Acclimation to Ozone Affects Host/Pathogen Interaction and Competitiveness for Nitrogen in Juvenile *Fagus sylvatica* and *Picea abies* Trees Infected with *Phytophthora citricola*

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Abstract: In a two-year phytotron study, juvenile trees of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) were grown in mixture under ambient and twice ambient ozone (O₃) and infected with the root pathogen *Phytophthora citricola*. We investigated the influence of O₃ on the trees’ susceptibility to the root pathogen and assessed, through a ¹⁵N-labelling experiment, the impact of both treatments (O₃ exposure and infection) on belowground competitiveness. The hypotheses tested were that: (1) both *P. citrica* and O₃ reduce the belowground competitiveness (in view of N acquisition), and (2) that susceptibility to *P. citrica* infection is reduced through acclimation to enhanced O₃ exposure. Belowground competitiveness was quantified via cost/benefit relationships, i.e., the ratio of structural investment in roots relative to their uptake of ¹⁵N. Beech had a lower biomass acquisition and captured less ¹⁵N under enhanced O₃ and *P. citrica* infection alone than spruce, whereas the latter species appeared to profit from the lower resource acquisition of beech in these treatments. Nevertheless, in the combined treatment, susceptibility to *P. citrica* of spruce was increased, while beech growth and ¹⁵N uptake were not further reduced below the levels found under the single treatments. Potential trade-offs between stress defence, growth performance, and associated nitrogen status are discussed for trees affected through O₃ and/or pathogen infection. With respect to growth performance, it is concluded that O₃ enhances susceptibility to the pathogen in spruce, but apparently raises the defence capacity in beech.

Key words: Ozone (O₃), European beech (*Fagus sylvatica*), Norway spruce (*Picea abies*), competition, pathogen resistance, N-15 labelling.

Introduction

Already, in 1973, Heagle stressed the importance of unraveling the interactions between plant responses to air pollutants and pathogen defence. Nevertheless, even after three decades of continued research (Manning and von Tiedemann, 1995; von Tiedemann and Firsching, 1998; Karnosky et al., 2002), such interactions have not been clarified to an extent that would allow understanding of ecophysiological principles regarding effects, in particular, of ozone (O₃) and *Phytophthora* species on forest trees (Matyssek and Sandermann, 2003; Laurence and Anderson, 2003).

O₃ is considered to be the potentially most phytotoxic air pollutant (e.g., Lefohn, 1992; Skárby et al., 1998). During the last decade, surface O₃ concentrations have increased on average by 1 – 2% each year (Stockwell et al., 1997), and O₃ levels are expected to stay high during the forthcoming decades (Fowler et al., 1999; Fabian, 2002; Ashmore, 2005). Adverse O₃ effects on trees include foliar injuries, premature leaf loss, reduced growth (Matyssek and Sandermann, 2003; Matyssek and Innes, 1999), and limited belowground carbon allocation (Anderson, 2003; Samuelson and Kelly, 2001). At the same time, O₃ is known to elicit plant responses typically associated with pathogen defence, including biosynthesis of lignin, increased phenylalanine ammonia-lyase (PAL) activity, as well as accumulation of phenolic compounds and pathogen-related proteins (Heagle, 1973; Heller et al., 1990; Sandermann et al., 1998; Matyssek and Sandermann, 2003). Cahill and McComb (1992) compared defence responses of two *Eucalyptus* species of contrasting susceptibility to *P. cinnamomi* and concluded that inhibition of the pathogen in the resistant species was due to a distinct induction of PAL which, as a consequence, initiated lignin synthesis and accumulation of phenolic compounds in the infected tissue.

*P. citrica* is an oomycete known to cause root rot across a broad range of plant hosts (Erwin and Ribeiro, 1996), including European beech (*Fagus sylvatica*; Fleischmann et al., 2002, 2004; Werres, 1995). Although affecting beech on a regional scale (Jung and Blaschke, 1996), *P. citrica* has not yet been found to infect Norway spruce (*Picea abies*) in the field (Nechwatal and Oßwald, 2001). Nevertheless, under laboratory conditions, spruce seedlings infected with *P. citrica* die or sustain severe root injury (Nechwatal and Oßwald, 2001). Therefore, development of juvenile spruce trees beyond the seedling stage is likely to be affected by *P. citrica* infection.

