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REGULAR ARTICLE

Contrasting ozone × pathogen interaction as mediated through competition between juvenile European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*)

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Abstract Based on the growth-differentiation balance theory (GDB) and the influence of tropospheric ozone (O_3) on plants, we hypothesized that pre-conditioning with elevated O_3 reduces adverse effects of the root rot pathogen *Phytophthora citricola* Sawada. To this end a 2-year phytotron study with juvenile European beech (*Fagus sylvatica* L.) and (*Picea abies* [L.] Karst.) grown in mixture was performed. The hypothesis was tested on phenological, leaf and root morphological as well as physiological aspects of plant performance. Contrasting with spruce, elevated O_3 limited leaf and root biomass development, photosynthetic performance and N uptake of beech. The growth limitation by O_3 conveyed increased resistance in beech against the pathogen. Conversely, spruce displayed enhanced susceptibility in the combined O_3/*P. citricola* treatment. The hypothesis was supported in the case of beech rather than spruce. Nevertheless, conclusions support GDB regarding the trade-off between growth and stress defense, although compliance appears to be species-specific.

Keywords Growth-differentiation balance theory (GDB) · *Phytophthora citricola* · Root rot pathogen · Tropospheric ozone (O_3) concentration · Global change

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Introduction

In 1953, Loomis stated about plant growth that "*Plants which are making a relatively rapid growth with a minimum of differentiation have thin leaves, long internodes, and high moisture content. The roots are poorly developed, the stems weak, the plants contain minimum quantities of various essential oils, gums, and other differentiation products*" (Loomis 1953a). Such plant performance was related to low resistance against abiotic stressors such as drought, heat or frost. In a companion paper, Loomis (1953b) suggested progressive cell differentiation to facilitate the formation of secondary metabolites which may be relevant in biotic stress defense. The principle derived from these statements was named by Loomis (1953b) as the growth-differentiation balance, deciding about plant vigour and/or stress defence, in particular, of parasites, in terms of a trade-off in resource allocation.

Such a view on allocation became the basis of the "Growth-Differentiation Balance Theory", hereon GDB. This theory has been elucidated by Herms and Mattson (1992) and adapted, enhanced and tested (Stamp 2003; Matyssek et al. 2005a, b; Bennett et al. 2006; Glynn et al. 2007). Basically, GDB provides a framework to predict plant resource allocation between growth and stress defense-related metabolism as well as functional differentiation over a range of environmental conditions (Loomis 1932, 1953a; Herms and Mattson 1992; Stamp 2003; Matyssek et al. 2005a, b). Within the framework of GDB, the present work aims at testing whether growth limitation in trees induced by tropospheric ozone (O_3) reduces their susceptibility to root pathogens.

Anthropogenic enhanced tropospheric O_3, which is the potentially most phytotoxic air pollutant (Lefohn 1992; Skärby et al. 1998; Matyssek and Sandermann 2003), falls under Grime's definition of stress (Grime 1977), being one of the evolutionary pressures for reduced growth. GDB predicts that any environmental factor that slows growth more than it slows photosynthesis will increase the resource pool for secondary metabolites and will promote structural and functional differentiation processes (Loomis 1953a, b; Herms and Mattson 1992; Arendt 1997). In fact, O_3 is known to reduce plant biomass development and in parallel to induce plant responses typically associated with pathogen defense, such as biosynthesis of lignin or increased phenylalanine ammonia-lyase (PAL) activity (Heagle 1973; Sandermann et al. 1998; Matyssek and Sandermann 2003). Moreover, the often constrained root development under enhanced O_3 (Andersen 2003, Pritsch et al. 2005) may result in a higher proportion of older and suberized roots which may display enhanced resistance against root pathogens such as *P. citricola*.

Phytophthora species are oomycetes that cause several plant diseases, including late blight of potato and tomato (*P. infestans*; Agrios 2005) or sudden oak death in the northwestern United States (*P. ramorum*; Rizzo and Garbelotto 2003). The species *P. citricola* is known to incite root rot across a broad range of tree hosts (among others: *Abies, Juglans, Pinus, Pseudotsuga* and *Quercus*; Erwin and Ribeiro 1996), including European beech (*F. sylvatica*; Werres 1995; Jung and Blaschke 1996; Fleischmann et al. 2002, 2004). To our knowledge, nothing is known about effects of *P. citricola* on field grown trees of Norway spruce (*P. abies*). Nevertheless, under laboratory conditions, spruce seedlings infected by this pathogen suffered from severe, sometimes even lethal root injury (Nechwatal and Oßwald 2001).

Based on GDB and the influence of tropospheric O_3 on plant (root) growth and secondary metabolism (Matyssek and Sandermann 2003), we hypothesized that exposure to elevated O_3 concentrations reduces adverse effects of *P. citricola* infection on trees, in particular, on their roots. This hypothesis was examined in a 2-year phytotron study on juvenile European beech and Norway spruce grown in mixture under fluctuating O_3 levels, either representing the ambient ($1\times O_3$) or twice-ambient O_3 regime ($2\times O_3$) of a nearby forest site (Matyssek et al. 2007). During summer of the first growing season, half of the trees in either regime were inoculated with the root pathogen *P. citricola*. Beech and spruce were expected to be differentially affected by the pathogen under $2\times[O_3]$ due to their different O_3 sensitivities as observed in a previous experiment with similar design (Grams et al. 2002; Kozovits et al. 2005a, b): Spruce had turned out to be virtually resistant to elevation of $[O_3]$ in contrast to beech, which developing several stress symptoms. In a previous account on the same spruce/beech systems (Luedemann et al. 2005) reported here, we focused on root physiological aspects of competition for nitrogen (N). O_3 rather than infection with *P. citricola* had limited the competitiveness of beech for N. In spruce, only the combined effect of O_3 and *P. citricola* had reduced whole-tree biomass and at the same time increased the competitiveness for N. In the present investigation, we examined the interacting effects of O_3 and *P. citricola* in the view of GDB (as stated in the hypothesis above), on the level of phenological, leaf and root-morphological as well as physiological aspects of plant performance.

