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REGULAR ARTICLE

Effects of ozone and *Phytophthora citricola* on non-structural carbohydrates of European beech (*Fagus sylvatica*) saplings

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Abstract A lysimeter study was performed to monitor long term effects of chronic ozone enrichment on saplings of European beech (*Fagus sylvatica* L). After 3 years of ozone exposure a root infection with *Phytophthora citricola* Swada was established in the fourth year to study the interaction between elevated ozone and the root infection on the carbon budget of beech saplings. By using quantitative PCR no differences in root infection with *P. citricola* were observed between the ozone treatments. In contrast to the first 3 years of ozone exposure, sucrose and starch concentrations in leaves were diminished in ozone treated plants in the fourth year. The root infection reduced sucrose concentrations in leaves. Starch reserves of the heterotrophic biomass were not affected by any treatments. Thus 4 years of ozone exposure and 1 year of *P. citricola* root infection had only limited effect on carbohydrate metabolism in beech saplings.

Keywords Fagus sylvatica · Lysimeter · Non-structural carbohydrates · *Phytophthora citricola* · Starch · Sucrose

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Introduction

In times of climate change it becomes more and more important to study its effect on plant–parasite interaction. This applies in particular to long life trees susceptible to certain pests and diseases. A secondary action of climate change is the increase in tropospheric ozone concentrations. Several models predict a substantial increase in surface ozone concentrations for the northern hemisphere (Meleux et al. 2007; Racherla and Adams 2008; Zeng et al. 2008). According to these models the main factors responsible for increasing ozone levels are enhanced emissions of soil-NO_x as well as of biogenic isoprene due to higher temperatures in combination with altered solar irradiation. Ozone is considered to be one of the most phytotoxic air pollutants (Paoletti et al. 2007; Skärby et al. 1998). The primary ozone damage in plants takes place in the leaf apoplast, where ozone enters through the stomata. Amongst

others, ozone affects the primary carbon metabolism of plants, e.g. the reduction of net primary production due to stomatal closure and / or damage of the leaf mesophyll (King et al. 2005), changes in the source-sink balance of plants (Andersen 2003), and the reduction of carbon allocation to roots (Samuelson and Kelly 2001). In mature beech trees a chronic elevated ozone exposure reduced both sucrose and starch concentrations in sun leaves (Blumenröther et al. 2007). The response of shade leaves to elevated ozone was not consistent between 2 years with contrasting climatic conditions.

The interaction of ozone with various plant diseases was summarized by Manning and v. Tiedemann (1995). In general, severity of bacterial diseases was reduced under elevated ozone, while necrotrophic leaf pathogens were favoured. However, a weak and transient reduction of *Apiognomonia errabunda* infection in beech leaves exposed to elevated ozone was reported (Bahnweg et al. 2005). The severity of several soilborne diseases of forest trees increased under ozone exposure (Fenn et al. 1990; Leininger et al. 1990; Manning and v. Tiedemann 1995), possibly as a consequence of a reduced carbon allocation to below ground biomass under elevated ozone (Andersen 2003; Samuelson and Kelly 2001).

The oomycete *Phytophthora citricola* Swada causes root and to some extent collar rot on several deciduous tree species such as oak (Jung et al. 2000) and beech (Jung et al. 2005). While mature trees and older saplings suffer a slow decline over several years, 4 months old seedlings of Fagus sylvatica were killed by the pathogen within a couple of days (Fleischmann et al. 2002, 2005). The susceptibility of beech seedlings decreases fast during ontogeny. Three year old seedlings tolerate a *P. citricola* infection for two vegetation periods (Fleischmann et al., submitted). These investigations showed that *P. citricola* transiently reduced carbon assimilation of beech. Furthermore, *P. citricola* infection diminished the uptake of mineral nutrients as well as the nutrient status of leaves (Fleischmann et al. 2004; Wang et al. 2003).

At the Helmholtz Zentrum München a lysimeter experiment was conducted since 2003 to study the impact of chronic ozone exposure on European beech saplings (Pritsch et al. 2008; Schloter et al. 2005). In addition, inoculation with the root pathogen *P. citricola* was performed in 2006 to investigate possible interactions between ozone and the plant disease. Our investigations focused on the effects of ozone and *P. citricola* root infection on non-structural carbohydrates (NSC) of beech. The NSC pool of beech consists mainly of the polysaccharide starch, the disaccharide sucrose, the monosaccharides glucose and fructose, and to a low extent the oligosaccharides raffinose and stachyose as well as the cyclic inositol (Hoch et al. 2003). In this present study we tested the hypotheses, that (1) carbon allocation to roots is reduced under elevated ozone; (2) the susceptibility of beech towards the root pathogen *P. citricola* increases under elevated ozone, as less carbon resources are available for plant defence reactions.