Since infection with *P. citrica* causes malfunctioning and rot of the roots, constrained belowground competitiveness of infected plants is to be expected. Likewise, O₃ causes trees to reduce their carbon allocation to the soil compartment and,
again, restricted competitiveness for belowground resources may result (Andersen, 2003). However, acclimation to O₃, which can elicit defence responses in plants (Sandermann et al., 1998), might reduce the susceptibility of trees to pathogen attack. With the objective of clarifying belowground competitiveness, the ¹⁵N uptake of juvenile beech and spruce trees that experienced disturbance by O₃ exposure and/or *P. citricola* inoculation was studied, in a phytotron experiment, through an interspecific competitive set-up (cf. Grams et al., 2002; Kozovits et al., 2005a, b). The hypotheses tested were that: (1) both *P. citricola* and O₃ reduce the belowground competitiveness of beech and spruce (in view of N acquisition), and (2) that susceptibility to *P. citricola* infection (and, therefore, impeded belowground competitiveness) is reduced through acclimation to enhanced O₃ exposure. Belowground competitiveness was quantified (according to Grams et al., 2002; Kozovits et al., 2005a) via cost/benefit relationships, i.e., the ratio of structural investment in (fine) roots relative to their uptake of ¹⁵N.

**Materials and Methods**

**Plants, climate conditions, and treatments**

In spring 2001, 1- and 2-year-old seedlings of European beech (*Fagus sylvatica* L., seed source 810-24 Bad Griesbach) and Norway spruce (*Picea abies* [L.] Karst., seed source 840-27 Altötting) were planted in containers (area of 0.7 × 0.4 m, soil depth of 0.3 m) which had been filled with untreated soil (dystric cambisol, Ah-B horizon, 540 m a.s.l.; see Kreutzer et al., 1991) from Höglwald Forest near Augsburg, Germany (Table 1 summarizes the sequence of experimental procedures). Twenty trees (arranged in rows as 4 × 5 individuals) were planted in an alternating pattern into each of 64 containers (1-to-1 beech/spruce mixtures, Fig. 1). To account for potential edge effects, all assessments were conducted only on the six central individuals in each container (cf. Grams et al., 2002).

After a first growing season (year 2001) in a climate-controlled greenhouse, 32 containers were selected for uniform tree height and transferred into four walk-in phytotrons (size ca. 2.8 × 3.4 m, at GSF National Research Centre for Environment and Health, Neuherberg near Munich, Germany) during the growing seasons of 2002 and 2003. During the winter months, plants were kept outside. Each phytotron contained four Plexiglas chambers (size: ca. 0.8 × 1.1 × 1.0 m) and adequate ventilation (150 m³ h⁻¹) to avoid significant temperature increase.