Materials and methods

Plants, climate conditions, and O_3 regimes In April 2001, 1- and 2-year-old seedlings of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* [L.] Karst.) were planted in containers (area of 0.56 m × 0.37 m, soil depth of 0.30 m) which had been filled with untreated forest soil. Twenty trees (arranged by rows of 4 × 5 individuals) were planted in an alternating pattern into each of 64 containers (1-by-1 beech/spruce

mixture, Fig. 1, for details see Luedemann et al. 2005). To diminish potential edge effects, all assessments were conducted on the six central individuals in each container.

Table 1 details the sequence of experimental procedures as follows: After 7 months (April to October 2001) in a climate-controlled greenhouse, 32 containers were transferred into four walk-in phytotrons (at Helmholtz Zentrum München, formerly GSF National Research Centre for Environment and Health, Neuherberg/Munich, Germany) during the growing seasons of 2002 and 2003. Each phytotron contained four Plexiglas chambers (size: ca. 0.8 m×1.1 m×1.0 m) with adequate ventilation (150 m^3 h^{-1}) to warrant the phytotron temperature regime inside the sub-chambers (cf. Kozovits et al. 2005a; Payer et al. 1993). During the winter months, plants were kept outdoors (Table 1).

In the phytotrons, the seasonal courses of air temperature, air humidity and of the ambient O_3 regime ($1\times O_3$), recorded in 1998 and 1999 at the study site "Kranzberg Forest" (see Pretzsch et al. 1998; Matyssek et al. 2007), were reproduced in the phytotrons on an hourly basis throughout the seasonal courses of 2002 and 2003, respectively. $1\times O_3$ provided the basis for the experimental $2\times O_3$ regime, so that plants were exposed to either $1\times O_3$ (serving as control) or $2\times O_3$, being restricted to <150 nl l^{-1} (see Table 2).

On July 30 2002, after exposure to O_3 for 10 weeks, plants were inoculated with the root rot pathogen *P. citricola* (see below "*Inoculation with P. citricola*"), resulting in four treatments: "control" (ambient [O_3]), "+O_3" (2× ambient [O_3]), "+Phy" (ambient [O_3] and *P. citricola* inoculation) and "+O_3/+Phy" (2× ambient [O_3] and *P. citricola* inoculation; see Table 1). Please see Luedemann et al. (2005) for further details on experimental conditions.

Inoculation with P. citricola On July 30, 2002, the soil was inoculated with the root rot pathogen *P. citricola* Sawada isolate Bu137/7N. The isolate originated from a declining beech grown in a mixed beech/spruce forest as it is typical for southern Germany (Fleischmann et al. 2002). To stimulate the production of zoosporangia and the release of zoospores, containers were flooded on July 30, 2002 and June 16, 2003 for 48 h each. The successful infection of both beech and spruce trees was confirmed and quantified with real-time quantitative PCR (Luedemann et al. 2005).

Plant biomass assessment Throughout the experiment the six central trees of each container were harvested at five different dates in 2002 and 2003 (Table 1). At the end of the experiment in September 2003, three containers (i.e. nine trees per species) in the control and +O_3 treatment each and five containers (i.e. 15 trees per species) in the +Phy and +O_3/+Phy treatment each were harvested. Tree biomass was separated by organs: foliage, stems and branches (hereafter referred to as "shoot axes"), and roots (separated into fine roots, ≤2 mm in diameter and coarse roots, >2 mm in diameter). Dry mass (DM) of each biomass fraction was determined.

Root architecture assessment Upon harvest, the roots of the six central trees per container were maintained under refrigeration (4°C) in demineralised water until photographic recording (cybershot DSC F707, Sony,

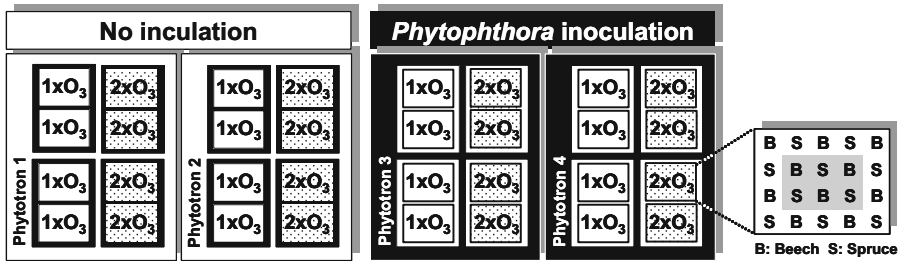

Fig. 1 Experimental set-up in the four phytotrons of the Helmholtz Zentrum München—German Research Center for Environmental Health (adapted from Luedemann et al. 2005). Each phytotron comprised four Plexiglas sub-chambers for individual O_3 fumigation. In phytotron 3 and 4 soil was inoculated with the root rot pathogen *P. citricola*. Two planting containers with mixed beech/spruce cultures were placed into each of the four Plexiglas sub-chambers per phytotron. The experimental set-up in chambers 1 and 3 was reproduced in chambers 2 and 4. Planting pattern of beech (B) and spruce (S) in a container is shown to the right, including the highlighted position of the six central study trees

Table 1 Experimental sequence

Date	Description	Number of containers			
Apr 2001	Planting 1-year-old beech and 2-year old spruce in 64 containers with forest soil (20 plants/container)	64			
Apr 2001 - Oct 2001	Plants growing inside a climate controlled glasshouse under ambient ozone concentrations				
Nov 2001 - Apr 22, 2002	Plants kept outdoors				
Apr 23, 2002	34 containers were selected for uniform tree height 1st harvest: 2 containers used for initial biomass assessment 32 containers transferred into phytotrons (16 in each O_3 treatment)	34 1xO3: 32 2xO3: 32 16		16	
Jul 30, 2002	2nd harvest: 4 containers used (2 from each O_3 treatment)	–Phy 14	+Phy	–Phy 14	+Phy
Jul 31, 2002	Inoculation with *P. citricola* in containers of +Phy and +O_3/+Phy treatments	6	8	6	8
Sep 18, 2002	3rd harvest: 8 containers used (2 containers per treatment)	4	6	4	6
Oct 28, 2002 - Apr 27, 2003	Plants kept outdoors				
Apr 28, 2003	Transfer of containers back into phytotrons				
Jul 14, 2003	4th harvest (assessing ^{15}N atom% prior to labeling): 4 containers used (1 container per treatment)	3	5	3	5
Jul 16, 2003	Enhancement of *P. citricola* infestation				
Jul 30, 2003	^{15}N-labeling with double-labeled $^{15}NH_4{}^{15}NO_3$				
Sep 25, 2003	Final harvest of 16 containers: 3 of control, 5 of +Phy, 3 of +O_3 and 5 of +O_3/+Phy	3	5	3	5
	Treatments	**Control**	**+Phy**	**+O_3**	**+O_3/+Phy**

Tokyo, Japan, 5 Mpixels in TIFF format). Images of roots were taken applying three soft light sources (one from below and two from above) to avoid shadows. Further analysis of root length, number of root tips and average diameter of roots were performed using the WinRhizo software (Version 3.9, Regent Instruments Inc., Quebec City, Canada).

