}\) to −1.25 MPa.

In the phytotrons, photosynthetic photon flux density (PPFD) was measured above-crown using a photodiode (Type G1118, Hamamatsu, Japan) in each container, because differences in light exposure were small between leaves of the young beech trees, \( g_{\text{night}} \) was set to 13% of \( g_{\text{O}_3,\text{max}} \) corresponding to the value of \( g_{\text{min}} \). The fraction \( f_{\text{soil}} \) was always set to 1 because containers were well watered throughout the experiment. Calculation of CU was performed for the 1 \( \times O_3 \) and 2 \( \times O_3 \) regime (Table 7), employing maximum \( g_s \) levels as measured separately in each treatment. All other responses of \( g_s \) to environmental factors were calculated based on the functions used by Emerson et al. (2000), which were parameterised as given in Table 2.

Modelled time courses of \( g_s \) were validated with gas exchange data which had continuously been assessed at “Kranzberger Forst” between 23 July–11 September.
2002 and 30 May–5 September 2003, with two stationary, climate-controlled branch cuvette systems (Walz, Effeltrich, Germany; Götz, 1996) which tracked the leaf gas exchange under ambient climate conditions. CU was calculated from \( g_s \) as derived from these measurements and compared to modelled CU.

As \( O_3 \) concentrations used for modelling CU were measured inside the canopy and close to the experimental branches, only boundary layers around leaves were relevant in the diffusion pathway of ozone prior to entry into stomata. Boundary layer thickness and conductance were calculated according to von Willert et al. (1995). Frequency analysis of wind velocity was derived from measurements at a reference position at 3 m above the canopy throughout the growing seasons. Data from the above-canopy reference position were used to estimate the frequency distribution of wind velocity inside the canopy, employing information about vertical wind profiles from Winterhalter (1998). Inside-canopy mean wind velocity was reduced by 80% relative to the above-canopy reference position. Mean wind velocity in the phytotrons of 0.2 m s\(^{-1}\) was determined using a hot-wire anemometer (Lambrecht 642, D). As leaf boundary layers turned out to be, on average, in a similar range in the phytotrons and the field (see Section 3), they were neglected in calculations of \( O_3 \) uptake and comparisons between juvenile and adult trees.

2.5. Statistics

Student’s \( t \)-test for one-sample (Table 7) and Student’s \( t \)-test for independent samples (two-tailed; Tables 3–5) were performed using SPSS 12.0 (SPSS, Inc., Chicago, USA). Comparisons and tests are specified in table and figure legends as appropriate. Data management and calculation of linear regression for validation of ozone flux was done using Diadem 8.1 (National Instruments, Austin, USA).

3. Results

3.1. Phenology, growth and physiology

The enhanced \( O_3 \) regime accelerated leaf loss in the phytotrons by 15–25% compared to 1 × \( O_3 \) in the first experimental year. In the field experiment, \( O_3 \)-induced leaf loss was most pronounced in the first year of free-air fumigation. Autumnal senescence was accelerated by 7–10 days as compared to the 1 × \( O_3 \) regime (Nunn et al., 2002). During the subsequent years, this effect became less distinct. Higher percentage of leaf injury (Table 3) was found in the phytotron (19%) than in the field study (1.7%) under 2 × \( O_3 \). In the phytotrons, plants under 2 × \( O_3 \) showed a significantly higher percentage of injured foliage area (1999: \( p = 0.07 \); 2000: \( p < 0.01 \)) than did plants under the 1 × \( O_3 \) regime (Table 3). In the field experiment, no difference was detectable between the two \( O_3 \) regimes in the sun crown, whereas in the shade crown no visible leaf injury was observed (Table 3). In the phytotron study, Matyssek et al. (2004) reported initiation of leaf injury at the end of June in both experimental years at CU of 2–4 mmol \( O_3 \) m\(^{-2}\). Initiation of symptom development occurred in the field study in the sun-exposed leaves of adult beech between the end of May and mid-June in each experimental year. This corresponded to CU between 1.8 and 9.4 mmol \( O_3 \) m\(^{-2}\).

Throughout the three experimental years of the field experiment, no significant difference in radial stem growth was detectable between the two \( O_3 \) regimes (H. Pretzsch, pers. comm.). In the phytotrons, 2 × \( O_3 \) significantly (\( p < 0.01 \)) decreased the radial stem increment of young beech only during the second experimental year (data not shown).