**Table 1** Experimental sequence

<table>
<thead>
<tr>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr 2001</td>
<td>Planting 1-year-old beech and 2-year-old spruce into 64 containers filled with forest soil (20 plants/container)</td>
</tr>
<tr>
<td>Apr 2001 – Oct 2001</td>
<td>Plants growing inside a climate-controlled glasshouse under ambient ozone regime</td>
</tr>
<tr>
<td>Nov 2001 – Apr 22, 2002</td>
<td>Plants kept outdoors</td>
</tr>
<tr>
<td>Apr 23, 2002</td>
<td>Selection of 34 containers for uniform tree height 1st harvest – 2 containers used for initial biomass assessment</td>
</tr>
<tr>
<td></td>
<td>Transfer of 32 containers into phytotrons (beginning of O₃ fumigation)</td>
</tr>
<tr>
<td>Jul 30, 2002</td>
<td>2nd harvest – 4 containers used, 2 from control and 2 from +O₃ treatment (28 containers remaining in phytotron)</td>
</tr>
<tr>
<td>Jul 31, 2002</td>
<td>Inoculation with <em>P. citricola</em> in containers of +Phy and +O₃/+Phy treatment</td>
</tr>
<tr>
<td>Sep 18, 2002</td>
<td>3rd harvest – 8 containers used, 2 containers per treatment (20 containers remaining in phytotron)</td>
</tr>
<tr>
<td>Oct 28, 2002 – Apr 27, 2003</td>
<td>Plants kept outdoors</td>
</tr>
<tr>
<td>Apr 28, 2003</td>
<td>Plants transferred back into phytotrons (O₃ fumigation experiment continued)</td>
</tr>
<tr>
<td>Jul 14, 2003</td>
<td>4th harvest (assessing ¹⁵N atom% prior to labelling) – 4 containers used, 1 container per treatment (16 containers remaining in phytotron)</td>
</tr>
<tr>
<td>Jul 16, 2003</td>
<td>Enhancement of <em>P. citricola</em> infestation</td>
</tr>
<tr>
<td>Jul 30, 2003</td>
<td>¹⁵N labelling with double-labelled NH₄NO₃</td>
</tr>
<tr>
<td>Sep 25, 2003</td>
<td>Final harvest – 16 containers (3 of control, 3 of +O₃, 5 of +Phy, 5 of +O₃/+Phy treatment)</td>
</tr>
</tbody>
</table>

**Fig. 1** Experimental set-up in the phytotrons of the GSF National Research Centre for Environment and Health. Each phytotron comprised four Plexiglas sub-chambers (for individual O₃ fumigation) with (Phytotron 3 and 4) or without *Phytophthora* inoculation (Phytotron 1 and 2). 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. Spacing of beech (B) and spruce (S) seedlings in a planting container is shown to the right. The position of the studied trees (six central trees per container) is highlighted.
in the sub-chambers (cf. Kozovits et al., 2005 a; Payer et al., 1993).

The varying climate conditions and O₃ regimes recorded in 1998 and 1999 at the study site "Kranzberg Forest" (see Pretzsch and Dursky, 2002; Nunn et al., 2002) were reproduced in the phytotrons on an hourly basis throughout the seasonal courses of 2002 and 2003. Plants were exposed to either ambient (1 × O₃) or experimentally enhanced O₃ levels (2 × O₃; restricted to < 150 nl l⁻¹). Air temperature, photosynthetic photon flux density (PPFD), relative humidity, CO₂ concentration, the two O₃ regimes, and resulting AOT 40 (sum of the hourly O₃ concentrations above 40 ppb during daytime hours, Fuhrer and Achermann, 1999) are presented on a monthly basis in Table 2.

Three tensiometers (Model T5, UMS, Munich, Germany) in each container continuously monitored soil moisture at a depth of 11 cm and triggered irrigation with de-ionized water whenever soil water tension reached 400 hPa. In accordance with previous experiments, plantations were supplied with 1 l of double-concentrated Hoagland solution each (Hoagland and Arnon, 1950; cf. Kozovits et al., 2005 a) four times during the growing season of 2002 to provide tissue nutrient levels similar to those found in trees of Bavarian forests (cf. Kreutzer et al., 1991). During the second growing season (2003), the nutrient solution was added three times, substituting the fourth fertilization by the ¹⁵N tracer application (see below). Beech foliar N concentration at the end of the experiment averaged 19.2 mg/g, which is considered low but not deficient (Wolff and Riek, 1999; Bergmann, 1993). Spruce foliar N concentration was about 15.5 mg/g, regarded as a representative average level (Bergmann, 1993). The average or low foliar N levels suggest this nutrient to be of relevance in competition.