Leaves flush phenology assessment The phenology of the leaves and twigs was observed in weekly intervals to assess seasonal plant development. As suggested by Häberle (1995), we classified beech leaves by six developmental stages: (0) buds closed, (1) buds swollen, (2) first leaf visible, folded (3) leaves unfolded but hanging, (4) leaves horizontally positioned, advanced length growth, axes still hanging, and (5) leaf and shoot development completed. For spruce we used the seven following stages: (0) buds closed, (I) bud break, (II) new axis shorter than 1 cm, (III) beginning of axis length growth, new axis <3 cm, (IV) advanced length growth, new axis longer than 3 cm, (V) needles of new shoots slightly spread, and (VI) elongation growth completed.

Leaf chlorophyll fluorescence The photosystem II operating efficiency ($F_q'/F_m'=(F_m'-F)/F_m'$, for nomenclature see Baker 2008) of leaves was measured on five shade and sun leaves each per container twice a month from June through October 2002 and from July through September 2003 by means of a PAM 2000 fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Measurements were taken during days with PPFD >500 µmol m^{-2} s^{-1} on light-adapted leaves. During measurements, PPFD in the phytotrons was kept constant.

Assessment of maximum rate of RuBP carboxylation ($V_{C,max}$) and maximum rate of electron transport (J_{max}) CO_2 response curves of the net CO_2 exchange rate (i.e. A/c_i curves) of sun exposed leaves were

Table 2 Monthly means of air temperature (T air), photosynthetic photon flux density (PPFD), relative air humidity (RH), CO_2 concentration, ambient (1×O_3; control and +Phy see "Materials and methods" for details and treatments coding) and twice-ambient O_3 concentrations (2×O_3; +O_3 and +O_3/+Phy), AOT40 as well as SUM0 values in the phytotrons throughout the growing season 2002 (April 23 to October 28) and 2003 (April 28 to September 25). Table adapted from Luedemann et al. 2005

Month	Day/ Night	T air [°C]	PPFD	RH	[CO_2]	1×O_3	2×O_3	AOT40 1×O_3	AOT40 2×O_3	SUM0 1×O_3	SUM0 2×O_3
			[µmol m^{-2} s^{-1}]	[%]	[ppm]	[ppb]	[ppb]	[µl l^{-1} h]	[µl l^{-1} h]	[µl l^{-1} h]	[µl l^{-1} h]
2002											
May	Day	16.4	427.2	54.0	391.1	38.3	69.7	3.3	16.0	59.29	101.71
	Night	12.1	0	73.1	408.1	24.1	43.0	–	–		
June	Day	19.7	473.0	58.4	398.4	34.7	63.7	2.2	13.0	101.42	179.59
	Night	14.9	0	78.6	430.7	18.9	35.8	–	–		
July	Day	20.1	428.8	61.5	382.3	33.4	60.4	1.6	11.0	140.54	250.80
	Night	15.5	0	82.3	421.9	14.4	26.8	–	–		
August	Day	20.6	463.0	54.2	387.5	43.4	76.0	4.2	15.5	186.33	331.04
	Night	14.6	0	77.8	439.8	15.7	27.6	–	–		
September	Day	16.3	386.6	62.2	371.0	25.0	42.6	0.2	3.4	212.54	376.10
	Night	12.0	0	82.9	410.7	12.1	21.2	–	–		
October	Day	13.7	311.9	70.5	392.5	21.3	37.7	0.1	2.5	237.23	420.10
	Night	10.0	0	84.9	403.3	15.7	28.2	–	–		
2003											
May	Day	17.1	424.6	60.4	380.3	36.2	72.4	2.7	15.2	40.63	80.60
	Night	12.4	0	77.4	409.4	21.4	41.6	–	–		
June	Day	17.6	460.2	58.5	386.3	37.7	75.0	3.0	19.5	89.07	176.34
	Night	13.6	0	79.8	430.9	23.9	45.7	–	–		
July	Day	20.4	415.0	58.5	383.0	34.2	70.8	3.0	18.2	134.18	267.90
	Night	15.8	0	77.1	413.8	21.5	40.4	–	–		
August	Day	19.4	392.4	61.7	395.4	32.1	64.3	1.9	13.8	174.34	347.58
	Night	15.2	0	80.9	438.9	18.3	35.3	–	–		
September	Day	18.1	420.5	63.2	393.7	31.0	61.8	1.6	10.4	204.34	405.86
	Night	14.3	0	80.2	435.1	15.3	27.1	–	–		

measured in beech and spruce by means of programmable gas exchange equipment (HCM-1000, open flow CO_2/H_2O porometer equipped with infra-red gas analysers, H. Walz, Effeltrich, Germany). Subsequently, J_{max} and $V_{C,max}$ were calculated from the CO_2 response curves according to von Caemmerer and Farquhar (1981). Measurements in 2002 were taken in the case of beech during June 17th through July 17th and during August 3rd through August 19th, and in the case of spruce during June 19th through July 21st and during Sept. 2nd through Sept. 23rd. In 2003, beech trees were measured during 13th June through 21st July and during 12th August through 12th Sept., and spruce trees during 22nd July through 11th August and during Sep. 2nd through Sept. 23rd. In beech, assessments were performed on fully light exposed leaves. Correspondingly in spruce, early assessments were performed on light-exposed current-year needles.