Materials and methods

Experimental design of the lysimeter study

The experimental setup of the beech—forest soil lysimeters including the free-air ozone exposure device was described in detail by Winkler et al. (2009a) as well as by (Pritsch et al. 2008) and (Schloter et al. 2005).

An overview about the experimental procedures is given in Fig. 1. In brief, eight lysimeters with a surface of 1 m^2 were filled with natural forest soil in 1999. In autumn 2002 each lysimeter was planted with four 3-year-old *Fagus sylvatica* L. saplings. The surrounding of the lysimeter cylinders was filled with the same soil. It was planted with beech

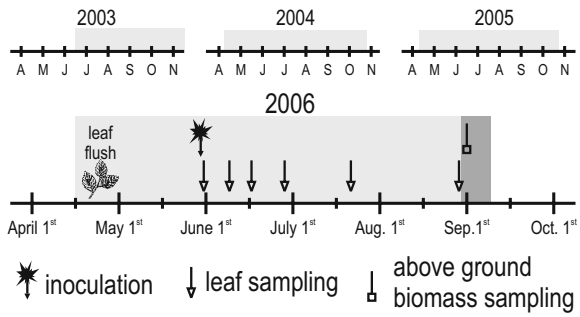

Fig. 1 Time diagram of the experimental procedure. Three year old beech saplings were planted autumn 2002. *Light grey bars* indicate the time of elevated ozone exposure; the *dark grey bar* indicated the time for below ground sampling. For details see Winkler et al. (2009a)

saplings (four saplings per square meter) as well to provide a homogeneous stand climate. In August 2006 the trees formed a closed stand with an average height of 1.91±0.06 m (mean±se).

A free-air exposure device was used to treat four of the lysimeters with two times ambient ozone concentrations during the vegetation periods of 2003 to 2006. However, elevation of ozone was restricted to levels lower than 150 nL L^{-1} to avoid acute ozone damage of leaves. A soil infestation with the root pathogen *P. citricola* was performed as an additional treatment at the end of May 2006 (see below; Fig. 2). To ensure that *P. citricola* did not naturally occur within the lysimeter soil, the absence of *Phytophthora* species in the soil as well as in root samples of the surrounding plantation had been verified by leaf baiting and re-isolation in June 2003. At the end of August 2006, the experiment was terminated and the total biomass above and below ground of each lysimeter was harvested. The above ground biomass was partitioned into first and second flush leaves of the sun (specific leaf area SLA=13.7±0.4 kg m^{-2}) and the shade crown (SLA= 25.0±0.6 kg m^{-2}), respectively, the main shoot axis and the lateral shoot axes. Below ground biomass was harvested in layers of 20 cm of soil depth and was further partitioned into coarse roots (diameter >1 mm) and fine roots (diameter ≤1 mm). Biomass was determined after drying the samples at 65°C to constant weight. For a detailed description of the harvest of lysimeter soil and plant biomass see Winkler et al. (2009b).

Soil infestation

For soil infestation *P. citricola* (isolate BoGa, isolated from a declining mature beech tree in Munich, Germany) was grown in sterile substrate consisting of vermiculite, wheat grain, calcium carbonate and V8-juice for 4 weeks according to Jung et al. (1996). Four days before inoculation, the *P. citricola* culture medium was washed with four volumes of distilled water to remove excess of nutrients and to induce the germination of oospores. At the day of inoculation the culture medium was mixed 2:1 (v/v) with soil from the lysimeters. Three holes (4 mm in diameter, 40 cm deep) were drilled into the soil at 15 cm from each beech tree stem. Sixty ml of inoculum were poured into each hole and covered with about 1 cm of soil. *P. citricola* inoculation was performed on two lysimeters per ozone treatment (Fig. 2). The remaining lysimeters were mock inoculated with a mixture of soil and sterile culture media. Although the weather was rainy at the days of inoculation each lysimeter was additionally watered with 12.5 L of distilled water at the day of inoculation as well as the day after, to allow zoospores of *P. citricola* to infect the beech roots.

During the final harvest three root samples per sapling were taken from each 20 cm-soil layer down to a depth of 100 cm. These roots were carefully washed with distilled water and visually examined for visible root rot symptoms (root necrosis, browning). Subsequently these samples were used for re-isolation of *P. citricola* and for quantification of *P. citricola DNA*. To test the viability of *P. citricola* at the end of