In the phytotrons, the 2 × \( O_3 \) regime significantly reduced the net \( CO_2 \) assimilation rate (\( J_{CO_2} \)) and \( g_s \) of young beech relative to those under 1 × \( O_3 \) (\( p < 0.01 \)) only in the second year (Table 4). In the field, \( J_{CO_2} \) did not differ significantly between the \( O_3 \) regimes, however, in the shade crown, \( J_{CO_2} \) tended to be lower under 2 × \( O_3 \) (\( p = 0.09 \)) relative to 1 × \( O_3 \); \( g_s \) was significantly decreased under 2 × \( O_3 \) in the shade leaves (\( p < 0.05 \)), whereas such an effect was smaller in the sun leaves (\( p = 0.07 \); Table 4). In the phytotrons, \( J_{CO_2} \) and \( g_s \) under 2 × \( O_3 \) (and 1 × \( O_3 \) in the first year) reached levels which were only about 35–50% of those found in sun leaves of adult trees but similar to the levels of shade leaves in adult beech. Only in the second experimental year, beech in the phytotrons reached, under 1 × \( O_3 \), about 70% of \( J_{CO_2} \) and \( g_s \) found in sun leaves of adult

<table>
<thead>
<tr>
<th>Year</th>
<th>Phytotron study</th>
<th>Field study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 × ( O_3 )</td>
<td>2 × ( O_3 )</td>
</tr>
<tr>
<td>First</td>
<td>3.9 ± 0.8</td>
<td>16.5 ± 6.7</td>
</tr>
<tr>
<td>Second</td>
<td>3.7 ± 1.0</td>
<td>19.0** ± 6.2</td>
</tr>
<tr>
<td>Third</td>
<td>0.8 ± 0.8</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

Student’s \( t \)-test two-tailed for differences between the 1 × \( O_3 \) and 2 × \( O_3 \) regimes in each experiment separately: **\( p < 0.01 \).
respectively. Student’s t-test two-tailed for differences between the 1 × O₃ and 2 × O₃ regimes in each experiment separately: *p < 0.05; **p < 0.01.

beech (Table 4). Young beech in the phytotrons had a slightly higher uptake capacity for ozone (higher gₛ) relative to maximum JₑCO₂ than had adult trees in the field (Table 4).

At the end of the growing season of 2000, mean total ascorbate concentrations (when dry weight based; Table 5) were about 50% higher in sun leaves of adult beech trees in the field as compared to leaves of young trees in the phytotrons. When based on leaf surface area, such levels were elevated by 300% in adult trees (p = 0.037). Glutathione levels were, on a dry weight basis, about 15% lower in adult trees at the end of the growing season of 2000 (Table 5). Related to surface area, such levels were about 170% higher (p = 0.087) in the adult trees. In contrast, mass-related tocopherol concentrations were about 44% lower in adult beech (p = 0.019) compared to young beech in the growing season of 1999 (Table 5), whereas on an area basis, the levels were about 6% higher in adult beech.

3.2. O₃ flux model validation

In 2002, CU based on gas exchange measurements in the branch cuvettes amounted to 4.0 mmol m⁻² (data not shown), whereas modelled CU, using the original parameterisation of Emberson et al. (2000) yielded an underestimation of 32%. After adapting the temperature response function of gₛ (fₙₐⁱₜₚₑ) to stand conditions and implementing a function for gₛₙᵣᵦᵣ (Table 2), modelling amounted to 3.5 mmol O₃ m⁻² and thereby confined the underestimation to 11% (linear regression measurement vs. modelling: y = 0.854x + 1.10; R² = 0.79). In 2003, measurement-based CU amounted to 6.4 mmol m⁻² (Fig. 2), whereas modelling based on the original parameterisation of Emberson et al. (2000) led to an overestimation of 73%. The use of the new parameterisation from 2002 resulted in a CU of 8.9 mmol m⁻² (overestimation of 39%). When the new parameterisation was extended by the site-specific soil moisture function (Fig. 2), modelled CU yielded 7.4 mmol m⁻² and confined overestimation to +16% (linear regression measurement vs. modelling: y = 0.850x + 10.4; R² = 0.66).

