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Effects of elevated pO_3 on carbon cycle between above and belowground organs of trees

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Abstract: Translocation of carbohydrate from leaves to roots via phloem and reallocation from roots to leaves via xylem regulate the allocation of carbon (C) between above and belowground organs of trees. To quantitatively analyze effects of elevated ozone concentrations pO_3 on the internal cycle of C, juvenile beech and spruce were grown in phytotrons and exposed to ambient and elevated pO_3 (i.e. twice-ambient O_3 levels, restricted to < 150 ppb) for two growing seasons. The translocation of C in the phloem and xylem was quantitatively studied by investigating the phloem/xylem-loading of sugars, the differentiation of stem conductive tissue and the hourly water flow through the stem. Results in the present study shown, elevated pO_3 significantly decreased C translocation from shoot to roots in beech by reducing both sugar concentration in the phloem and conductive phloem area. Elevated pO_3 also significantly decreased C reallocation from the roots to the shoot in beech by reducing both of sugar concentration in the xylem and transpiration rate. The adverse effects of elevated pO_3 on C translocation in the phloem and xylem, however, were small in spruce. Contrasting to beech, spruce is less sensitive to elevated pO_3, regarding to phloem differentiation and sugar concentrations in the phloem and xylem.

Keywords: beech; carbon cycle; elevated pO_3; phloem; spruce; xylem

Introduction

Plant leaves fix carbon dioxide (CO_2) from the atmosphere by the enzyme ribulose-1,5-bisphosphate-carboxylase/oxygenase and build carbohydrates as primary products of photosynthesis. Since the photosynthates will be needed not only in foliage, but also for respiration, growth and development of other plant organs (Kozlowski *et al.*, 1991), internal availability of carbohydrates depends on an efficient regulation between different organs and tissue. Here, the long-distance transport of sugars (the main form of carbohydrate transport) in the phloem and xylem plays a central role for regulation of carbon (C) allocation between above and belowground organs in trees (Heizmann *et al.*, 2001). The cycling of C, from leaves to roots via the phloem and from the roots to the leaves via the xylem, maybe also controls leaf photosynthesis and C metabolites (Farrar and Williams, 1991; Chiou and Bush, 1998). A quantitative investigation on C translocation between shoot and roots in plants, however, is litter reported.

Ozone (O_3) is in low concentration stets natural composition of the atmosphere. O_3 in the troposphere mainly originates from photochemical reactions of volatile organic compounds (VOCs) with nitrogen oxides (NOx) released from anthropogenic and natural sources (Stockwell *et al.*, 1997). During the past century, the concentration of O_3 (pO_3) in the troposphere near the ground has increased in the northern hemisphere to currently by a factor of about two to four times (Stockwell *et al.*, 1997). High pO_3 is considered one of the most detrimental environmental factors for plant growth and development (Rennenberg *et al.*, 1996a; Matyssek and Innes, 1999). High pO_3 can not only affect CO_2 uptake by altered stomatal conductance, but also dark and light reactions of photosynthesis, by reducing the activity and concentration of Rubisco as well as the light reaction efficiency (Grams *et al.*, 1999; Matyssek and Innes, 1999). Thereby, elevated pO_3 contributes to a decrease in photosynthesis and assimilate production(Lux *et al.*, 1997; Matyssek and Innes, 1999). On the other hand, O_3-induced membrane injury and collapse of mesophyll as well as phloem cells may restrict sugar phloem-loading and assimilate transport out of the leaves, because a decrease in carbohydrate contents of belowground organs was often observed (Rennenberg *et al.*, 1996b; Grantz and Farrar, 1999; Andersen, 2003; Liu *et al.*, 2004). However, effects of elevated pO_3 on C translocation in the phloem and xylem of plants remain unclearly.

In the present study, we quantitatively analyzed the effects of elevated pO_3 on C cycle between above and belowground organs of trees. For this aim, translocation rate of C in the phloem and xylem was calculated through the concentrations of sugars in the phloem exudates and xylem sap, C transport velocity, the area of stem conductive tissue as well as transpiration rate of the whole plants. Beech (*Fagus sylvatica* L.) and spruce (*Picea abies* L. Karst.), the most important tree species in Central Europe with different sensitivity to elevated pO_3 (Skaerby *et al.* 1998; Matyssek and Innes, 1999), were chosen for the

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experiments.