At the end of July 2002, in two of the four phytotrons a total of 16 containers were inoculated with the root pathogen *P. citricola* (Table 1, for details see below), while each phytotron contained four Plexiglas chambers for additional O₃ fumigation (two each with 1 × O₃ or 2 × O₃, each chamber with two planting containers, 20 plants/container; Fig. 1). In this way, four O₃/*P. citricola* regimes were established: (1) 1 × O₃/non-inoculated (in the following referred to as "control"). (2) 2 × O₃/non-inoculated = "+O₃", (3) 1 × O₃/*P. citricola*-inoculated = "+Phy" and (4) 2 × O₃/*P. citricola*-inoculated = "+O₃/+Phy". This set-up was

### Table 2: Monthly mean values of air temperature (T air), photosynthetic photon flux density (PPFD), relative humidity (RH), CO₂ concentration, 1 × O₃, 2 × O₃, AOT40 in the phytotrons throughout the growing seasons of 2002 (April 23 to October 28) and 2003 (April 28 to September 25). The ambient O₃ regime was representative for southern Germany (cf. Nunn et al., 2002)

<table>
<thead>
<tr>
<th>Month</th>
<th>Day/night</th>
<th>T air (°C)</th>
<th>PPFD (μmol m⁻² s⁻¹)</th>
<th>RH (%)</th>
<th>CO₂ (μl l⁻¹)</th>
<th>1 × O₃ (nl l⁻¹)</th>
<th>2 × O₃ (nl l⁻¹)</th>
<th>AOT40 (μl l⁻¹ h)</th>
<th>AOT40 (μl l⁻¹ h)</th>
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<td>427.2</td>
<td>54.0</td>
<td>391.1</td>
<td>38.3</td>
<td>69.7</td>
<td>3.3</td>
<td>16.0</td>
</tr>
<tr>
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<td>12.1</td>
<td>0</td>
<td>73.1</td>
<td>408.1</td>
<td>24.1</td>
<td>43.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Jun day</td>
<td>19.7</td>
<td>473.0</td>
<td>58.4</td>
<td>398.4</td>
<td>34.7</td>
<td>63.7</td>
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<td>13.0</td>
</tr>
<tr>
<td></td>
<td>night</td>
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<td>0</td>
<td>78.6</td>
<td>430.7</td>
<td>18.9</td>
<td>35.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Jul day</td>
<td>20.1</td>
<td>428.8</td>
<td>61.5</td>
<td>382.3</td>
<td>33.4</td>
<td>60.4</td>
<td>1.6</td>
<td>11.0</td>
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<td>15.5</td>
<td>0</td>
<td>82.3</td>
<td>421.9</td>
<td>14.4</td>
<td>26.8</td>
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<td>–</td>
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<tr>
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<td>20.6</td>
<td>463.0</td>
<td>54.2</td>
<td>387.5</td>
<td>43.4</td>
<td>76.0</td>
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<td>15.5</td>
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<tr>
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<td>0</td>
<td>77.8</td>
<td>439.8</td>
<td>15.7</td>
<td>27.6</td>
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<td>–</td>
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<tr>
<td></td>
<td>Sep day</td>
<td>16.3</td>
<td>386.6</td>
<td>62.2</td>
<td>371.0</td>
<td>25.0</td>
<td>42.6</td>
<td>0.2</td>
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<td>82.9</td>
<td>410.7</td>
<td>12.1</td>
<td>21.2</td>
<td>–</td>
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<tr>
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<td>Oct day</td>
<td>13.7</td>
<td>311.9</td>
<td>70.5</td>
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<td>21.3</td>
<td>37.7</td>
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<tr>
<td>2003</td>
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<td>17.1</td>
<td>424.6</td>
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<td>409.4</td>
<td>21.4</td>
<td>41.6</td>
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<td>–</td>
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<tr>
<td></td>
<td>Jun day</td>
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<td>58.5</td>
<td>386.3</td>
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<td>75.0</td>
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<td>79.8</td>
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<td>45.7</td>
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<td>–</td>
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<tr>
<td></td>
<td>Jul day</td>
<td>20.4</td>
<td>415.0</td>
<td>58.5</td>
<td>383.0</td>
<td>34.2</td>
<td>70.8</td>
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<td>18.2</td>
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<tr>
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<td>413.8</td>
<td>21.5</td>
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<tr>
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<td>80.9</td>
<td>438.9</td>
<td>18.3</td>
<td>35.3</td>
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<td>–</td>
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<td>Sep day</td>
<td>18.1</td>
<td>420.5</td>
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<td>1.6</td>
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<tr>
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<td>14.3</td>
<td>0</td>
<td>80.2</td>
<td>435.1</td>
<td>15.3</td>
<td>27.1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
independently replicated across the four phytotrons (i.e., 2 + 2 phytotrons, Fig. 1). Mycorrhization was present in all containers (Pritsch et al., 2005).