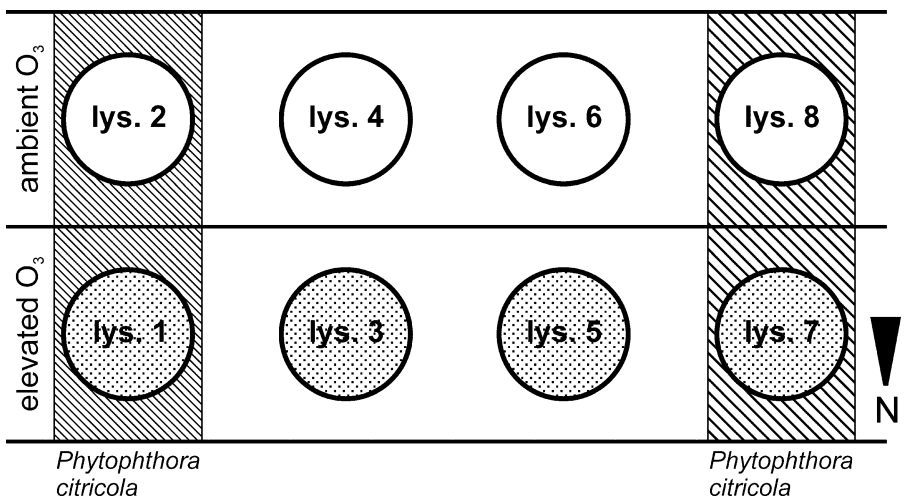

Fig. 2 Schematic drawing of the lysimeter experimental site. *Lysimeters (lys.) with odd numbers* were treated with two-times ambient ozone, while *lysimeters with even numbers* were not exposed. In lysimeters *1*, *2*, *7* and *8* the *Phytophthora citricola* infection was performed. Lysimeters *3* to *6* were mock inoculated with sterile inoculation media

the experiment rhizosphere soil was sampled up to a depth of 100 cm. A leaf baiting test (Jung et al. 2000) was conducted to re-isolate *P. citricola* from these soil samples. Baiting leaves as well as short root sections (ca. 1 cm) of the above root samples were plated onto Phytophthora-selective PARPNH-agar (Tsao and Guy 1977). Outgrowing hyphae were transferred to V8-agar and determined according to morphological characteristics as *P. citricola*.

Quantification of *P. citricola* DNA in roots

Infection of the roots system was quantified by means of TaqMan real-time quantitative PCR (qPCR) using a *P. citricola* specific set of primers and a fluorescent probe (Böhm et al. 1999). For that purpose the remaining parts of the fine root samples, used for re-isolation, were pooled per tree and soil layer. This resulted in one root sample per sapling and soil layer. Samples were freeze dried and ground to a fine powder in a MM20 ball mill (Retsch, Haan, Germany). DNA was extracted and further purified from 20 mg milled roots with the Plant DNeasy Mini-kit (Qiagen, Hilden, Germany) and subsequently with the Wizard DNA Clean-up System (Promega, Mannheim, Germany) according to the guidelines of the manufacturers. DNA extracts were diluted 1:10 in sterile water. The amount of *P. citricola* DNA within the DNA extracts was quantified on a SDS 7700 Sequence Detection System (Applied Biosystems, Frankfurt, Germany) using the ABSOLUTE qPCR ROX Mix (ABgene, Hamburg, Germany). The qPCR was performed as three technical replicates with 40 cycles of denaturation (94°C for 15 s) and combined annealing/extension (60°C for 60 s). A dilution series of genomic DNA extracted from pure *P. citricola* mycelium (isolate BoGa) was used to calculate a linear standard curve of log[*P. citricola* DNA] vs. CT-value. The PCR efficiency was calculated as $E=10^{(-1/\text{slope})}$. The mean CT-value of the technical replicates was used to calculate the amount of *P. citricola* DNA within a sample.

Analysis of non-structural carbohydrates

The leaf samples for the analysis of NSC (soluble sugars and starch) were taken at 12:00 CET at six dates as indicated in Fig. 1. Except of the final harvest, where also shade leaves and leaves of the second flush were analysed, only first flush leaves of the sun crown were sampled. The samples for starch analysis of main shoot axes, lateral shoot axes, coarse roots and fine roots were taken during the first day of the final harvest [end of August 2006]. All root samples analysed for starch concentration belonged to the soil layer 0–20 cm. Extraction of soluble sugars and starch was performed on 20 mg of freeze-dried and finely ground plant material according to Schloter et al. (2005). In brief, samples were extracted for three times consecutively with hot (85°C) water. After centrifugation supernatants were combined and used for high performance liquid chromatography (HPLC) analysis. Subsequently, starch was determined as glucose equivalents after enzymatic digestion of the remaining plant pellet with amylase and amyloglucosidase. HPLC analyses were performed with a CARBOsep CHO-820 calcium column (Transgenomic, Glasgow, UK). The column was maintained at 85°C and double distilled water was used as mobile phase with a flow rate of 0.6 mL min^{-1}. Total carbohydrate content of plant organs was calculated by multiplying the carbohydrate concentration with the biomass of the corresponding organ.