1 Materials and methods

1.1 Plants and treatments

2- and 3-year-old seedlings of beech and spruce (about 0.2 m high) were planted in May 1998 in containers (0.70 m × 0.40 m × 0.30 m, volume=84 L) filled with natural forest soil, arranged in rows of 4 × 5 individuals. In mid-April 1999, the containers were transferred to the walk-in phytotrons (2.8 m × 3.4 m) of the National Research Centre for Environment and Health (GSF) near Munich, Germany. For the following two growing seasons of 1999 and 2000, the plants were exposed to ambient pCO_3 and elevated pO_3 (i.e. twice-ambient pO_3 levels, maximum restricted to < 150 ppb). In the phytotrons, monthly mean day values of photosynthetic photo flux were about 430—480 μmol/(m^2·s); monthly mean air temperature and relative humidity amounted to about 18—20℃ and 57%—63% at day, 13—15℃ and 77% —84% at night. These climate conditions correspond to those of an air quality study at the Kranzberg Forest near Freising Germany (Pretzsch *et al.*, 1998). Liquid fertilizer (partial strength Hoagland solution) was added five times during each growing season to ensure a non-limiting nutrient support in the containers (Liu *et al.*, 2004).

1.2 Assessment of transpiration

Leaf gas exchanges of the trees were measured using porometry (HCM-1000 CO_2/H_2O diffusion porometer, H. Walz, Effeltrich, Germany) during the period from August 24 to 31, 2000 (total 189.7 h). Transpiration rate was calculated on the basis of the transpiratory water loss of leaves. By relating the leaf area-based transpiration rate to the foliage area of the whole plant, mean transpiration rate of the whole-tree (n=5—6) was calculated to assess C translocation through the stem xylem.

1.3 Collection of xylem sap

Shoots of 8—10 individuals of beech or spruce from each treatment were cut directly above the soil. Small pieces of bark and cambium were removed from the cut end. The stripped end was rinsed thoroughly with double-distilled water in order to diminish contamination with cellular constituents. The shoot was fitted into a pressure chamber (Soilmoisture, Santa Barbara, USA). By rising the pressure in the chamber, the xylem sap was collected (The first protruding solution was discarded), immediately frozen in liquid N_2, and stored at -80℃ until biochemical analysis.

1.4 Collection of phloem exudates

Phloem exudates were collected by the modification of the EDTA-technique (King and Zeevart, 1974) described by Rennenberg *et al.* (1996b). During the collection of xylem sap, small bark pieces of beech and spruce were removed from the shoot cut end of the harvested trees (n=8—10). After determination of fresh weight, the pieces were placed in exudation solution and incubated at room temperature for 5 h. The phloem exudates were frozen in liquid N_2 and stored at -80℃ until biochemical analysis.

1.5 Analysis of sugar concentrations

Sugar concentrations of the xylem sap and phloem exudates were measured colorimetrically after derivation with Anthrone reagent at 578 nm (Carroll *et al.*, 1956). Glucose was used as a standard.

1.6 Determination of transport velocity of sugars in the phloem

Labeled ^{14}C-sucrose (Moravek Biochemicals, Richland, California, USA; specific radioactivity 492 mCi/mmol) was fed directly into the stem phloem of 5—6 potted beech and spruce using the "bark flap technique" (Rennenberg *et al.*, 1996b). After incubation, bark segments of the stem below the fed point at a length of 1 cm were removed from the stem basis to apex, and immediately transferred into scintillation vials containing 2 ml of 20 mmol/L EDTA. After 24 h, bark segments were removed out of the vials, 4 ml scintillation fluid (OptiPhase Hisafe 2, Wallac Oy, Finland) were added into each vial. ^{14}C-radioactivity of the solutions was measured using a liquid scintillation counter (Wallac System 1409, Wallac Oy, Turku, Finland). Samples were counted at 65% —90% efficiency and corrected for quenching. Transport velocity of sugars in the phloem was calculated through the front position of labeled sucrose and incubation time.