**Plant biomass assessment**

Six central trees per container were harvested on five different dates throughout the experiment in 2002 and 2003 (Table 1). At the end of the first growing season in September 2002, 9 plants per species and treatment were harvested (corresponding to 3 central plants of each species in three planting containers per treatment). At the end of the experiment, in September 2003, 9 trees per species in the control and +O3 treatment each and 15 trees per species in the +Phy and +O3/+Phy treatment each were harvested. Tree biomass was separated by organs: foliage (divided into current-year and older needles for spruce and first and second flush for beech), stems, and branches (in sum as non-green aboveground biomass, hereafter referred to as “shoot axes” biomass), and roots. The latter were further separated into fine roots (<2 mm in diameter) and coarse roots (>2 mm in diameter). Dry mass (DM) was determined of each biomass fraction. After harvest, DNA was extracted from 20 mg of dried and pulverized root material using the Plant DNeasy Minikit (Qiagen, Hilden, Germany) as master mix for the PCR reaction. The DNA was further purified using the Wizard DNA purification system (Promega, Mannheim, Germany) and finally resolved in 100 μl of water. *P. citricola* DNA was quantified using a nested PCR, with the first PCR step being performed on a conventional thermocycler (T-Gradient, Biometra, Göttingen, Germany). In the first PCR step (30 cycles, 94°C for 20 s, 59°C for 30 s, and 67°C for 20 s), 2 μl DNA were amplified with the primers P5 and P6 (each 0.3 μM) together with the Taqman probe F3 (0.2 μM; Böhm et al., 1999) using the ABSolute QPCR ROX Mix (ABgene, Hamburg, Germany) as master mix for the PCR reaction. The Ct values and initial DNA concentration were calculated with the ABI prism sequence detection system software (version 1.6.3).

**Application of 15N tracer**

On July 30, 2003, three trees per species and O3 treatment each were harvested to determine their atom % 15N value prior to the 15N label application (2nd harvest, Table 1). The remaining 16 containers (three of the control and +O3 treatment each and five containers per +Phy and +Phy/+O3 treatment) were irrigated with 3 l of a 0.24 mM double-labelled NH4NO3 solution (99% 15NH415NO3, Campro Scientific, Berlin, Germany). In this way, 76.45 mg/m2 of 15N was applied and each container received 21.41 mg of 15N.

**15N mass spectrometry analysis**

All biomass fractions of dried plant material were ground to a fine powder and analyzed in combined element analyzers (EA3000, Euro Vector instruments and software, Milan, Italy and NA 1108, Carlo Erba, Milan, Italy) and isotope ratio mass spectrometers (Isoprime, GV-Instruments, Manchester, UK, and Delta_plus, Thermo Electron Corp., Bremen, Germany) for their N concentration and 15N atom % using identical reference material on all machines.

**Calculation of 15N uptake**

Uptake of 15N was calculated as

$$\text{15N uptake} = \left( \frac{\text{atom}\%^{15N}_{\text{after}} - \text{atom}\%^{15N}_{\text{before}}}{100 \cdot \text{N}_{\text{cont}}} \right) \cdot \text{N}_{\text{cont}}$$

where 15N uptake is the amount of 15N taken up by the trees between the addition of the 15N label and the harvest at the end of the experiment, expressed as grams; atom%15N, is the atom % of 15N in plant biomass at the harvest, atom%15N before is the 15N atom % before 15N label employment and N_cont is the total amount of N (all isotopes) in the plant expressed as grams at the time of harvest.