^{15}N tracer application and quantification of its uptake Three trees per species and O_3 treatment each were harvested to determine their atom% ^{15}N prior to the ^{15}N label application (2nd harvest, Table 1). The remaining containers were irrigated with 3 L of a 0.24 mM double-labelled NH_4NO_3 solution (99% $^{15}NH_4{}^{15}NO_3$, Campro Scientific, Berlin, Germany). Harvested plant material was analyzed in combined element analysers (NA 1108, Carlo Erba, Milan, Italy or EA3000, Euro Vector instruments, Milan, Italy) and isotope ratio mass spectrometers (Deltaplus, Thermo

Electron Corp., Bremen, Germany or IsoPrime, GV-Instruments, Manchester, UK) for their N concentration and ^{15}N atom %. The same reference material was used on both instruments. Subsequently, we calculated the uptake of ^{15}N as $^{15}N_{uptake}=(atom\%^{15}N_{after} - atom\%^{15}N_{before})/100 \bullet N_{cont}$, where $^{15}N_{uptake}$ is the amount of ^{15}N taken up by the trees between the addition of the ^{15}N label and the harvest at the end of the experiment (expressed as grams); atom% $^{15}N_{after}$ is the atom % of ^{15}N in plant biomass at the harvest, atom% $^{15}N_{before}$ is the ^{15}N atom % before ^{15}N label employment and N_{cont} is the sum of ^{14}N and ^{15}N in the plant dry matter expressed as grams at the harvest. For further details see Luedemann et al. (2005).

Statistical analysis Parameters were examined by two-way ANOVA with the factors ozone and *P. citricola* infection for statistical significance at 5%. All data were checked for skewed distribution and transformed appropriately whenever required. In addition, data on biomass fractions (i.e. data with highest integrative power) were used to test for a chamber (phytotron) effect. One (spruce needle biomass) out of the 40 tested parameters displayed such an effect with a *P*-value of 0.04. All tests were conducted with SPSS 12.0 (SPSS Inc., Chicago, IL). Hereafter, we distinguish the expressions "ozone" and "*P. citricola*" from "+O_3" and "+Phy", using the former terminology when indicating the two-way ANOVA factors, but the latter notation when referring to individual treatments, alone or in combination (+O_3/+Phy), relative to the control.

Results

At the final harvest, the total biomass of beech tended to be reduced under all treatments relative to the control (Fig. 2a), i.e. by about 50% under +O_3 and about 30% under +Phy. No additional reduction was found in the combined +O_3/+Phy treatment relative to +O_3 (Fig. 2a). Ozone significantly reduced the root/shoot biomass ratio in beech, with the decline being proportionally larger in fine-root than coarse-root biomass. Except for shoot axes of beech, analysed plant fractions displayed significant limitations in biomass formation under elevated ozone. Inoculation with *P. citricola* resulted in considerable decrease in the biomass formation of beech, failing, however, to reach statistical significance, either at the whole-tree or the organ level (Fig. 2a, Table 3).

Conversely, ozone and *P. citricola* limited the biomass development of spruce only when acting in combination (significant ozone × *P. citricola* interaction, Table 3, Fig. 2b), except for a negative effect of enhanced ozone on needle biomass and a raise in needles to shoot axes biomass ratio in the presence of the pathogen (Fig. 2b, Table 3).

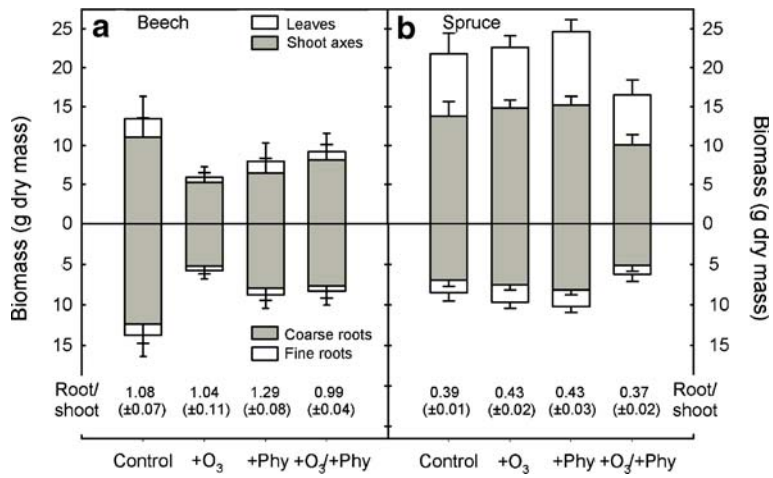

Fig. 2 Biomass of beech (**a**) and spruce (**b**) at the final harvest. Aboveground biomass is represented increasing from the zero line upwards whereas belowground biomass is represented increasing from the zero line downwards. Data on total above- and belowground biomass was reproduced from Luedemann et al. 2005. *Gray* fills represent shoot axes and coarse root biomass. Data are presented as means ± SE, $n=9$ for control and +O_3 and $n=15$ for +Phy and +O_3/+Phy

Table 3 Probability levels of two-way ANOVA for tree biomass, root parameters, ^{15}N uptake, J_{max} and $V_{C,max}$ of beech (a) and spruce (b), considering the factors "ozone", "*P. citricola*", and their interaction