1.7 Determination of conductive phloem area

Since only living phloem element can transport metabolites, area of active phloem of cross-sectional bark tissue was microscopically determined. At the harvest, stem segments of beech or spruce (n=8—10) were cut off directly over the soil, transferred into the fix solution containing buffered 4% glutaraldehyde, infiltrated by an embedding methacrylate monomer, and polymerized at 55℃ for 24 h. The embedded samples were sectioned at 3 μm using a rotary microtome (Leica 2040 Supercut, Leica, Germany) and stained with water soluble aniline blue (Currier and Strugger, 1956). The microsections were observed for autofluorescence by means of a fluorescence microscope (Axiophot, Zeiss, Germany) equipped with the filter combination (BP 395-440, FT 460 and LP 470). The area of conductive phloem was calculated by microscopic measurement of the distances between cambium and sieve elements closed by callus (Liu, 2003):

$$\text{Conductive phloem area} = \pi (R - W_{\text{bark}} + W_{\text{phloem}})^2 - \pi (R - W_{\text{bark}})^2$$

$$\pi = 3.1416$$

where R is the mean radius of stem segments of each treatment (n = 5—6); W_{bark} is the width of cross-sectional bark tissue (mm) (n = 8—10); W_{phloem} is the width of conductive phloem (mm) (n = 30—50).

1.8 Calculation of translocation rate in the phloem

Translocation rate of sugars in the phloem of beech and spruce was calculated with following equation:

$$T_{phloem} = C_p \times V \times A \times D/P \ (\mu mol\ glucose/(h \cdot plant))$$

where, C_p is the sugar concentration in the phloem exudates (μmol glucose/g bark fw); V is the transport velocity of sugars in the phloem (cm/h); A is the area of conductive phloem (cm^2); D is the density of fresh bark tissue (g/cm^3; supposed: $D \approx 1$); P is the percentage of conductive phloem area on cross-sectional bark area (%).

Translocation rate of sugars in the xylem of beech and spruce was calculated with following equation:

$$T_{xylem} = C_x \times T_s \ (\mu mol\ (glucose)/(h \cdot plant))$$

where, C_x is the concentration of sugars in xylem sap (μmol glucose/ml xylem sap); T_s is the mean transpiration rate of the whole plant (ml H_2O/(h·plant)).

1.9 Data analysis

Statistical analysis was performed using SPSS 10.0 software program (SPSS Science, Chicago, IL). Two way analysis of variance (ANOVA) was used to test the effects of elevated pO_3 on the rate of transpiration, the concentrations of sugars in the phloem exudates and xylem sap, the area of conductive phloem, and the rate of translocation. If significant differences were found, Tukey's multiple comparisons were used to determine the difference between treatments. The differences were considered significant at $P < 0.05$.

2 Results and discussion

2.1 Sugar concentrations in the phloem exudates and xylem sap

As shown in Fig.1a, sugar concentration (expressed on a bark fresh-weight basis) in the phloem exudates of beech decreased significantly compared to control. This result supports the opinion that elevated pO_3 limited sugar phloem-loading of plants (Rennenberg *et al.*, 1996a; Grantz and Farrar 1999; Andersen, 2003). Contrasting to the findings in beech phloem, sugar concentration in the phloem exudates of spruce under elevated pO_3 tended to a small increase.

In the xylem sap of beech, sugar concentration was reduced significantly by elevated pO_3 resembled in the phloem exudates (Fig.1b). This could mean that limited C allocation from leaves to roots reduced carbohydrate contents in roots (Grantz and Farrar, 1999; Liu *et al.*, 2004), resulting in a reduced availability of re-allocable C in the roots which may generate a decrease xylem-loading of sugar. In spruce, however, sugar concentration in the xylem sap tended to be increased by elevated pO_3 like in the phloem exudates. The resemblances of response in sugar concentrations between the phloem and xylem in beech and spruce may mirror the close coupling of assimilate transport between the xylem and phloem (Sauter and Witt, 1997; Heizmann *et al.*, 2001). The differences of response in sugar concentrations between beech and spruce indicated that beech is more sensitive to elevated pO_3 than spruce, the findings were identical with previous studies (Skärby *et al.*, 1998; Grams *et al.*, 1999; Matyssek and Innes 1999; Liu *et al.*, 2004).

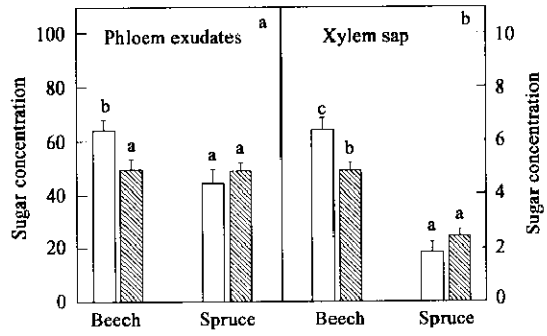

Fig.1 Concentration of sugars in the phloem exudates (a) (μmol glucose/g bark fw) and in the xylem sap (b) (μmol glucose/ml xylem sap) of beech and spruce exposed to elevated pO_3 (means ±SE for n=8—10, ANOVA)