In addition, modelled gₛ values closely predicted the actually recorded time courses of stomatal conductance, as exemplified for a week in August 2002 and 2003 each (Fig. 3a, b). In 2002, the model did reproduce the actual diurnal course (maximum gₛ = 300 mmol m⁻² s⁻¹) under humid conditions, and in 2003 under severe drought (Staatsforstverwaltung, 2004). The reduction of gₛ

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**Table 4**

<table>
<thead>
<tr>
<th>Year</th>
<th>Phytotron study</th>
<th>Field study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 × O₃</td>
<td>2 × O₃</td>
</tr>
<tr>
<td>JₑCO₂ max (µmol m⁻² s⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>4.3 ± 0.5</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Second</td>
<td>8.2** ± 0.6</td>
<td>4.7 ± 0.7</td>
</tr>
<tr>
<td>gₛ max (µmol m⁻² s⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>87.0 ± 10.9</td>
<td>102.1 ± 10.1</td>
</tr>
<tr>
<td>Second</td>
<td>165.7** ± 12.4</td>
<td>86.3 ± 7.6</td>
</tr>
</tbody>
</table>

In the field, values represent the mean of two years, because no difference between the years occurred. Student’s t-test two-tailed for differences between the 1 × O₃ and 2 × O₃ regimes in each experiment separately: *p < 0.05; **p < 0.01.

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**Table 5**

Mean levels of ascorbate, glutathione and tocopherol in leaves of young beech in phytotrons and adult beech in the field as related to leaf dry weight or leaf surface area

<table>
<thead>
<tr>
<th></th>
<th>Phytotrons: young beech (µmol g dw⁻¹)</th>
<th>Field: adult beech trees (µmol g dw⁻¹)</th>
<th>Phytotrons: young beech (µmol m⁻²)</th>
<th>Field: adult beech trees (µmol m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbate</td>
<td>2.04 ± 0.3</td>
<td>3.07 ± 0.4</td>
<td>93.8* ± 17.8</td>
<td>279.5 ± 32.3</td>
</tr>
<tr>
<td>Glutathione</td>
<td>0.36 ± 0.04</td>
<td>0.31 ± 0.03</td>
<td>14.2 ± 0.4</td>
<td>24.2 ± 3.1</td>
</tr>
<tr>
<td>Tocopherol</td>
<td>1.32* ± 0.18</td>
<td>0.74 ± 0.13</td>
<td>60.6 ± 8.3</td>
<td>64.3 ± 11.1</td>
</tr>
</tbody>
</table>

Ascorbate and glutathione were measured in August of the first experimental year in the phytotrons and the field, respectively (means ± SE: n = 2). Tocopherol was measured monthly from June through September in the first experimental year (means ± SE: n = 8) in the phytotrons and the field, respectively. Student’s t-test two-tailed for differences between young and adult trees: *p < 0.05.
(maximum $g_s = 90 \text{ mmol m}^{-2} \text{s}^{-1}$) in 2003 was met by the model as well (Fig. 3b). Under these latter conditions, the model predicted, however, stomatal opening in the early morning, which was actually delayed by about 1–2 h. Also, modelling predicted minimum $g_s$ in the afternoon when, in fact, stomata were open (Fig. 3b). Hence, the precision of modelling was restricted under the drought conditions of 2003.

In addition to stomatal conductance and $O_3$ concentrations, $CU$ is restricted by the leaf boundary layer. Since mean wind velocity was very low, both in the phytotrons and inside the forest canopy (Table 6), we concluded that boundary layer thickness was high and of similar order of magnitude at both locations. Nevertheless, a tendency of locally enhanced but spatially fluctuating wind velocities in phytotrons, facilitating turbulently decreased boundaries and increased $O_3$ uptake, is likely, as the canopy of plantations extends during plant growth (cf. Payer et al., 1993).

### 3.3. Estimates of ozone uptake

In the phytotrons, $CU$ during the first experimental year was 1.9 times higher under $2 \times O_3$ compared to $1 \times O_3$ (15.0 mmol m$^{-2}$; Table 7). In the second experimental year, lower stomatal conductance (Table 4) and a shorter growing season (plants were harvested on 31 August) resulted in 25% less ozone uptake under $2 \times O_3$ than during the first year. However, trees under $1 \times O_3$ displayed $O_3$ uptake to be enhanced by 50% due to higher $g_s$ as compared to the first year (Table 4) so that $CU$ became similar under both $O_3$ regimes. In the first experimental year, $CU$ in September, however, accounted only for 2–5% of total $CU$. In the sun crown of adult beech, $CU$ was 1.2–1.6 times higher under $2 \times O_3$ (20.6–21.7 mmol m$^{-2}$; Table 7) compared to $1 \times O_3$. In the third experimental year, leaves under $1 \times O_3$ took up 39% more ozone compared to the preceding years. In the shade crowns of adult beech, leaves displayed approximately 30–50% of $CU$ of the leaves in sun crowns under the $1 \times O_3$ and $2 \times O_3$ regimes, respectively.