**Quantification of competitiveness**

Competitiveness was quantified by cost/benefit ratios that relate biomass investment (here: root mass) to resource gain (here: 15N uptake). Such ratios were postulated earlier (Küppers, 1984; Sprugel et al., 1991) and experimentally tested in preceding studies (Grams et al., 2002; Kozovits et al., 2005b; Reiter et al., 2005).

**Statistical analysis**

Analysis of variance (ANOVA) was used to test for potential chamber and container effects on assessed tree parameters. As analysis yielded absence of significant chamber and container effects, individual trees were used as experimental units during subsequent statistical treatment. All data were checked for skewed distribution and transformed appropriately whenever required. For the specific comparison between +Phy and +O3/+Phy *P. citricola* DNA amount in fine roots, a t-test was employed for examination of statistical significance. All other investigated parameters were examined by two-way ANOVA with the factors ozone and *P. citricola* infection for a significance level of 5% (Zar, 1999). Tests were conducted with SPSS 12.0 (SPSS Inc., Chicago, IL). Hereafter, we distinguish the expressions “ozone” and “*P. citricola*” from “+O3” 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 (+O3/+Phy), relative to the control.
Table 3  
P. citricola infection of fine roots harvested in September 2003. Infection was quantified with real-time quantitative PCR and expressed as ng P. citricola DNA per g fine root. *-Test between +Phy and +O₃/+Phy yielded p = 0.845 for beech and 0.097 for spruce.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+O₃</th>
<th>+Phy</th>
<th>+O₃/+Phy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beech</td>
<td>0.00</td>
<td>0.00</td>
<td>1.47</td>
<td>1.26</td>
</tr>
<tr>
<td>Spruce</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Results

P. citricola infection

The success of the P. citricola infection was confirmed in all P. citricola-treated plant containers throughout the entire experiment (Table 3). P. citricola was re-isolated from baiting leaves taken after the first flooding treatment, although the frequency of re-isolation was lower from soil samples taken during winter and upon the second flooding. P. citricola could neither be re-isolated from the control nor the +O₃ containers. The total amount of P. citricola DNA found in fine roots of beech was higher than in spruce. Ozone did not affect the infection rate of beech, but tended to increase the amount of P. citricola DNA in spruce fine roots (Table 3). In coarse roots, P. citricola was detectable only when the fine root infection was high (data not shown).

Whole plant biomass and N status

At the end of the experiment, the total biomass of beech was lower across the treatments relative to the control (Fig. 2a). Growth under +O₃ was about 50% lower, whereas the presence of P. citricola caused a reduction of 30%. Remarkably, the +O₃/+Phy treatment did not further reduce the biomass production of beech (Fig. 2a). Conversely, ozone and P. citricola limited biomass development of spruce only in combination (significant ozone x P. citricola interaction, Table 4; Fig. 2b).

Beech root biomass changed in proportion to whole tree biomass, with similar significances for all factors (Table 4). P. citricola and ozone alone did not significantly change spruce root biomass (Table 4b), while a significant negative ozone x P. citricola effect on spruce roots and whole tree biomass was observed (Table 4a).

According to Imo and Timmer (1992), the relation of whole plant N concentration versus N content allows conclusions on plant N use for growth (Fig. 3). N balanced growth is represented in total plant biomass (i.e., of beech and spruce) per container, independent of treatments. During the 56-day period since the application of the ¹⁵N label to the soil (July 30 through September 25, 2003), beech trees of the control took up twice the amount of ¹⁵N compared with those of the different treatments (Fig. 4a; main effect of ozone, Table 4a). Since ¹⁵N was added towards the end of the growing season, only a minor portion of the total uptake of ¹⁵N was invested into leaves (max. of 6.1% under +O₃/+Phy, Table 5a). The amount of ¹⁵N invested into shoot axes ranged between 25 and 38%. Investment into roots was highest under +O₃ and +Phy (70.6 and 70.4%, respectively) and lowest under +O₃/+Phy (56.2%).