Open bars, ambient pO_3; grey bars, elevated pO_3; divergent lettering indicates statistically significant differences at P<0.05 (ANOVA); molecular mass of glucose is 180.2 g/mol

2.2 Area of conductive phloem

In beech stem, the area of conductive phloem was decreased significantly by elevated pO_3 (Fig.2), because an appreciable number of sieve elements were blocked by callus than in ambient air (Liu, 2003). In spruce as in beech, conductive phloem area under elevated pO_3 was also smaller than control. Thereby, the conductive functionality of the phloem can be constrained by callus deposition in the sieve elements (Polle *et al.*, 2000; Matyssek and Sandermann, 2003). This may adjust, in the phloem, the translocation capacity to restrict assimilate delivery from the foliage (Matyssek *et al.*, 2002). The limiting effects of elevated pO_3 on the width of conductive phloem tissue in trees were consistent with previous findings (Guenthardt-Goerg *et al.*, 1993; Matyssek *et al.*, 2002).

2.3 Transport velocity of sugars in the phloem

Transport velocity of sugars in the phloem varied with tree species. In spruce phloem, sugar transport velocity was significant higher than in beech phloem. The downward transport velocity in beech phloem amounted to (32.0 ± 2.9) cm/h and in spruce phloem

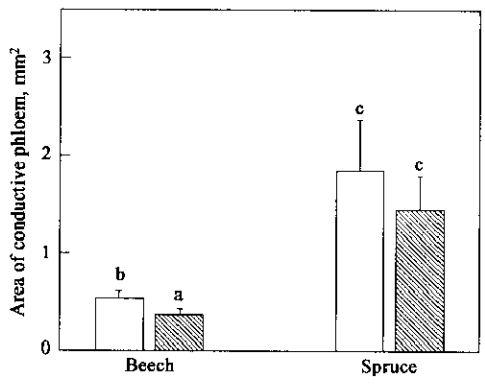

Fig.2 Effects of elevated pO_3 on the area of conductive phloem of beech and spruce stem
Open bars. ambient pO_3; grey bars. elevated pO_3; divergent lettering indicates statistically significant differences at $P<0.05$ (means $\pm SE$ for $n = 5 - 6$, ANOVA)

to 81.6 ± 9.0 cm/h (mean ± *SD* for $n = 5—6$).

2.4 Transpiration rate

In beech, the mean hourly water flow through the stem xylem per plant was decreased by elevated pO_3 compared to gaseous control regime (Fig.3), because elevated pO_3 affected water loss of leaves (transpiration rate) by reducing stomatal conductance and, even generated a visible injury to leaves (Grams *et al.*, 1999). Remarkably, transpiration rate of spruce under elevated pO_3 was increased clearly contrasted to beech (Fig.3). Although spruce often displays much lower sensitivity to pO_3 (Skärby *et al.*, 1998; Matyssek and Innes, 1999; Liu *et al.*, 2004), physiological reasons for the response in transpiration rate can not be interpreted.

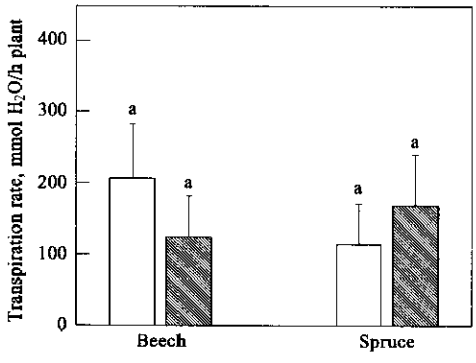

Fig.3 Effects of elevated pO_3 on transpiration rate of the whole plant of beech and spruce
Transpiration rate per leaf area (cm^2) was measured on the basis of the transpiratory water loss of leaves; by relating the leaf area-based transpiration rate to the foliage area of the whole plant, mean transpiration rate of the whole-tree (n=5—6) was calculated; open bars. ambient pO_3; grey bars. elevated pO_3

2.5 Translocation rate of sugars in the phloem and xylem

In beech phloem, the mean hourly translocation rate of sugars per plant was significantly reduced by elevated pO_3 (Fig.4a), which was consistent with negative responses of both sugar concentration in the phloem and conductive phloem area. Consequently, carbohydrates apparently missed in belowground organs of plants (Grantz and Farrar, 1999; Anderson, 2003; Liu *et al.*, 2004). In spruce phloem, however, sugar translocation rate stayed at the level of control (Fig.4a). The downward translocation in spruce was impacted more by changes in conductive phloem area.

