The Role of Antioxidative Defense in Determining Ozone Sensitivity of Norway Spruce (*Picea abies* (L.) Karst.) Across Tree Age: Implications for the Sun- and Shade-Crown

By

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K e y   w o r d s : *Picea abies* (L.) Karst., tree age, cumulative ozone uptake, ozone flux, antioxidants, needle morphology.

S u m m a r y

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We compared ascorbate contents of current-year *Picea abies* needles with respect to tree age and crown position. In order to exclude microclimatic influences potted seedlings were transferred into the upper canopy of a 45 year-old stand. In the sun- and the shade-crown 100-needle dry weight was significantly lower in seedlings than in adult trees, whereas the opposite was found for specific leaf area. Such age dependent changes in needle morphology also influenced the detoxification capacity: In general, the contents of ascorbate per unit needle surface area increased with increasing tree age, a fact that could not be observed when needle dry-weight based ascorbate concentrations were compared in both canopy positions. As O_3 injury results from the balance between uptake and detoxification processes, the overall tree response to O_3 can be expressed as the ratio between the amount of potentially available ascorbate and the O_3 flux into the needles. Our data show that the contents of the potentially available ascorbate per nmol O_3 taken up per second increased with tree age. In general, the amount of potentially available ascorbate per nmol O_3 taken up was lower in the shade- than in the sun crown which points towards a higher risk of O_3 damage in the shade-crown

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I n t r o d u c t i o n

Due to technical and logistic reasons most of the information on ozone (O_3) effects on forest trees is based on chamber studies conducted on seedlings (REICH 1987, PYE 1988, SANDERMANN & al. 1997, MATYSSEK & INNES 1999). However, a few studies indicated that the sensitivity to O_3 of seedlings may differ from that of adult trees (SAMUELSON & EDWARDS 1993, FREDRICKSEN & al. 1995, 1996, GRULKE & MILLER 1994, KELLY & al. 1995, KOLB & al. 1997). For example, relationships between cumulative O_3 uptake and net photosynthesis clearly indicate that seedlings are more sensitive to O_3 exposure than adult Norway spruce [*Picea abies* (L.) Karst.] trees (WIESER & al. 2002, Fig. 1).
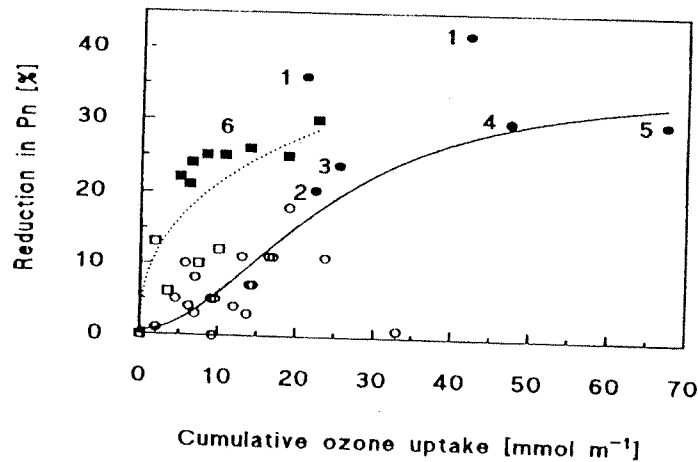

Fig. 1. Percent reduction in net photosynthesis (Pn) in current-year needles of *Picea abies* mature trees (●, O) and seedlings (■, □) after 11 to 253 days of exposure to mean O_3 concentrations ranging from 0 to 137 nl l^{-1}. Each data point represents the mean of 4 to 20 individual twigs. Open symbols: not statistically significant different from O_3-free controls; solid symbols: mean O_3 concentrations eliciting statistically significant reductions ($P < 0.05$) were as follows: mature trees (1) 137 nl l^{-1}, (2) 115 nl l^{-1}, (3) 127 nl l^{-1}, (4) 121 nl l^{-1}, (5) 132 nl l^{-1}, and seedlings: (6) 100 nl l^{-1}. (Modified after WIESER & al. 2002).

The response to O_3 however, is not only dependent on O_3 uptake, but also on morphological differences (FREDRICKSEN & al. 1995, FERDINAND & al. 2000) as well as physiological and biochemical processes within the needles (TAYLOR & HANSON 1992, WELLBURN & WELLBURN 1996). In field grown trees at the timberline ecotone 100-needle dry weight significantly increased with tree age, while the opposite was found for specific leaf area (TEGISCHER & al. 2002, WIESER & al. 2002). These morphological changes affected the interpretation of measured antioxidant contents. On a total needle surface area basis the contents of ascorbate and glutathione increased with tree age, a fact that could not be observed when needle dry-weight based antioxidant concentrations were compared (TEGISCHER & al. 2002).