Parameter	Ozone	*P. citricola*	Ozone × *P. citricola*
(a) **Beech**			
Shoot biomass	0.217	0.696	0.101
Root biomass	0.025*	0.491	0.062
Root to shoot	0.028*	0.282	0.100
Coarse root biomass	0.036*	0.556	0.051
Fine root biomass	0.026*	0.378	0.138
Fine to coarse roots	0.038*	0.104	0.816
Leaf biomass	0.025*	0.834	0.281
Shoot axes biomass	0.337	0.670	0.081
Leaves to shoot axes	0.000***	0.731	0.558
Whole tree biomass	0.074	0.409	0.054
Root diameter	0.174	0.372	0.044 *
Root length	0.071	0.663	0.164
Tips per root length	0.130	0.736	0.845
J_{max} 2002 Jun/July	0.877	–[a]	–[a]
J_{max} 2002 Aug/Sept	0.055	0.118	0.475
J_{max} 2003 Jun/July	0.767	0.389	0.254
J_{max} 2003 Aug/Sept	0.037*	0.058	0.045*
$V_{C,max}$ 2002 Jun/July	0.939	–[a]	–[a]
$V_{C,max}$ 2002 Aug/Sept	0.040*	0.893	0.842
$V_{C,max}$ 2003 Jun/July	0.690	0.964	0.062
$V_{C,max}$ 2003 Aug/Sept	0.058	0.188	0.077
^{15}N uptake per root tip	0.021*	0.215	0.955
(b) **Spruce**			
Shoot biomass	0.070	0.437	0.028*
Root biomass	0.120	0.334	0.006**
Root to shoot	0.623	0.615	0.065
Coarse root biomass	0.101	0.411	0.014*
Fine root biomass	0.315	0.192	0.001**
Fine to coarse roots	0.693	0.368	0.006**
Needle biomass	0.018 *	0.868	0.034*
Shoot axes biomass	0.154	0.240	0.035*
Needles to shoot axes	0.691	0.016 *	0.115
Whole tree biomass	0.071	0.383	0.014*
Root diameter	0.882	0.265	0.005**
Root length	0.372	0.318	0.000***
Tips per root length	0.102	0.174	0.832
J_{max} 2002 Jun/July	0.738	–[a]	–[a]
J_{max} 2002 Aug/Sept	0.298	0.325	0.505
J_{max} 2003 Jun/July	0.047 *	0.951	0.622
J_{max} 2003 Aug/Sept	0.024 *	0.542	0.351
$V_{C,max}$ 2002 Jun/July	0.508	–[a]	–[a]
$V_{C,max}$ 2002 Aug/Sept	0.143	0.290	0.693
$V_{C,max}$ 2003 Jun/July	0.370	0.475	0.725
$V_{C,max}$ 2003 Aug/Sept	0.780	0.904	0.290
^{15}N uptake per root tip	0.012 *	0.001 **	0.001**

[a] Plants not yet inoculated with *P. citricola*

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Leaf flushing was delayed in 2003 in both species as compared with 2002. Ozone as a single factor had no significant effect on leaf phenology in either species (Fig. 3). Leaf flush in beech started earlier in 2003 under $+O_3$/+Phy (significant *P. citricola* x ozone interaction) but then evolved more slowly than in the other treatments. In 2003, phenological development of spruce was significantly accelerated by *P. citricola*, in particular towards the end of flushing in June.

In beech, ozone was the major factor significantly lowering the PSII operating efficiency (F_q'/F_m') in shade leaves from July 30, 2002 onwards, and partially also in light-exposed leaves during the 2002 growing season. In 2003, only shade leaves were affected. In the first month upon *P. citricola* inoculation on July 31, 2002, the PSII operating efficiency in beech leaves was reduced (Fig. 4a, b). Spruce was affected neither by ozone nor by the pathogen, while displaying, during both years, higher PSII operating efficiencies in the light-exposed foliage as compared with beech (Fig. 4d).

In beech, irrespective of the treatment, J_{max} was found to be about twice as high as $V_{C,max}$ (Fig. 5a, b). During 2003, both J_{max} and $V_{C,max}$ levels were reduced by about 50 % in August/September relative to the earlier assessments in June/July. The opposite trend was found in spruce during 2002 (rather than 2003) with higher parameter levels observed on current-year needles in August/September than earlier during the season (Fig. 5c, d). Ozone lowered J_{max} in beech in August/September (significant in 2003) and positively affected J_{max} of spruce in 2003 (Fig. 5c, d, Table 3). Likewise, $V_{C,max}$ was significantly reduced by ozone in beech during Aug/Sept 2002. No significant effect by *P. citricola* on either species was observed.

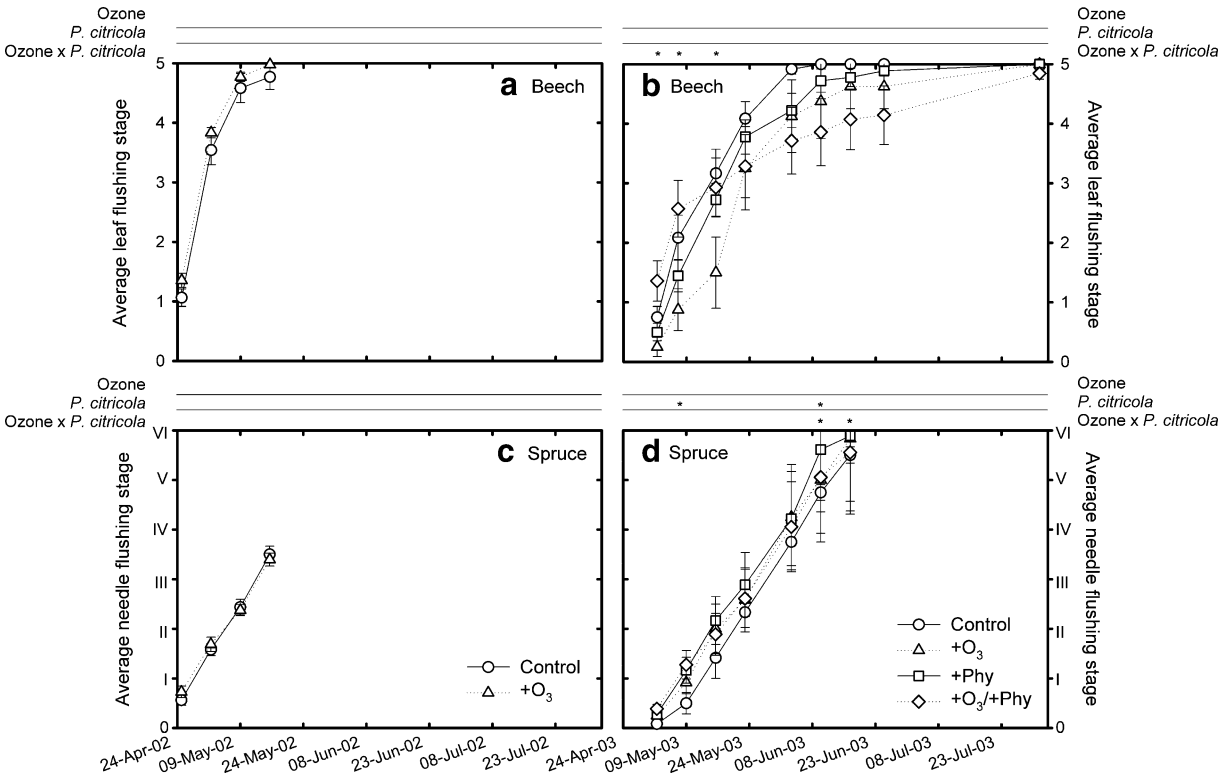

Fig. 3 Flushing of beech (**a**, **b**) and spruce (**c**, **d**) trees during the 2002 (**a**, **c**) and 2003 (**b**, **d**) growing periods. Data are presented as means ± SE (n=12 to 24). Significant effects ($P<$ 0.05) of the factors 'ozone', '*P. citricola*' and their interaction ('ozone x *P. citricola*') are indicated by *. Please see "Materials and methods" for description of flushing stages