Under $1 \times O_3$ in the first experimental year, $CU$ in the phytotrons was similar to $CU$ of sun leaves of adult beech during the first two years. In the second year, young beech in phytotrons took up 22–70% more ozone than did the sun leaves of the field trees (Table 7). Under $2 \times O_3$, $CU$ of phytotron plants, in both experimental years, was significantly higher or as high
Table 6
Frequency distribution of horizontal wind velocity and corresponding boundary layer thickness (calculated according to von Willert et al., 1995) inside the canopy at the study site "Kranzberger Forst" (a) during the growing seasons of 2001 and 2002 and (b) in the phytotrons inside the canopy at the study site "Kranzberger Forst" (a) and Phytotrons (b).

<table>
<thead>
<tr>
<th>Wind velocity (m s⁻¹)</th>
<th>Boundary layer (mm)</th>
<th>Years 2001 and 2002 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.0–0.16</td>
<td>2.83–2.24</td>
<td>11.5 ± 0.2</td>
</tr>
<tr>
<td>0.20–0.48</td>
<td>2.00–1.29</td>
<td>65.1 ± 1.3</td>
</tr>
<tr>
<td>0.52–0.60</td>
<td>1.24–1.15</td>
<td>11.8 ± 0.3</td>
</tr>
<tr>
<td>0.64–0.80</td>
<td>1.12–1.00</td>
<td>8.1 ± 0.5</td>
</tr>
<tr>
<td>&gt;0.84</td>
<td>0.98–0.77</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Mean</td>
<td>1.42</td>
<td>100</td>
</tr>
<tr>
<td>0.39 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytotrons</td>
<td>2.00</td>
<td>100</td>
</tr>
<tr>
<td>mean 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Wind velocities were measured at about 3 m above-canopy. Inside-canopy wind velocities were approximated according to Winterhalter (1998) through multiplication by 0.2.

as in the sun crown of adult trees (Table 7). CU of leaves of young beech in the phytotrons was consistently about 3–4 times higher than CU in the shade leaves of adult beech (Table 7).

4. Discussion

In the literature, most studies have found seedlings to be more sensitive to ozone than adult trees (Gruulke and Miller, 1994; Frederiksen et al., 1996a, b), and only for Quercus rubra a higher sensitivity of adult trees was reported (Samuelson and Kelly, 1997). In the present study, ozone impact was also greater on young beech in the phytotrons than on adult beech in the field. Under 2 × O₃, radial stem increment was not reduced in the field, whereas a significant decrease was found in the phytotrons during the second year of fumigation. JCO₂ and gₛ were significantly decreased in young beech under 2 × O₃ in the second year. Stomatal closure in response to enhanced ozone levels has been found in open-top chambers (Bortier et al., 2000; Matyssek and Sandermann, 2003). In the field, however, adult beech showed minor stomatal closure only in the shade crown in response to ozone. In both experimental years, young beech displayed a higher extent of leaf injury under both O₃ regimes compared to the adult trees, along with a distinct increase of injured leaf area under 2 × O₃, which was absent in the field.

Under natural stand conditions, juvenile trees tended to have lower gₛ as compared with adult trees when shaded underneath the stand canopy (Kolb and Matyssek, 2001). This finding was supported by the phytotron experiment, contrasting with hypothesis 1.

In the present study, we found CU of young beech in the phytotrons to be higher than in the shade leaves and at least as high as in sun leaves of adult beech in the field, even though SUM0 was about 21% lower in the phytotron experiment as compared to the field, which supported hypothesis 2. The reason for the similarity in CU is that, in modelling of CU at the whole-branch level in the field, a gₛ was employed that accounted for transient gₛ levels under fluctuating, natural light conditions (Table 2). The high gₛ given in Table 4 for sun-exposed branch parts reflects gₛ measured under light-induced, steady-state conditions. In addition, the ratio in CU between distal vs. proximal parts of sun branches can be up to a factor of 3 as demonstrated in Matyssek et al. (2004).