As O_3 injury results from the imbalance between uptake and detoxification processes, WIESER & al. 2002 suggested a ratio between the amount of potentially available antioxidants under consideration and the O_3 flux into the needles as a measure for the physiologically active ozone dose. Their data showed that the potentially available nmol ascorbate per nmol O_3 taken up per second increased with tree age. This corroborated a higher susceptibility to O_3 in young trees as compared to adult ones (WIESER & al. 2002).

Changes in needle morphology and antioxidant concentrations of field grown trees differing in size and age may also be attributed to microclimatic differences within a stand, such as temperature, humidity, and irradiance. The latter is probably the most important one. For example low irradiance may not only reduce stomatal opening and hence also O_3 uptake (WIESER & HAVRANEK 1993, WIESER & al. 2000) but also net photosynthesis. On the other hand, low irradiance potentially reduces defense capacities, since fully light exposed needles generally contain more antioxidants (POLLE 1997). In order to exclude the impact of microclimatic differences within the canopy on needle physiology and biochemistry, potted 4-year old seedlings of *Picea abies* were grown in the upper and lower canopy of a 45-year old forest stand. The goal of this study was to examine gas exchange parameters and antioxidant contents of current-year Norway spruce needles with respect to tree age and crown position.

Material and Methods

Three-year-old spruce seedlings were planted in 1998 into 70 l containers (20 seedlings per container) containing a natural forest soil (see for details GRAMS & al. 2002). In spring 1999 five containers each were transferred into the upper sun (22 m height) and shade crown (17 m height) at the "free-air ozone fumigation plot" at Kranzberg Forest near Munich, Germany (HÄBERLE & al. 1999, KARNOSKY & al. 2001, WERNER & FABIAN 2002). Seedlings were watered regularly in order to avoid soil water stress.

For the assessment of antioxidants current-year needles were sampled under uniform light conditions at both canopy positions from five trees per age class (in the case of seedlings 1 per container) in September 1999. Reduced ascorbate and dehydroascorbate were quantified on lyophilized needle material as described by TAUSZ & al. 1996 and related to needle dry weight. In order to focus on the combined effect of O_3 uptake and detoxification capacity, antioxidant levels were also related to total needle surface area.

Measurements of needle gas exchange were performed in situ on neighboring twigs in all study trees by means of a portable gas exchange system (LCA3; ADC, Hoddesdon, UK) equipped with a PLC(C) leaf chamber. Gas exchange parameters were calculated according to VON CAEMMERER & FARQUHAR 1981 and related to total needle surface area estimated with glass beads (THOMPSON & LEYTON 1971).

Finally, 100-needle dry weight and specific leaf area were determined from the twigs used for the assessment of needle gas exchange. Accompanying measurements performed immediately before harvest indicated that there were no significant differences in needle water potential between seedlings and adult trees, neither in the shade-, nor in the sun-crown. Corresponding means for the sun- and the shade-crown were -1.6 ± 0.1 MPa and -1.6 ± 0.2 MPa, respectively. Predawn measurements assessed in the trees the day after harvest averaged -1.2 ± 0.2 MPa in the sun-crown and -1.3 ± 0.2 MPa in the shade-crown.

Differences between crown positions and tree age classes were evaluated using analysis of variance (ANOVA) followed by post-hoc comparisons according to the Duncan's LSD-test.

Results and Discussion

In both, seedlings and adult trees apparent net photosynthesis and stomatal conductance to water vapor were higher in the sun- than in the shade-crown, while the opposite was found for the CO_2 concentration in the mesophyll internal air spaces (Table 1). These observed differences in the photosynthetic machinery between the sun- and the shade-crown can mainly be attributed to differences in irradiance (PPFD) during the measurement period (sun-crown 1500 ± 200 µmol m^{-2} s^{-1}, shade-crown and 56 ± 19 µmol m^{-2} s^{-1}). Air and needle temperature as well as water vapor pressure deficit, by contrast, did not differ significantly between the two canopy positions (data not shown). Age dependent differences however, were not statistically significant, neither in the sun- nor in the shade-crown (Table 1).

