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Tree age dependence and within-canopy variation of leaf gas exchange and antioxidative defence in *Fagus sylvatica* under experimental free-air ozone exposure

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Ozone effects on leaf gas exchange and antioxidative systems of beech across tree age and canopy level were investigated in a free air exposure system.

Abstract

We characterized leaf gas exchange and antioxidative defence of two-year-old seedlings and 60-year-old trees of *Fagus sylvatica* exposed to ambient ($1\times O_3$) or two-fold ambient ($2\times O_3$) O_3 concentrations (maximum of 150 ppb) in a free-air canopy exposure system throughout the growing season. Decline in photosynthesis from sun-exposed to shaded conditions was more pronounced in adult than juvenile trees. Seedling leaves and leaves in the sun-exposed canopy had higher stomatal conductance and higher internal CO_2 concentrations relative to leaves of adult trees and leaves in shaded conditions. There was a weak overall depression of photosynthesis in the $2\times O_3$ variants across age classes and canopy positions. Pigment and tocopherol concentrations of leaves were significantly affected by canopy position and tree age, whereas differences between $1\times O_3$ and $2\times O_3$ regimes were not observed. Glutathione concentrations were significantly increased under $2\times O_3$ across both age classes and canopy levels. Seedlings differed from adult trees in relevant physiological and biochemical traits in ozone response. The water-soluble antioxidative systems responded most sensitively to $2\times O_3$ without regard of tree age or canopy position.

Keywords: Ozone; *Fagus sylvatica*; Beech; Tree age; Leaf gas exchange; Antioxidants; Pigments; Glutathione

1. Introduction

Tropospheric ozone (O_3) is one of the most important air pollutants affecting forest trees (Lefohn, 1991; Sandermann et al., 1997). Most information on O_3 effects on trees is based on chamber studies conducted with seedlings (e.g. Reich, 1987; Pye, 1988; Sandermann

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et al., 1997; Matyssek and Innes, 1999). Given the differences in morphological and physiological characteristics, seedlings are however uncertain surrogates for adult forest trees (Kelly et al., 1995; Kolb et al., 1997; Kolb and Matyssek, 2001). Such differences may strongly influence responses to O_3 as was previously shown for *Picea abies* (Wieser et al., 2002a). In addition, comparisons between seedlings and adult trees are often confounded by the differences in microclimate, which exist between seedlings (near the soil surface) and the canopy of adult trees. Only few studies addressed this issue by exposing seedlings at the level of forest canopy (Kolb and Matyssek, 2001; Wieser et al., 2002b, 2003). Even under comparable microclimatic field conditions, leaf based traits were found to significantly differ between seedlings and adult trees (Wieser et al., 2002b, 2003). It has been hypothesised that these differences can cause differences in ozone sensitivity, which may be related to the ratio of ozone uptake *versus* detoxification capacity (Wieser et al., 2002a, 2003). However, there is a lack of direct comparisons of responses between seedlings and adult trees to ozone stress because of the difficulty of exposing tall trees to experimentally enhanced O_3 regimes (Werner and Fabian, 2002; Matyssek and Sandermann, 2003).

Trees respond to O_3 stress by mechanisms of avoidance and defence, such as restriction of O_3 uptake by stomatal narrowing and detoxification by biochemical reactions (Wieser et al., 2002a). In addition to developmental aspects, different O_3 sensitivity of field grown trees of different size and age may also be attributed to microclimatic differences in e.g. irradiance, temperature and/or humidity. For example, in *Fagus sylvativa* low irradiance does not only reduce stomatal opening and hence O_3 uptake, but also net photosynthesis (Wieser et al., 2003). On the other hand, concentrations of antioxidants – potentially important to detoxify ozone and secondary products – are often higher in sun leaves or upper canopy foliage (Wieser et al., 2003; Garcıa-Plazaola et al., 2004). Hence, it is difficult to predict the potentially confounding effects of canopy position on the uptake and detoxification of O_3.

Therefore, the present study aimed at a characterisation of leaf gas exchange and antioxidative defence of seedlings and adult trees of *F. sylvatica* exposed to ambient and experimentally enhanced O_3 concentrations in a free-air canopy fumigation system (Nunn et al., 2002; Werner and Fabian, 2002). Two-year-old seedlings were grown in the upper sun-exposed canopy and near the forest floor (where conditions were similar to the lower shade canopy) in a 60-year-old beech stand (Pretzsch et al., 1998) throughout the growing season 2003. This unique experimental set-up allowed us to address the following questions: (1) Do canopy position and tree age modify physiological and biochemical leaf traits of *F. sylvatica* potentially relevant to ozone response? (2) Does O_3 sensitivity depend on canopy position and tree age? (3) Does ozone exposure in the field affect antioxidative defence in *F. sylvatica* leaves – if so – (4) are there significant interaction effects with canopy position and tree age? Emphasis was on photosynthesis (as an integrating indicator of potential adverse O_3 effects and a pre-requisite of defence), stomatal conductance (which determines ozone uptake), as well as pigments and antioxidative compounds (as a measure of defence capacity).

2. Materials and methods

2.1. Study site and experimental free-air O_3 exposure system

The study was carried out at the Kranzberg Forest near Munich, Germany (485 m a.s.l., 48°25′08″ N, 11°39′41″ E, Pretzsch et al., 1998). During the growing season 2003 60-year-old beech trees (*Fagus sylvatica* L.) were exposed to either ambient ($1\times O_3$) or two-fold ambient O_3 levels ($2\times O_3$), the latter regime being generated a free-air canopy O_3 exposure system (Nunn et al., 2002; Werner and Fabian, 2002). To prevent acute O_3 injury under $2\times O_3$, maximum O_3 concentrations were restricted to 150 ppb.

Five adult trees were studied under each O_3 regime ($1\times O_3$ and $2\times O_3$) between April 15 through September 15, 2003. Additionally, two-year-old beech seedlings were planted into 30 L containers (6 plants each) with forest soil from the site (Grams et al., 2002). To ensure similar microclimate for the leaves of seedlings and adult trees, nine containers at each O_3 regime were transferred into the upper sun-exposed layer (25 m height) of the stand canopy. The canopy was accessible through scaffolding and a research crane (Matyssek and Häberle, 2002). In parallel, the same number of containers was exposed to both O_3 regimes at the forest floor, where light conditions were comparable to those in the shade canopy of adult trees. The soil in the containers was kept well watered (up to field capacity) at all times. Foliage of adult trees was measured at the canopy top (“sun canopy”) and about 2–3 m below the uppermost canopy layer (“shade canopy”).

For biochemical analyses leaves of 5 adult trees and 3–6 seedlings (depending on availability of plant material) under each O_3 regime were harvested on September 15, 2003. Leaves were taken between 11:00 and 13:00 hours solar time and frozen in liquid nitrogen immediately (within seconds). Further processing (freeze-drying, pulverisation) was done as suggested in Tausz et al. (2003).

Gas exchange measurements were carried out on September 15, 2004 on leaves of 3–5 adult trees (depending on availability and accessibility of foliage) and 7 seedlings at each O_3 regime and canopy level.