In the field, low light conditions and somewhat reduced O₃ levels in the shade crown constrained CU.

In modelling ozone uptake, we found that in humid years the correlative model for stomatal conductance (Jarvis, 1976; Emberson et al., 2000) had an adequate accuracy in modelling diurnal courses of gₛ and CU in adult beech when site-specific parameterisation was used. However, in dry years adult beech not only displayed reduced maximum gₛ but also changed the diurnal patterns of gₛ. Such changes could not be reproduced by the response function of gₛ to soil drought as provided by the model approach by Emberson et al. (2000), because the soil moisture deficit measured in the field displayed a gradual decrease over weeks (Fig. 1) rather than pronounced diurnal fluctuations. In addition, rapid response in gₛ to precipitation occurred (data not shown), although the soil moisture deficit remained.

Table 7
CU of the phytotron and field study during the experimental years; field CU was corrected for the ozone distribution in the canopy using passive sampling data.

<table>
<thead>
<tr>
<th>O₃ uptake, CU (mmol m⁻²)</th>
<th>Projected leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phytotron study</td>
</tr>
<tr>
<td></td>
<td>1 × O₃</td>
</tr>
<tr>
<td>First</td>
<td>15.0ᵃ</td>
</tr>
<tr>
<td>Second</td>
<td>22.4ᵇ</td>
</tr>
<tr>
<td>Third</td>
<td>18.4ᵇ</td>
</tr>
</tbody>
</table>

Means ± SE: n = 20 per treatment, crown zone and year; Student’s t-test for one-sample: p < 0.05; same letters mark absence of significant differences in CU between phytotron and field conditions for the 1 × O₃ and 2 × O₃ regime, respectively. Phytotron study: experimental time 2 May–26 September (31 August second year); field study: growing season from 1 May to 15 October.
unchanged, when water was taken up instantly by the fine roots underneath the soil surface. Cermak et al. (1993) reported that tall beech trees under drought stress responded to irradiation within minutes — a phenomenon that cannot be accounted for by the model. Nevertheless, when comparing different modelling approaches of stomatal conductance, van Wijk et al. (2000) concluded the approach of Jarvis (1976) to be suitable for implementing the responsiveness to soil water content, given an adequate parameterisation. We suggest the CU model (Emerson et al., 2000) in dry years to be combined with response functions of $g_s$ to soil moisture and precipitation. Due to the exceptional drought in 2003 (Staatsforstverwaltung, 2004), CU in sun leaves amounted to 86–116% of the mean CU of the three preceding years, although the ozone exposure was about 45–51% higher in 2003 (M. Löff, Unpublished observation). Schaub et al. (2003) reported a significant decrease in leaf injury for Prunus serotina seedlings under drought conditions as compared to the well-watered treatment. CU was higher under irrigation due to a higher stomatal conductance in the latter trees. Young ozone-treated beech was protected from visible leaf injury and growth reductions by drought as compared to the well-watered ozone treatment (Dixon et al., 1998). In the present study, CU in 2003 calculated with the adapted soil function ($f_{soil}$) (Fig. 2) was reduced by 23%, due to stomatal responses to drought, as compared to CU under non-limiting soil conditions in the previous humid years. The distinct reduction of CU in the field by drought was in support of hypothesis 2.

Nocturnal $g_s$ has been discussed as an important factor contributing to ozone injury in plants (Musselman and Minnick, 2000). In birch, night-time rather than daytime exposure to the otherwise same O$_3$ regime caused reduction in whole-plant biomass production and changes in allocation, although $g_s$ was lower at night (Matyssek et al., 1995). Massman (2004) found that 15% of the daily CU occurred in a vineyard at night. Since partially open stomata at night have been reported in more than 120 plant species (Musselman and Minnick, 2000), we calculated CU on a 24-h basis, although one needs to caution that the extent of stomatal opening at night may depend on the species or genotype, the stage of ontogeny or the site conditions (e.g. effects of drought and nutrition). The incorporation of a module for nocturnal $g_s$ into the ozone-flux model resulted, in 2002, in a nocturnal O$_3$ uptake of 7.6% of total CU, although this level was lower in 2003 (3.1%) because of the prolonged drought. As adverse effects of nocturnal CU cannot be ruled out, taking into account the supposedly lower detoxification capacity at night (Matyssek et al., 1995), night-time should be covered in modelling physiologically relevant O$_3$ doses.