Table 1. Apparent net photosynthesis (Pn), stomatal conductance to water vapor (g_{H2O}), CO_2 concentration in the mesophyll internal air spaces (Ci) in current-year needles of seedling and adult *Picea abies* trees in response to canopy location. Data are means of 5 trees ± SD. Values with different letters indicate significant differences at P < 0.05.

Position	Pn [µmol m^{-2} s^{-1}]	g_{H2O} [mmol m^{-2} s^{-1}]	Ci [µmol mol-1]
Sun-crown			
seedling	3.43±1.0a	65.4±10.2a	268±12a
adult tree	3.02±4.4a	50.4±11.2a	253±11a
Shade-crown			
seedling	0.001±0.002b	28.6±12.5b	339±21b
adult tree	0.08±0.24b	15.8±4.5b	349±0b

Data obtained from current-year needles also demonstrated significant differences in needle morphology with respect to crown position and tree age. In both seedlings and adult canopy trees 100-needle dry weight was significantly higher in the sun- than in the shade-crown, while the opposite was found for specific leaf area (Table 2). Nevertheless, even though their light environment was the same, seedling needles had a significantly lower 100-needle dry weight and a higher specific leaf area than needles of adult canopy trees (Table 2). Similar age dependent changes in the morphology were also observed in sun exposed needles of seedling, sapling, and adult *Picea abies* trees (TEGISCHER & al. 2002), and thus strongly suggesting a development from a kind of shade-type towards a more sun-type foliage with increasing tree age and size (WIESER & al. 2002).

Such age related differences in needle morphology probably may also influence the detoxification capacity under ambient pollution impact. However, there were no significant differences between *Picea abies* trees of different age in whole needle ascorbate contents when based on the needle dry weight neither in the sun- (TEGISCHER & al. 2002, WIESER & al. 2002) nor in the shade-crown (Fig. 2). Fur-

thermore, the redox state of the ascorbate pool (reduced ascorbate expressed as % of the sum of ascorbate and dehydroascorbate) also remained constant and was 70 ± 10 % in all the tree age classes investigated.

Table 2. 100-needle dry weight and specific leaf area of current-year needles of seedling and adult *Picea abies* trees in response to canopy location. Data are means of 5 trees ± SD. Values with different letters indicate significant differences at P < 0.05.

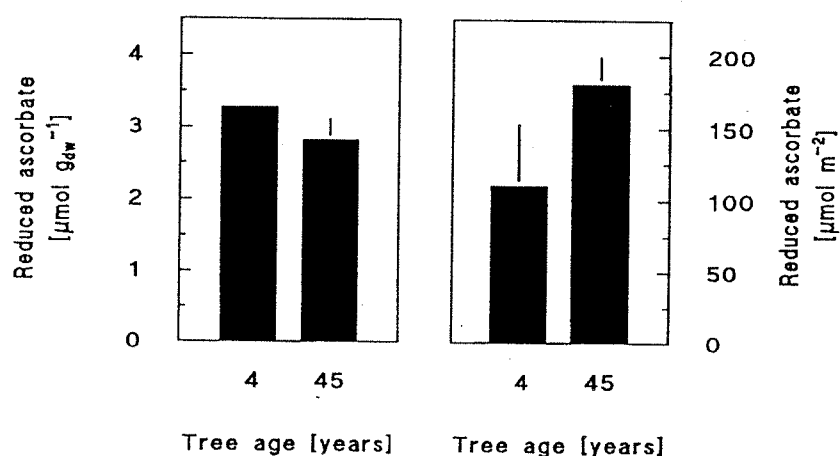| Position	100-needle dry weight [mg]	Specific leaf area [$cm^2\ g^{-1}$]
Sun-crown		
seedling	70±16 a	174±24 a
adult tree	331±68 b	86±8 b
Shade-crown		
seedling	41±4 a	298±53 c
adult tree	279±85 bc	156±27 d

Fig. 2. Reduced ascorbate related to needle dry weight (left) and total needle surface area (right) in current-year needles of 4 and 45-year old *Picea abies* trees in the shade crown at 17 tree height. Data are means of 5 trees ± SD

How ontogeny modifies tree response to O_3 is not known and the short-coming in scaling O_3 effects from seedlings to adult trees and from chamber to stand conditions is well documented (HOGSETT & ANDERSSEN 1998). Results obtained in *Picea abies* (WIESER & al. 2002, Fig. 1) and *Fagus sylvatica* (BAUMGARTEN & al. 2000) clearly indicate that variations in the ontogenetic response are not sufficiently described by cumulative O_3 uptake and O_3 flux, respectively. As the threshold O_3 flux inducing statistically significant effects on net photosynthesis in *Picea abies* approached 2.14 to 2.45 nmol m^{-2} s^{-1} per unit of total needle surface area for an exposure duration ≥ 70 days and ≥ 23 in adult trees and seedlings, respectively several other factors might be important in determining symptom expression including mechanisms of maintenance and repair as well as

antioxidant defense mechanisms (WIESER & al. 2002). Ascorbate as the major cellular antioxidant in the aqueous phase is a good representative for these mechanisms (SMIRNOFF 1996).