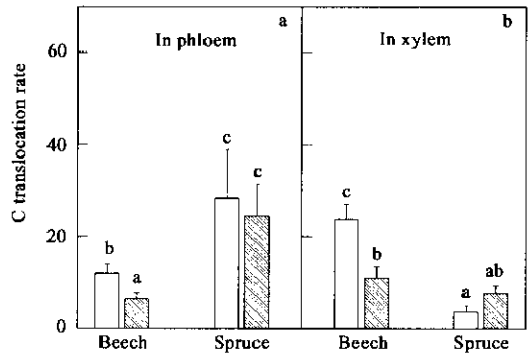

Fig.4 C translocation rate (μmol glucose/ (h·plant)) in the phloem (a) and xylem (b) of beech and spruce exposed to ambient or elevated pO_3 (means $\pm SE$ for n=5—6, ANOVA)
Translocation rate in phloem was quantified by measuring (1) the concentration of sugars in the phloem exudates, (2) transport velocity of sugars in the phloem, (3) conductive phloem area of stem and its percentage on the bark cross-sectional area. Translocation rate in xylem was calculated using the rate of transpiration and the concentration of sugars in xylem sap; open bars. ambient pO_3; grey bars. elevated pO_3; divergent lettering indicates statistically significant differences at $P<0.05$ (ANOVA); molecular mass of glucose is 180.2 g/mol

In beech xylem, mean hourly translocation rate of sugars per plant under elevated pO_3 was significantly lower than in ambient air (Fig.4b), resembled in the phloem. In contrast, the upward translocation rate in spruce xylem was enhanced distinctly by elevated pO_3. The changes in C translocation rate in the xylem of both tree species were consistent with responses of both sugar concentrations and transpiration rate (Matyssek and Sandermann, 2003). As soluble sugar contents in roots, under elevated pO_3, were significantly reduced in beech, but remained in spruce at the level of control (Liu *et al.*, 2004), the responses in C translocation rate of beech and spruce also reflected the availability of re-allocable C in roots.

3 Conclusions

In the present study, effects of elevated pO_3 on C cycle via the phloem and xylem of beech and spruce were investigated quantitatively. Elevated pO_3 significantly reduced sugar concentrations in the phloem exudates of beech, and constrained the conductive functionality of the phloem by inducing callus deposition in the sieve elements, resulting in a decreased area of conductive phloem. Thereby, C translocation from leaves to roots via the phloem was significantly reduced in beech. Elevated pO_3 also significantly reduced C translocation from the roots to

the shoot via the xylem of beech by reducing the both of sugar concentration in xylem sap and transpiration rate of the whole plant.

In spruce, elevated pO_3 had minor effects on both of sugar concentration in the phloem exudates and phloem differentiation. Hence, C translocation rate from shoot to roots via the phloem stayed at the levels of control. C translocation from the roots to the shoot via the xylem tended to be clearly increased by elevated pO_3 due to the increases in both of sugar concentration in the xylem sap and water flow through the stem xylem (transpiration rate).

From these results it is concluded that elevated pO_3 significantly reduced C cycle between above and belowground organs of beech. Spruce resembled beech in the response pattern of C translocation rate in the phloem to elevated pO_3. Spruce, however, contrasted with beech in terms of the response patterns of C translocation rate in the xylem. Therefore, effects of elevated pO_3 on C cycle between above and belowground organs varied with tree species. Contrasting to beech, spruce was less sensitive to the elevated pO_3, regarding the phloem differentiation and the concentration of sugars in the phloem and xylem.

References:

Andersen C P, 2003. Source-sink balance and carbon allocation below ground in plants exposed to ozone [J]. New Phytol, 157: 213—228.

Carroll N V, Longley R W, Roe J H, 1956. The determination of glycogen in liver and muscle by use of anthrone reagent[J]. J Biol Chem, 220: 583—593.

Chiou T J, Bush D R, 1998. Sucrose is a signal molecule in assimilate partitioning[J]. Plant Biol, 95(8): 4784—4788.

Currier H B, Strugger S, 1956. Aniline blue and fluorescence microscopy of callus in bulb scales of *Allium cepa* L [J]. Protoplasma, 45: 552—559.

Farrar J F, Williams M L, 1991. The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source-sink relations and respiration [J]. Plant, Cell and Environ, 14: 819—830.