Beech fine roots tended to be thicker and shorter across the treatments in relation to the control (Fig. 6). Although the interaction between *P. citricola* and ozone was significant regarding root diameter, ozone did not change the response of beech roots to pathogen infection (Fig. 6, Table 3). In general, fine roots of spruce were thicker than in beech. Spruce fine-roots under $+O_3$ and +Phy were thinner and longer as compared with those of the control (Fig. 6), whereas the combined $+O_3$/+Phy treatment restricted root length compared with the individual treatments (significant O_3 × *P. citricola* interaction, Table 3).

In both species, the number of root tips was a function of root length and was not affected by either treatment (Fig. 7, Table 3). Nevertheless, the physiological activity of beech roots with respect to nitrogen uptake, assessed as ^{15}N uptake per root tip was significantly reduced by ozone (Fig. 8a). However, in spruce the combined $+O_3$/+Phy treatment stimulated ^{15}N uptake per root tip, as indicated by a significant interaction of ozone and *P. citricola* (Table 3, Fig. 8b).

Discussion

Based on GDB and the known influences of tropospheric O_3 on tree growth, we hypothesized acclimation to elevated O_3 levels to counteract adverse effects of *P. citricola* infection on tree performance. We expected beech and spruce to be differentially affected by the pathogen upon continued exposure to $2\times O_3$ due to the different O_3 sensitivities of the two species (Lippert et al. 1996; Grams et al. 1999; Kozovits et al. 2005a, b).

In the present study, we found limited fine root and leaf biomass production of juvenile beech under elevated O_3 (cf. Lippert et al. 1996; Winkler et al. 2009) to be related to lowered photosynthetic performance as reflected by PSII operating efficiency, J_{max} and $V_{C,max}$ along with delayed leaf flushing in spring (Figs. 3, 4 and 5). Conversely, biomass production of beech in the presence of the root rot pathogen *P. citricola* was reduced in the absence of decreased photosynthetic performance. Beech trees growing under the combined $+O_3$/+Phy treatment even performed

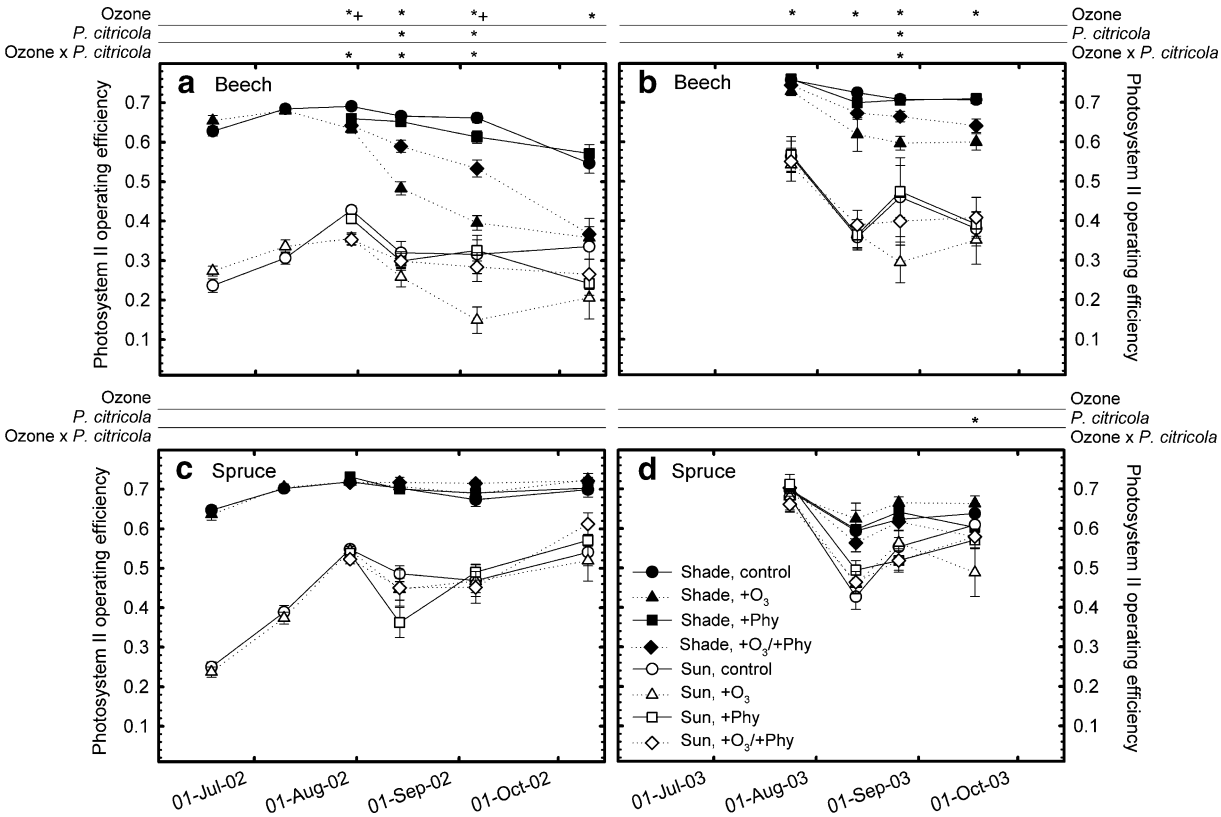

Fig. 4 Photosystem II operating efficiency (Fq'/Fm') in beech (**a**, **b**) and spruce leaves (**c**, **d**) during 2002 (**a**, **c**) and 2003 (**b**, **d**) growing periods. Data are presented as means ± SE, n=7 to 79. Significant effects (P<0.05) of the factors 'ozone', '*P. citricola*' and their interaction ('ozone × *P. citricola*') for leaves in the shade and sun crown are indicated by * and +, respectively