However, the quantification of O_3 detoxification rates is difficult. Model calculations estimated the role of apoplastic ascorbate as a first line of defense resulting in between 20 and 60 % detoxification of O_3 in herbaceous plants (ZHENG & al. 2000). When apoplastic ascorbate scavenges O_3, dehydroascorbate has to be re-translocated to the symplast and regenerated therein (HOREMANS & al. 2000). This transport and/or the glutathione-dependent ascorbate regeneration may be the rate-limiting step, as suggested in a study on *Fagus* (LUWE 1996). Furthermore, evidence on the degree of protection afforded by extracellular ascorbate seems still inconclusive (JAKOB & HEBER 1998, TURCSÁNY & al. 2000). Since in a recent study total leaf ascorbate was nearly as good a predictor for ozone resistance as the apoplastic fraction (ZHENG & al. 2000), we decided to use total needle ascorbate per unit needle surface area as an approximate metric for potential detoxification capacity.

In sun-exposed needles, the content of reduced ascorbate per unit needle surface area was significantly lower in younger trees compared to older ones (TEGISCHER & al. 2002). Pollutant uptake in the shade-crown may contribute considerably to whole tree uptake (WIESER & al. 2000), and defense capacities may be even lower in shade-crown (POLLE 1997). Our results showed that shade-crown needles of young trees also contained less ascorbate per unit needle surface area than needles from adult trees (Fig. 2). In addition, there were no statistically significant differences in stomatal conductance to water vapor between seedlings and adult trees, neither in the shade- nor in the sun-crown (cf. Table 1). Thus, if we assume that significant effects on net photosynthesis only occur above a threshold O_3 flux of 2.45 nmol m^{-2} s^{-1} per unit of total needle surface area in seedlings and adult trees, respectively (WIESER & al. 2002), the amount of potentially available ascorbate per nmol O_3 taken up per second tended to increase with tree age in the shade- (Fig. 3) and in the sun-crown (WIESER & al. 2002). At an average the amount of potentially available ascorbate per nmol O_3 taken up was 90 % lower in the shade- than in the sun-crown (WIESER & al. 2002) and thus suggesting a higher risk of O_3 damage in the shade-crown of *Picea abies* trees.

These data strongly suggest a higher susceptibility to O_3 in seedlings as compared to adult ones, a fact that could not be observed when needle dry-weight based antioxidant concentrations were compared. However, it is important to emphasize that these examples make no provision for the effects of O_3 on the contents of antioxidants *per se*!

In conclusion, the knowledge of O_3 uptake is essential to predict age- and size-related differences in the response to O_3. But information about the amount of available antioxidants per unit leaf area and their age related changes with tree age and size may be of equal importance in the development of air quality standards. Although our approach (cf. also WIESER & al. 2002) strongly suggests that age related differences in O_3 susceptibility of *Picea abies* can be related to differences

in the amount of potential available antioxidants, there is a further need to examine age related differences in the response to O_3 in other forest tree species.
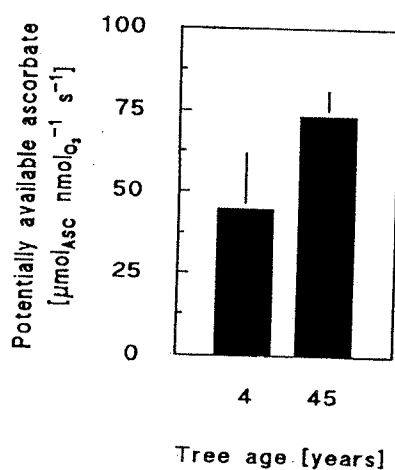

Fig. 3. The amount of potentially available ascorbate per unit of O_3 taken up at the threshold flux of 2.45 nmol m^2 s^{-1} eliciting significant effects on the maximum net photosynthesis under natural conditions in current-year needles of 4 and 45-year old *Picea abies* trees in the shade crown at 17 tree height. Data are means of 5 trees ± SD.

Acknowledgements

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