Statistical analysis

All statistical analyses were performed with SPSS 16.0.1 (SPSS Inc., USA). All data were ln-transformed to obtain normal-distribution. To test the effects of elevated ozone exposure and *P. citricola* infection on non-structural carbohydrates of sun leaves during the vegetation period, the repeated measure module of the general linear model (GLM) was used, adjusted for interaction effects of independent variables with time (sampling date). To test the effects of ozone and *P. citricola* on the parameters assessed at single dates during the vegetation period (NSC of sun leaves) as well as at the final harvest (root infection as well as starch concentration and starch content of different plant organs) a two-way ANOVA was performed using the univariate GLM with ozone and *P. citricola* as fixed factors and the lysimeter as a covariate.

Results

Infection of beech trees by *Phytophthora citricola*

The soil infestation experiment with the root pathogen *P. citricola* was conducted between end

of May and end of August 2006. During this time there was no visible indication of root infection on above-ground plant parts, such as stem necroses or wilt of leaves as described for beech seedlings infested with *P. citricola* (Fleischmann et al. 2002, 2005). At the end of the experiment—13 weeks after inoculation—the pathogen was re-isolated from soil samples of all four lysimeters inoculated with the pathogen. The presence of the pathogen was confirmed down to a soil depth of 40 cm corresponding with the depth of inoculation (data not shown). *P. citricola* was not re-isolated from any soil sample of the lysimeters number 3 to 5, which served as controls. There were no apparent root rot symptoms on the root samples taken for re-isolation and quantification of *P. citricola* DNA. However, it was possible to re-isolate *P. citricola* from 23 out of 48 root samples from the top soil layer (0–20 cm) of the four inoculated lysimeters, and from 15 out of 48 samples from the second soil layer (20–40 cm). It was not possible to re-isolate *P. citricola* from roots sampled at deeper soil layers or from roots of the control lysimeters.

Infection of roots was quantified by measuring *P. citricola* DNA by means of qPCR. Highest infection was monitored within a soil depth of 0–20 cm, while it was lower in a depth of 20–40 cm (Table 1). In agreement with the results of re-isolation, *P. citricola* was detected by qPCR neither in roots grown deeper than 40 cm, nor in roots of the control lysimeters. The variance of root infection within a lysimeter was high, and hence, no effect of the ozone treatment on *P. citricola* infection was observed. A significant effect of the covariant lysimeter was detected in the soil layer 0–20 cm (p= 0.023), with higher root infection in lysimeters number seven and eight [located in the west of the lysimeter array], and lower infection in lysimeter number 1 and 2.

The *P. citricola* treatment had no effect on root biomass of beech saplings. The root morphological parameters *specific root tip density* and *specific root length* within the soil layers 0–20 cm as well as 20–40 cm were significantly reduced (data presented in Winkler et al. 2009b).

Non-structural carbohydrates of beech leaves

Analyses of NSC were periodically performed on sun leaves from the time of inoculation until the end of the experiment. Sucrose concentrations in sun leaves (Fig. 3a) were significantly reduced by both the ozone treatment and the *P. citricola* infection with a significant interaction of the treatments according to the time series analysis. On the basis of the single sampling date, ozone significantly affected the leaf sucrose concentration at any date, while the *P. citricola* effect was significant 1 week after inoculation only. Starch concentrations of sun leaves (Fig. 3b) were significantly diminished by the ozone treatment but not by the root infection. However, at the single sampling date, the ozone effect was only significant at three out of six sampling dates.

Table 1 Quantification of *Phytophthora citricola* DNA in beech roots of different soil layers using *real-time* qPCR

Treatment	Lys.	Soil depth				
		0–20 cm		20–40 cm		40–100 cm
		Lys. mean	Treat mean	Lys. mean	Treat mean	Lys. mean
Ambient O_3 + *P. citricola*	2	8.3 (4.8)	19.6 (7.9)	1.2 (0.7)	9.1 (6.9)	n. d.
	8	30.8 (13.4)		16.9 (13.5)		n. d.
Elevated O_3 + *P. citricola*	1	5.85 (5.5)	22.1 (13.4)	2.9 (1.2)	3.9 (1.7)	n. d.
	7	38.5 (25.3)		4.9 (3.3)		n. d.

No *P. citricola* DNA was found in roots of the control lysimeters (lys.)