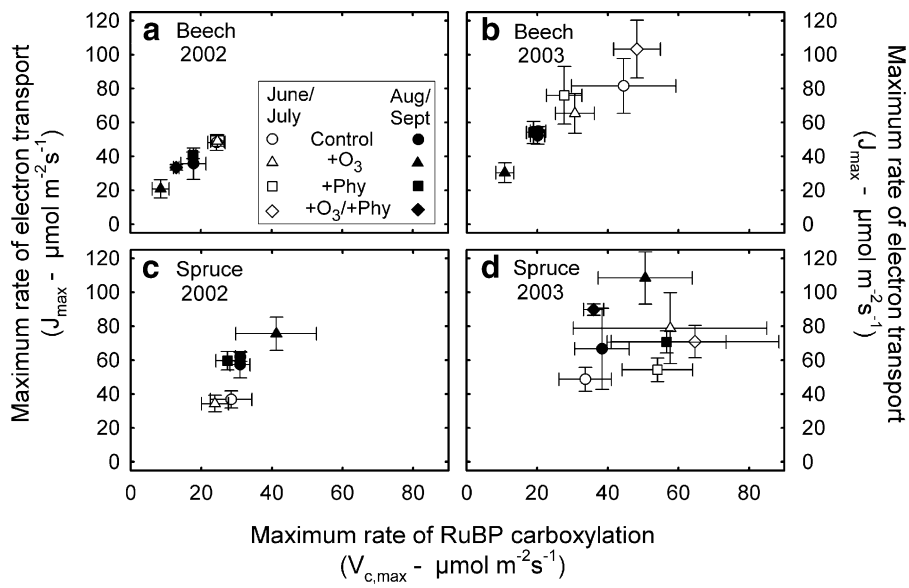

Fig. 5 Maximum rate of RuBP carboxylation ($V_{c,\,max}$) and electron transport rate at saturated light (J_{max}) of beech leaves (**a**, **b**) and spruce needles (**c**, **d**) during the 2002 (**a**, **c**) and 2003 (**b**, **d**) growing periods

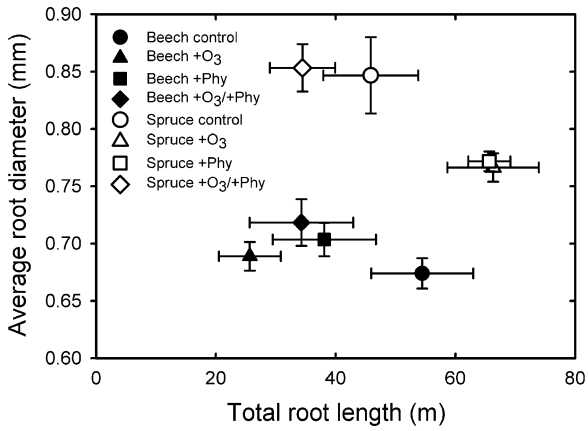

Fig. 6 Average root diameter vs. total root length in fine roots at the end of the experiment. *Closed* and *open symbols* correspond to beech and spruce trees, respectively. Data are presented as means ± SE, n=9 for control (*circles*) and +O_3 (*triangles*), and n=15 for +Phy (*squares*) and +O_3/+Phy (*diamonds*)

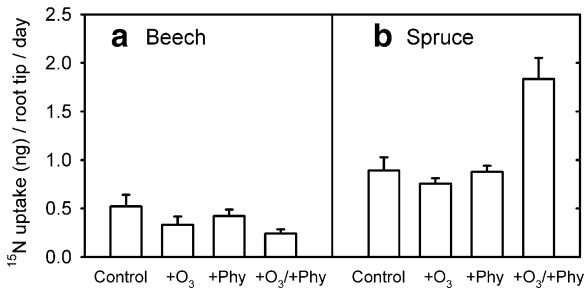

Fig. 8 Daily ^{15}N uptake per root tip of beech (**a**) and spruce (**b**). Data are presented as means ± SE, n=9 for control and +O_3, and n=15 for +Phy and +O_3/+Phy

better in terms of PSII operating efficiency in comparison to trees that were exposed only to +O_3. Such findings are consistent with increased biomass at the end of the experiment under +O_3/+Phy relative to +O_3 (Fig. 2). Hence, we conclude in the case of beech that additional metabolic efforts must have prevailed under +O_3/+Phy (such as enhanced respiration and antioxidative defense, cf. Berger et al. 2007) at the expense of growth to overcome the adverse impact represented by +O_3 per se. In addition, enhanced [O_3] may have caused raised levels of non-structural carbohydrates (NSC) in leaves due to sink strength

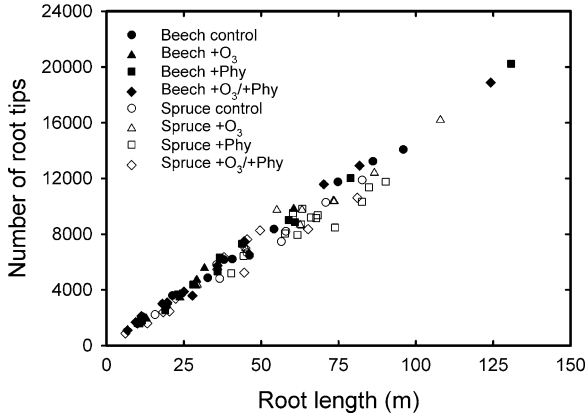

Fig. 7 Number of root tips vs. root length at the end of the experiment. *Closed* and *open symbols* correspond to beech and spruce trees, respectively. Data from control treatment are represented by *circles*, +O_3 by *triangles*, +Phy by *squares* and +O_3/+Phy by *diamonds*

limitation by constrained root growth and impaired phloem loading (Grantz and Farrar 2000) which would lead to feedback inhibition of photosynthesis in beech leaves. Indeed, such phloem impairment was also reflected by starch accumulation along leaf veins (Matyssek et al. 1992). Under infection with *P. citricola* this inhibition may be diminished by enhanced C needs for defense as well as repair (cf. Fig. 4). However, in an earlier, similar experiment we observed slightly reduced levels of NSC in beech leaves under elevated O_3 (Liu et al 2004). In contrast to beech neither biomass development nor photosynthetic performance of spruce trees were adversely affected by the pathogen or elevated O_3 treatment, reflecting the lower O_3 susceptibility of this latter species (Kozovits et al. 2005a; Grams et al. 2007). Moreover in the following year, J_{max} was even significantly enhanced by ozone during the assessment periods (Table 3).