Grams T E E, Anegg S, Haeberle K H *et al.*, 1999. Interactions of chronic exposure to elevated CO_2 and O_3 levels in the photosynthetic light and dark reactions of European beech (*Fagus sylvatica* L.)[J]. New Phytol, 144: 95—107.

Grantz D A, Farrar J F, 1999. Acute exposure to ozone inhibits rapid carbon translocation from source leaves of Pima cotton [J]. J Experim Botany, 50: 1253—1262.

Guenthardt-Goerg M S, Matyssek R, Scheidegger C *et al.* 1993. Differentiation and structural decline in the leaves and bark of birch (*Betula pendula*) under low ozone concentrations[J]. Trees, 7: 104—114.

Heizmann U, Kreuzwieser J, Schnitzler J P *et al.*, 2001. Assimilate transport in the xylem sap of pedunculate oak (*Quercus robur*) saplings[J]. Plant Biol, 3: 132—138.

King R W, Zeevart A D, 1974. Enhancement of phloem exudation from cut petioles by chelating agents[J]. Plant Physiol, 53: 96—103.

Kozlowski T T, Kramer P J, Pallardy S G, 1991. The physiological ecology of woody plants[M]. San Diego: Academic Press.

Liu X P, 2003. Regulation of C, N and S allocation in beech and spruce in pure and mixed culture under elevated pCO_2 and/or O_3[D]. Dissertation of University Freiburg. Wissenschafts-Verlag, Germany.

Liu X P, Kozovits A R, Grams T E E *et al.*, 2004. Competition modifies effects of enhanced ozone/carbon dioxide regimes on the carbohydrate and biomass partitioning in juvenile spruce and beech[J]. Tree Physiol, 24: 1045—1055.

Liu X P, Grams T E E, Matyssek R *et al.*, 2005. Effects of elevated pCO_2 and/or pO_3 on C-, N- and S-metabolites in the leaves of juvenile beech and spruce differ between trees grown in monoculture and mixed culture [J]. Plant Physiol Biochem, 43: 147—154.

Lux D, Leonard S, Mueller J *et al.*, 1997. Effects of ambient ozone concentrations on contents of non-structural carbohydrates in young *Picea abies* and *Fagus sylvatica* [J]. New Phytol, 137: 399—409.

Matyssek R, Guenthardt-Goerg M S, Maurer S *et al.*, 2002. Tissue structure and respiration of stems of *Betula pendula* under contrasting ozone exposure and nutrition [J]. Trees, 16: 375—385.

Matyssek R, Innes J L, 1999. Ozone—a risk factor for trees and forests in Europe? [J]. Water Air Soil Pollut, 116: 199—226.

Matyssek R, Sandermann H, 2003. Impact of ozone on trees: an ecophysiological perspective[J]. Prog Botany, 64: 349—404.

Polle A, Matyssek R, Guenthardt-Goerg M S *et al.*, 2000. Defense strategies against ozone in trees: the role of nutrition [M]. In: Plant responses to environmental pollution (Agrawal S. B., Agrawal. M., ed.). New York: Boca Raton. 223—245.

Pretzsch H, Kahn M, Grote R, 1998. The mixed spruce-beech forest stands of the "Sonderforschungsbereich" "Growth or Parasite Defence?" in the forest district Kranzberger Forest [J]. Forstwiss Centralbl, 117: 241—257.

Rennenberg H, Herschbach C, Polle A, 1996a. Consequences of air pollution on shoot-root interactions [J]. J Plant Physiol, 148: 296—301.

Rennenberg H, Schneider S, Weber P, 1996b. Analysis of uptake and allocation of nitrogen and sulphur compounds by trees in the field [J]. J Experim Botany, 47: 1491—1498.

Sauter J, Witt W, 1997. Structure and function of rays: storage, mobilisation, transport [M]. In: Trees-contributions to modern tree physiology (Rennenberg H., Escherich W., Ziegler H., ed.). Leiden: Backhuys Publ. 177—195.

Skaerby L, Ro-Poulsen H, Wellburn F A M *et al.*, 1998. Impacts of ozone on forests: a European perspective[J]. New Phytol, 139: 109—122.

Stockwell W R, Kramm G, Scheel H E *et al.*, 1997. Ozone formation, destruction and exposure in Europe and the United States[M]. In: Forest decline and ozone (Sandermann H., Wellburn A. R., Heath R. L., ed.). Berlin, Germany: Springer. 1—32.

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