No significant treatment effects were observed (values are given in ng *P. citricola* DNA per g root dry weight (means (se)). The standard curve used for quantification had a slope=−3.57 (r^2 =0.977) resulting in a PCR-efficiency of E=1.91

n. d. not detected

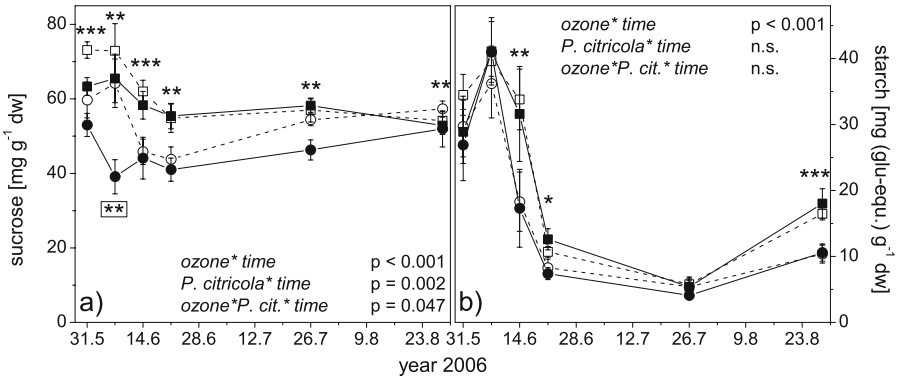

Fig. 3 Sucrose (**a**) and starch (**b**) concentrations of beech leaves under the influence of chronic ozone enrichment and a root infection with *Phytophthora citricola* measured in the growing period 2006. *Squares* ambient ozone; *circles* elevated ozone, *open symbols* (*dashed line*) healthy controls, *closed symbols* (*solid line*) inoculated with *P. citricola*. Values are given as mean per treatment ± standard error (n=8); *asterisks* indicate a significant ozone effect at single dates and *boxed asterisks* indicate a significant *P. citricola* effect at single dates, respectively (*p<0.05; **p<0.01; ***p<0.001) The statistics within the figures shows the p-values of the time series analyses for the effects ozone, *P. citricola* and ozone × *P. citricola* with time (*n.s.* not significant)

Starch concentrations and contents of different plant organs

After the final harvest at the end of August 2006 the starch concentration of different plant organs was determined (Table 2). Highest concentrations were found in sun leaves of the second flush, followed by sun and shade leaves, in this order, of the first flush. Similar values of starch concentrations were found in all parts of the heterotrophic biomass (lateral shoot axis, main shoot axis and coarse roots), with the exception of fine roots, where starch concentrations were lowest. A significant ozone effect on starch concentrations was observed in leaves (in second flush leaves only in tendency) but not in the heterotrophic biomass. The *P. citricola infection* had no effect on starch concentrations.

When the total starch content within the different plant organs was calculated, highest values were found in the main shoot axis followed by lateral shoot axis

Table 2 Starch concentration of different organs of beech saplings measured after the final harvest

	Leaves 2nd flush sun	Leaves 1st flush sun	Leaves 1st flush shade	Lateral shoot axis	Main shoot axis	Coarse roots (>1 mm)	Fine roots (<1 mm)
Treatment							
Amb. O_3	26.99 (2.89)	16.46 (0.92)	16.53 (2.78)	13.84 (2.21)	14.00 (1.48)	14.10 (2.22)	4.03 (0.69)
Amb. O_3 + *P. citricola*	25.68 (2.76)	18.03 (2.23)	19.24 (4.51)	18.71 (1.22)	15.06 (1.12)	14.91 (1.71)	3.93 (1.21)
Elev. O_3	21.22 (2.21)	10.36 (1.35)	14.68 (2.50)	14.63 (0.50)	16.28 (1.47)	16.24 (1.83)	3.10 (0.75)
Elev. O_3 + *P. citricola*	19.46 (3.40)	10.58 (1.30)	9.71 (0.86)	15.37 (1.79)	13.83 (1.97)	14.44 (1.94)	3.49 (0.78)
Statistical analysis							
Ozone	0.06	0.000	0.033	n.s.	n.s.	n.s.	n.s.
P. citricola	n.s.	n.s.	n.s.	0.072	n.s.	n.s.	n.s.
Lysimeter	n.s.	0.018	n.s.	0.097	n.s.	n.s.	0.021
Ozone × *P. citricola*	n.s.	n.s.	n.s.	0.057	n.s.	n.s.	n.s.

All starch concentrations are given as glucose equivalents per biomass [mg/g] (treatment means (standard error); (n=8)). In the section statistical analysis p-values for treatment, covariate and interaction effects are given. P-values less than 0.1 are shown in the table although effects were assumed to be significant with p<0.05

n.s. not significant

Table 3 Poole sizes of starch in different organs of beech saplings measured after the final harvest