The total amount of ¹⁵N uptake by spruce exceeded that of beech under impacts of +O₃ and/or +Phy (Fig. 4b). Under such latter conditions, spruce increased its uptake by more than 25% relative to the control and complementary to beech. Compared with beech, spruce invested somewhat less ¹⁵N into roots (55.3% of total uptake in the control, 54.1% at +O₃ and 51.3% at +Phy), in particular under +O₃/+Phy (43.8%, Table 5b). Evergreen spruce incorporated significant amounts of acquired ¹⁵N into current-year and old needles. The lower percentage (related to whole tree uptake) of ¹⁵N that was recovered belowground under +O₃/+Phy compared with the control in spruce was due mainly to a P. citricola effect (Table 4b).
To analyze the cost/benefit relationship of $^{15}$N uptake, as a measure of belowground competitiveness, the amount of $^{15}$N taken up per fine root biomass was calculated (Fig. 5). Only ozone had a negative main effect (Table 4a) on the belowground competitiveness of beech for $^{15}$N (Fig. 5a). However, both ozone and infection with \emph{P. citricola}, in particular, when in combination (+O$_3$/+Phy), did significantly increase the competitiveness of spruce for $^{15}$N (ozone, \emph{P. citricola}, and ozone × \emph{P. citricola} main effects; Table 4b, Fig. 5b). Considering the $^{15}$N uptake per total root biomass (Figs. 5c,d), beech was more efficient in the control compared with the other treatments (significant ozone effect, Table 4a). On the other hand, spruce had a higher $^{15}$N uptake per total root biomass under enhanced ozone and infection with \emph{P. citricola} (significant main effects, Table 4b), which was due to the increase under +O$_3$/+Phy (Fig. 5d).

\begin{table}
\centering
\begin{tabular}{llll}
\hline
\textbf{Measurement} & \textbf{Ozone} & \textbf{P. citricola} & \textbf{Ozone × P. citricola} \\
\hline
Whole tree biomass & 0.074 (*) & 0.409 & 0.054 (*) \\
Root biomass & 0.025 * & 0.491 & 0.062 (*) \\
N concentration & 0.007 ** & 0.052 (*) & 0.134 \\
N content & 0.251 & 0.678 & 0.027 * \\
$^{15}$N uptake & & & \\
\quad leaves & 0.892 & 0.262 & 0.152 \\
\quad leaves 2nd flush & 0.592 & 0.372 & 0.429 \\
\quad shoot axes & 0.355 & 0.314 & 0.111 \\
\quad fine roots & 0.508 & 0.666 & 0.267 \\
\quad coarse roots & 0.764 & 0.284 & 0.297 \\
\quad total & 0.005 ** & 0.282 & 0.419 \\
\quad belowground & 0.472 & 0.159 & 0.049 * \\
$^{15}$N uptake per root biomass & 0.015 * & 0.276 & 0.817 \\
$^{15}$N uptake per fine root biomass & 0.037 * & 0.726 & 0.982 \\
\hline
\end{tabular}
\caption{Significance levels of two-way ANOVA for whole tree biomass, root biomass, $^{15}$N uptake and partitioning (values relative to whole tree uptake), N concentration and N content, of beech (a) and spruce (b), considering the factors "ozone", "\emph{P. citricola}", and their interaction.}
\end{table}
ment. However, infection with *P. citricola* had no significant main effect because, under the combined treatment (+O3/+Phy), infection with the pathogen did not further reduce growth relative to that with +O3. This result for beech is in support of hypothesis 2 in that susceptibility to *P. citricola* was reduced through acclimation to increased O3 levels (ozone × *P. citricola* interaction: *p* = 0.054).