The findings on the photosynthetic performance and leaf phenology of juvenile trees under elevated O_3 are similar to effects in adult trees under field conditions (Nunn et al. 2005; Löw et al. 2007). Moreover, in agreement with adult beech trees exposed to 2×O_3 (Haberer et al. 2007; Matyssek et al. 2007), also in the present study elevated ozone did negatively influence the ^{15}N uptake rate on a root tip (as presented in Fig. 8) as well as on a root biomass basis (Luedemann et al. 2005). Spruce did not display negative effects on root length and morphology as well as on ^{15}N uptake, neither caused by elevated ozone nor pathogen infection. However, under +O_3/+Phy the ^{15}N uptake was considerably enhanced (Luedemann et al. 2005). Bearing in mind that trees grew in a competitive design experiment, the weaker belowground competitiveness of the one species (beech, Kozovits

et al. 2005b; Grams et al. 2007) might enhance the resource availability to the other competing species (spruce). Thus assuming unimpaired functionality of root tips, the increase of resource (N) availability might have raised the N uptake in spruce (cf. Schwinning 1996; Kozovits et al. 2005a; Grams and Andersen 2007; Grams and Matyssek 2009). Enhanced N uptake might have fostered concomitant occurrence of disease symptoms in spruce. Such an effect would be in line with the majority of available evidence as improved N supply may increase vulnerability to fungal diseases (Walters and Bingham 2007; Witzell and Martin 2008). Likewise, an N fertilization experiment with beech and spruce (Tomova et al. 2005) turned out defense-related compounds such as fungistatic phenolics to be limited in spruce even under a minor increase of N availability. Unlike beech, it appears that spruce has the capacity of adequately coping with oxidative stress, however, only if exerted either by ozone or the pathogen. Both stressors do elicit same primary mechanisms of defense (Matyssek and Sandermann 2003), rendering ozone a kind of "abiotic model pathogen" (Matyssek et al. 2005a, b). However, the metabolic effort of spruce in achieving the "ecological gain" of oxidative stress tolerance of one stressor may lead, as a kind of trade-off and upon continued strain, to "ecological costs" in terms of susceptibility to the other stressor (Heil and Baldwin 2002), in particular, if occurring with delay, and although same defense mechanisms may be involved. In the field, chronic O_3 stress can in fact precede pathogen infection, being the scenario that perhaps raises N demand, but concurrently impairs spruce. It must remain open, however, to what extent the susceptibility of adult spruce trees would be affected by the applied treatments, as to our knowledge impairment of spruce in the field by *P. citricola* has, at least to date, not become apparent.

In view of GDB, spruce tended to grow at a rate proportional to the resource availability, as the latter was increased due to the reduced growth of the competitor beech. As a consequence, spruce tended to become susceptible to the pathogen. Conversely, beech showing growth limitation under enhanced [O_3] conveyed increased resistance against the pathogen at concurrent inoculation (+O_3/+Phy)—i.e. in the absence of additional constraint by infection under elevated O_3. These findings are in agreement with Loomis (1953a, b) if it is presupposed that the decline in growth is larger, in proportion, than that of photosynthesis, i.e. the latter not being the primary factor limiting growth. It is conceivable that the enhancement of pathogen resistance in beech is mediated through mechanisms incited by elevated O_3, as the latter causes defense responses of the plant similar to those against pathogenic elicitors (Heagle 1973; Sandermann et al. 1998). Likewise, O_3-induced limited root growth in beech was paralleled by lower specific fine root length (SRL, data not shown). This may be caused by a higher abundance of older and/or suberized roots under enhanced O_3 which is, in addition, known to increase lignin concentrations in whole plant biomass and roots (Bonello et al. 1993; Booker 2000; Szantoi et al. 2007). As a result, roots of beech trees grown under elevated O_3 may display increased resistance against *P. citricola*. This interpretation is supported by findings of Cahill and McComb (1992) who compared defense responses of two Eucalyptus trees of contrasting susceptibility to *P. cinnamomi* and concluded that inhibition of the pathogen in the resistant species was related to a distinct induction of PAL which, as a consequence, initiated lignin synthesis and accumulation of phenolic compounds in the infected tissue.

In the present case, such an interpretation would hold for beech but not so for spruce, although the response of both tree species appears to be in line with GDB. One has to bear in mind, however, that direct testing of complex plant defense hypotheses such as GDB is not a trivial task if not impossible (Stamp 2004) which to some extent also applies to examining sub-hypotheses as presented here. Moreover, the present paper only indirectly allows for conclusions on plant defense metabolism as the involvement of secondary metabolites was not addressed. Nevertheless, the presented data on biomass development and physiological performance of the plants support our hypothesis that exposure to elevated O_3 reduces adverse effects of *P. citricola* infection on trees in the case of beech, but not so in the case of spruce.

The adaptive potential of plant and pathogen populations to a changing environment is of great importance for the prediction of future environmental scenarios (Garrett et al. 2006). Hence, Chakraborty et al. (2008) underline the necessity of integrating data on plant disease into models that project the fate of agricultural, forest and natural ecosystems under changing atmospheric conditions. Data presented in

this study, focusing on two ecologically and economically important Central-European trees, reflect contrasting responsiveness to atmospheric and biotic stressors and their interaction. Our conclusions coincide with GDB, which continues to be a promising instrument for modeling plant performance under stress scenarios of a changing environment (cf. Matyssek et al. 2005a, b; Gayler et al. 2008), even though the existence of a trade-off between growth and stress defense may be species-specific.

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