	Leaves 2nd flush sun	Leaves 1st flush sun	Leaves 1st flush shade	Lateral shoot axis	Main shoot axis	Coarse roots (>1 mm)	Fine roots (<1 mm)
Treatment							
Amb. O_3	0.21 (0.06)	0.64 (0.07)	0.63 (0.16)	2.19 (0.55)	2.77 (0.32)	2.35 (0.36)	0.18 (0.04)
Amb. O_3 + *P. citricola*	0.14 (0.03)	0.98 (0.23)	0.71 (0.17)	3.53 (0.58)	3.82 (0.56)	2.71 (0.47)	0.20 (0.07)
Elev. O_3	0.12 (0.02)	0.47 (0.10)	0.55 (0.18)	2.13 (0.27)	3.64 (0.56)	2.54 (0.36)	0.12 (0.04)
Elev. O_3 + *P. citricola*	0.21 (0.05)	0.43 (0.10)	0.33 (0.06)	2.50 (0.52)	2.94 (0.61)	2.31 (0.31)	0.15 (0.03)
Statistical analysis							
Ozone	n.s.	0.001	0.031	n.s.	n.s.	n.s.	0.07
P. citricola	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Lysimeter	n.s.	0.001	n.s.	n.s.	n.s.	n.s.	0.01
Ozone × *P. citricola*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

All starch contents are given as glucose equivalents per organ [g] (treatment means (standard error); (n=8)). In the section statistical analysis, p-values for treatment, covariate and interaction effects are given. P-values less than 0.1 are given in the table although effects were assumed to be significant with $p<0.05$

n.s. not significant

and coarse roots (Table 3). Total starch contents of leaves and fine roots were low because of their limited biomass. A significant ozone effect was found in leaves of the first flush as well as in fine roots. No effect of *P. citricola* on starch contents was observed.

Discussion

In the presented study we used a lysimeter experiment to study the impact of chronically elevated ozone exposure as well as a root infection with *P. citricola* on beech saplings. Usually, the focus of lysimeter studies is on soil processes like soil water relations or nutrient fluxes within the soil. With this experiment, the use of lysimeters was expanded to the field of plant ecophysiology and plant pathology. A lysimeter provides a unique tool to carry out soil infestation experiments under semi-natural conditions without the risk of spreading the inoculum into a natural ecosystem. Therefore, lysimeter studies can serve as a link between phytotron or greenhouse experiments with rather non-natural conditions and field studies on naturally occurring plant diseases.

A common practice in conducting soil infestation experiments with *Phytophthora* spp. is the transient flooding of the soil compartment to boost the infection pressure by the release of pathogen zoospores (Jung et al. 1996; Matheron and Mircetich 1985; Maurel et al. 2001). Besides technical problems with flooding a lysimeter of 2 m^3 of soil volume, such a flooding had counteracted the semi-natural approach of the lysimeter study. Therefore, we added only 25 L of water per lysimeter to trigger zoospore release of *P. citricola* as a compromise between biological needs and the semi-natural experimental procedure. Yet, the amount of *P. citricola* DNA measured in fine roots was comparable to greenhouse or phytotron experiments on beech seedlings, with periodical flooding (Fleischmann et al. 2005; Luedemann et al. 2005). Indeed the variance of root infection in the lysimeter study was higher compared to these studies. There was no detectable dispersion of *P. citricola* into the soil layers deeper than 40 cm, and pathogen was restricted to fine roots and rhizosphere soil at this depth. There were no significant differences in root infection rates between the ambient and the elevated ozone treatment; hence our second hypothesis on the interaction of *P. citricola* infection with the ozone exposure was not proved. Exceptional high temperatures and low precipitation from end of June to the beginning of August 2006 (mean temperature of 21°C and precipitation of 33 mm in July 2006) might have overlaid the ozone effects, as drought negatively affects both *P. citricola* growth and ozone flux into the leaf apoplast.

The analysis of NSCs of beech leaves focused on starch, serving as transitory carbon storage in chlor-

oplasts, and on sucrose, being transported within the phloem sap. In 2006 both NSCs were significantly reduced in ozone exposed leaves. A similar reduction was reported in leaves of the sun crown of mature beech trees exposed to elevated ozone (Blumenröther et al. 2007). *Fagus sylvatica* is considered to have a symplastic phloem loading (Eschrich and Fromm 1994; Gamalei 1991). As raffinose concentrations are low in beech leaves (Hoch et al. 2003), a passive phloem loading, following a concentration gradient between leaf mesophyll and sieve elements (Münch 1930; Turgeon 2006), is likely to take place in beech. Hence, lower sucrose concentrations in ozone exposed leaves can be interpreted as reduced phloem transport and deminished allocation of carbon to carbon sink tissue.

The low concentrations of starch at June 21st and July 26th were attributed to high temperatures and drought. A similar drought effect on leaf starch concentrations was observed during the extremely dry summer of 2003 (Schloter et al. 2005). Under these conditions net assimilation is low, due to stomatal narrowing, and almost all new assimilates can be exported out of leaves at once. Hence no transitory starch accumulates in leaves.