In the phytotrons, autumnal senescence occurred earlier than in the field, therefore, mainly the periods with high ozone concentrations in the field (May–August) were reproduced in the phytotrons. As a consequence, young beech took up the same amount of O$_3$ within an up to one month shorter growing season and experienced high ozone concentrations from bud break onwards. Matyssek and Sandermann (2003) suggested that not only CU, but also ozone peak concentrations are important for the extent of ozone impact and leaf injury. Incipient leaf injury was observed at CU of 4 mmol m$^{-2}$ in the phytotrons and at a range of 1.8–9.4 mmol m$^{-2}$ in the field. This range was consistent with findings by Baumgarten et al. (2000) who reported a CU of 6 mmol m$^{-2}$ prior to incipient leaf injury in adult beech trees and beech saplings in phytotrons. However, correlation between CU and leaf injury does not appear to be linearly correlated (Nunn et al., 2005), which agrees with findings in P. serotina, where the tendency of extending foliar injury was neither uniform nor linear with the decrease in tree size (Fredericksen et al., 1996a, b). In the field, leaf injury occurred only within the upper layer of the canopy (0.5 m), suggesting that ozone-induced leaf injury was enhanced under high light conditions, similar to observations by Vollenweider et al. (2003b). In 1999, LAI (leaf area index) in the phytotrons was 3.1 and 4.2 m$^2$ m$^{-2}$ under 1 $\times$ O$_3$ and 2 $\times$ O$_3$, respectively, compared to 5.6 m$^2$ m$^{-2}$ in the field stand (Grote and Reiter, 2004), suggesting that a larger part of the total crown area of the young trees was exposed to high light levels in the phytotrons, which could potentially account for the high amount of injured foliage area. In 2000, LAI amounted to 9.2 m$^2$ m$^{-2}$ under 1 $\times$ O$_3$ and 8.6 m$^2$ m$^{-2}$ under 2 $\times$ O$_3$ in the phytotrons, however, injured foliage area was as high as in the previous year (Table 3).

In a field experiment with P. serotina seedlings, Fredericksen et al. (1996b) observed lost coupling between $J_{CO_2}$ and $g_s$ in leaves with extended foliar injury. The rate of $F_{O_3}$ vs. $J_{CO_2}$ was higher in seedlings than in neighbouring adult trees so that less energy for sustaining detoxification was assumed to be available to the seedlings (Kolb and Matyssek, 2001). We found that young beech in the phytotrons displayed lower $J_{CO_2}$ in relation to the higher O$_3$ uptake as compared to adult trees (hypothesis 3, corroborated) so that probably fewer resources were available for detoxification and repair relative to adult trees (Kolb and Matyssek, 2001). This may be consistent with the lower N levels of leaves in the juvenile trees, although the nutritional status may determine the strategy of defence in variable ways (Maurer and Matyssek, 1997; Polle et al., 2000; Matyssek et al., 2002). Wieser et al. (2003) found beech seedlings to exhibit significantly lower levels of antioxidants, namely ascorbate, glutathione and tocopherol, on a surface area basis as compared to adult trees. A higher photo-protective capacity of adult Fagus sylvatica trees was indicated relative to the seedlings.
The area basis closely relates the O₃ uptake through the leaf surface to the defence capacity behind that surface. In the present study, adult beech trees in the field had higher levels of ascorbate and glutathione, on a surface area basis, and were, in this respect, better protected against oxidative stress than young beech trees (hypothesis 3, accepted). With respect to tocopherol, however, the detoxification capacity of young beech did not appear to be impaired relative to adult trees in the field.

In summary young beech in phytotrons, when exposed to similar ozone regimes as adult trees in the field, had a slightly higher ozone uptake in the first experimental year and appeared to be more susceptible to O₃ injury because of a lower flux-related detoxification capacity. Therefore, we conclude that both experimental conditions and inherent physiological properties contribute to an increased O₃ sensitivity of young beech in phytotrons, which underlines the need for considering both physical and physiological aspects when scaling sapling responses to ozone in chambers to the O₃ responisiveness of field-grown forest trees.

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