Starch concentration and content, respectively of the heterotrophic biomass at the final harvest were affected by neither the ozone nor the *Phytophthora* treatment. The only exception was a significant reduction of the starch content in fine roots under elevated ozone, as both starch concentrations and biomass of fine roots were diminished in tendency under elevated ozone. On the whole, plant level starch content was not affected, as fine roots contributed only by about 6.5% to total biomass. In a phytotron study with beech and spruce seedlings grown in monoculture or mixed culture, and treated with elevated ozone and CO_2 ,respectively, Liu et al. (2004) measured significant reductions of soluble sugars and starch concentrations under elevated ozone in shoot axis, coarse roots and fine roots of beech, but not in leaves. Furthermore, ozone effects were more pronounced under elevated CO_2 and were not visible in mixed cultures. These findings are in contrast to our results and underline the problem of comparability of trees of different ontogenetic stage, as well as between phytotron studies vs. field experiments as it was discussed by Nunn et al. (2005).

Overall, most of the presented starch concentrations in different plant organs were lower than the values published for mature beech trees (Hoch et al. 2003). While in leaves starch concentrations were almost equal, the values of beech saplings were five times lower in lateral axis and about two times lower in the stem as compared to mature trees. Again, this might be an ontogenetic effect, but also differences in the methodology for the determination of NSCs (HPLC method vs. enzyme coupled photometrical assay) might contribute to these different findings. The results on starch reserves in heterotrophic biomass do not support our first hypothesis regarding a reduced carbon allocation to roots under elevated ozone.

In conclusion, these present results are of limited significance, but underline the necessity to operate experiments on trees exposed to abiotic and biotic stresses for several years, as some plant responses might not occur within one vegetation period. The comparison with the first 3 years of this experiment (Pritsch et al. 2008; Schloter et al. 2005) showed that it took 4 years of ozone exposure to form a significant ozone effect at least on NSC of leaves.

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References

Andersen CP (2003) Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol 157:213–228 doi:10.1046/j.1469-8137.2003.00674.x

Bahnweg G, Heller W, Stich S et al (2005) Beech leaf colonization by the endophyte *Apiognomonia errabunda* dramatically depends on light exposure and climatic conditions. Plant Biol 7:659–669 doi:10.1055/s-2005-872943

Blumenröther MC, Low M, Matyssek R, Osswald W (2007) Flux-based response of sucrose and starch in leaves of adult beech trees (*Fagus sylvatica* L.) under chronic free-air O-3 fumigation. Plant Biol 9:207–214 doi:10.1055/s-2006-924565

Böhm J, Hahn A, Schubert R et al (1999) Real-time quantitative PCR: DNA determination in isolated spores of the mycorrhizal fungus *Glomus mosseae* and monitoring of *Phytophora infestans* and *Phytophthora citricola* in their respective host plants. J Phytopathol 147:409–416 doi:10.1111/j.1439-0434.1999.tb03842.x

Eschrich W, Fromm J (1994) Evidence for 2 pathways of phloem loading. Physiol Plant 90:699–707 doi:10.1111/j.1399-3054.1994.tb02526.x

Fenn ME, Dunn PH, Wilborn R (1990) Black stain root disease in ozone-stressed ponderosa pine. Plant Dis 74:426–430 doi:10.1094/PD-74-0426

Fleischmann F, Schneider D, Matyssek R, Oßwald WF (2002) Investigations on net CO_2 assimilation, transpiration and root growth of *Fagus sylvatica* infested with four different *Phytophthora* species. Plant Biol 4:144–152 doi:10.1055/s-2002-25728

Fleischmann F, Göttlein A, Rodenkirchen H et al (2004) Biomass, nutrient and pigment content of beech (*Fagus sylvatica*) saplings infected with *Phytophthora citricola, P. cambivora, P. pseudosyringae* and *P. undulata*. For Pathol 34:79–92

Fleischmann F, Koehl J, Portz R et al (2005) Physiological change of *Fagus sylvatica* seedlings infected with *Phytophthora citricola* and the contribution of its elicitin "Citricolin" to pathogenesis. Plant Biol 7:650–658 doi:10.1055/s-2005-872891

Gamalei Y (1991) Phloem loading and its development related to plant evolution from trees to herbs. Trees Structure Funct 5:50–64

Hoch G, Richter A, Korner C (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell Environ 26:1067–1081 doi:10.1046/j.0016-8025.2003.01032.x

Jung T, Blaschke H, Neumann P (1996) Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. Eur J Forest Pathol 26:253–272 doi:10.1111/j.1439-0329.1996.tb00846.x

Jung T, Blaschke H, Osswald W (2000) Involvement of soilborne *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. Plant Pathol 49:706–718 doi:10.1046/j.1365-3059.2000.00521.x

Jung T, Hudler G, Jensen-Tracy S et al (2005) Involvement of *Phytophthora* species in the decline of European beech in Europe and the USA. Mycologist 19:159–166 doi:10.1017/S0269915X05004052

King JS, Kubiske ME, Pregitzer KS et al (2005) Tropospheric O-3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO_2. New Phytol 168:623–635 doi:10.1111/j.1469-8137.2005.01557.x

Leininger TD, Winner WE, Alexander SA (1990) Root disease incidence in eastern white-pine plantations with and without symptoms of ozone injury in the Coweeta Basin of North-Carolina. Plant Dis 74:552–554 doi:10.1094/PD-74-0552

Liu WP, Kozovits AR, Grams TEE et al (2004) Competition modifies effects of enhanced ozone/carbon dioxide concentrations on carbohydrate and biomass accumulation in juvenile Norway spruce and European beech. Tree Physiol 24:1045–1055

Luedemann G, Matyssek R, Fleischmann F, Grams TEE (2005) Acclimation to ozone affects host/pathogen interaction and competitiveness for nitrogen on juvenile *Fagus sylvatica* and *Picea abies* trees infected with *Phytophthora citricola*. Plant Biol 7:640–649 doi:10.1055/s-2005-872902

Manning WJ, v Tiedemann A (1995) Climate change: potential effects of increased atmospheric Carbon dioxide (CO_2), ozone (O_3), and ultraviolet-B (UV-B) radiation on plant diseases. Environ Pollut 88:219–245

Matheron ME, Mircetich SM (1985) Pathogenicity and relative virulence of *Phytophthora* spp from walnut and other plants to rootstocks of English walnut trees. Phytopathology 75:977–981 doi:10.1094/Phyto-75-977

Maurel M, Robin C, Capron G, Desprez-Loustau ML (2001) Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulation, mineral nutrition and vulnerability to water deficit of five oak and chestnut species. For Pathol 31:353–369

Meleux F, Solmon F, Giorgi F (2007) Increase in summer European ozone amounts due to climate change. Atmos Environ 41:7577–7587 doi:10.1016/j.atmosenv.2007.05.048

Münch E (1930) Die Stoffbewegungen in der Pflanze. Fischer, Jena

Nunn AJ, Kozovits AR, Reiter IM et al (2005) Comparison of ozone uptake and sensitivity between a phytotron study with young beech and a field experiment with adult beech (*Fagus sylvatica*). Environ Pollut 137:494–506 doi:10.1016/j.envpol.2005.01.036

Paoletti E, Bytnerowicz A, Andersen C et al (2007) Impacts of air pollution and climate change on forest ecosystems—emerging research needs. ScientificWorldJournal 7:1–8

Pritsch K, Ernst D, Fleischmann F et al (2008) Plant and soil system responses to ozone after 3 years in a lysimeter study with juvenile beech (*Fagus sylvatica* L.). Water Air Soil Pollut Focus 8:139–154 doi:10.1007/s11267-007-9164-4

Racherla PN, Adams PJ (2008) The response of surface ozone to climate change over the Eastern United States. Atmos Chem Phys 8:871–885

Samuelson L, Kelly JM (2001) Scaling ozone effects from seedlings to forest trees. New Phytol 149:21–41 doi:10.1046/j.1469-8137.2001.00007.x

Schloter M, Winkler JB, Aneja M et al (2005) Short term effects of ozone on the plant–rhizosphere–bulk soil system of young beech trees. Plant Biol 7:728–736 doi:10.1055/s-2005-872987

Skärby L, Ro-Poulsen H, Wellburn FAM, Sheppard LJ (1998) Impacts of ozone on forests: A European perspective. In: 3rd new phytologist symposium on major biological issues resulting from anthropogenic disturbance of the nitrogen cycle, Lancaster, England, pp 109–122

Tsao P, Guy S (1977) Inhibition of *Mortierella* and *Pythium* in a *Phytophthora*-isolation medium containing hymexazol. Phytopathology 67:796–801

Turgeon R (2006) Phloem loading: how leaves gain their independence. Bioscience 56:15–24 doi:10.1641/0006-3568(2006)056[0015:PLHLGT]2.0.CO;2

Wang ZY, Göttlein A, Rodenkirchen H et al (2003) The influence of *Phytophthora citricola* on rhizosphere soil solution chemistry and the nutritional status of European beech seedlings. J Phytopathol 151:365–368 doi:10.1046/j.1439-0434.2003.00729.x

Winkler JB, Lang H, Graf W, Reth S, Munch JC (2009a) Experimental setup of field lysimeters for studying

effects of elevated ozone and below-ground pathogen infection on a plant-soil-system of juvenile beech (*Fagus sylvatica* L.). Plant Soil doi:10.1007/s11104-009-9936-x

Winkler JB, Fleischmann F, Gayler S et al (2009b) Do chronic aboveground O_3 exposure and belowground pathogen stress affect growth and belowground biomass partitioning of young beech trees (*Fagus sylvatica* L.)? Plant Soil, submitted

Zeng G, Pyle JA, Young PJ (2008) Impact of climate change on tropospheric ozone and its global budgets. Atmos Chem Phys 8:369–387