Compared with the control, neither +O3 nor +Phy significantly changed the growth of spruce, but under the combined treatment (+O3/+Phy) whole tree biomass was reduced by about 25%. Thus, regarding unchanged growth at +O3 and +Phy (and contrasting with beech), hypotheses 2 was not supported for spruce. Despite the different responses to ozone and/or *P. citricola*, both species increased their whole plant N concentration when growth was restricted (Fig. 3). Since N uptake in both species is well regulated (Gessler et al., 2004), enhanced N concentrations under the high ozone regime and *P. citricola* infection is likely due to metabolic demand and may indicate that N was used for purposes other than growth, perhaps for stress defence (cf. Herms and Mattson, 1992; Zangerl and Bazzaz, 1992). In general, both O3 and pathogens can induce a defence-related metabolism, including pathogen-related proteins, as well as an increase in enzyme activities such as PAL leading to accumulation of phenolic compounds and biosynthesis of lignins (Heller et al., 1990; Schmitt and Sandermann, 1990; Sandermann et al., 1998; Matyssek and Sandermann, 2003). Remarkably, the highest 15N uptake efficiency (N uptake per unit of root biomass) of spruce was found under probably high N demand for stress defence under the +O3/+Phy treatment (Fig. 5). Experiments with elicitors of chemical resistance showed that induction of resistance is N-dependent (Dietrich et al., 2004), and that resistance induction may reduce competitiveness (Dietrich et al., 2005).

**Fig. 4** Daily whole tree 15N uptake by beech (a) and spruce (b) trees during the 56 days of label application. Values are means ± SE, *n* = 9 for control and +O3 each, and *n* = 15 for +Phy and +O3/+Phy each. Ozone and Phytophthora effects and their interactions are given by * and **, corresponding to *p* < 0.05 and *p* < 0.01, respectively, and (*) for *p* < 0.10 (non-significant).

**Table 5** Partitioning of 15N in beech (a) and spruce (b) trees, taken up during the 56 days of label application (means ± SE, *n* = 9 for control and +O3 each, and *n* = 15 for +Phy and +O3/+Phy each)

<table>
<thead>
<tr>
<th>a Beech</th>
<th>Leaves</th>
<th>Leaves 2nd flush</th>
<th>Shoot axes</th>
<th>Fine roots</th>
<th>Coarse roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.6%</td>
<td>0.2%</td>
<td>31.9%</td>
<td>21.6%</td>
<td>43.6%</td>
</tr>
<tr>
<td></td>
<td>0.14 ± 0.05</td>
<td>0.01 ± 0.00</td>
<td>1.68 ± 0.56</td>
<td>1.14 ± 0.38</td>
<td>2.30 ± 0.77</td>
</tr>
<tr>
<td>+O3</td>
<td>2.2%</td>
<td>0.0%</td>
<td>27.2%</td>
<td>21.1%</td>
<td>49.4%</td>
</tr>
<tr>
<td></td>
<td>0.04 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>0.49 ± 0.16</td>
<td>0.38 ± 0.13</td>
<td>0.89 ± 0.30</td>
</tr>
<tr>
<td>+Phy</td>
<td>2.0%</td>
<td>2.7%</td>
<td>25.0%</td>
<td>24.6%</td>
<td>45.7%</td>
</tr>
<tr>
<td></td>
<td>0.05 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.64 ± 0.17</td>
<td>0.63 ± 0.16</td>
<td>1.17 ± 0.30</td>
</tr>
<tr>
<td>+O3/+Phy</td>
<td>4.6%</td>
<td>1.5%</td>
<td>37.6%</td>
<td>17.3%</td>
<td>39.1%</td>
</tr>
<tr>
<td></td>
<td>0.09 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td>0.74 ± 0.19</td>
<td>0.34 ± 0.09</td>
<td>0.77 ± 0.20</td>
</tr>
<tr>
<td>b Spruce</td>
<td>Old needles</td>
<td>Current year needles</td>
<td>Shoot axes</td>
<td>Fine roots</td>
<td>Coarse roots</td>
</tr>
<tr>
<td>Control</td>
<td>1.6%</td>
<td>19.3%</td>
<td>23.9%</td>
<td>22.8%</td>
<td>32.5%</td>
</tr>
<tr>
<td></td>
<td>0.09 ± 0.03</td>
<td>1.09 ± 0.36</td>
<td>1.35 ± 0.45</td>
<td>1.29 ± 0.43</td>
<td>1.84 ± 0.61</td>
</tr>
<tr>
<td>+O3</td>
<td>1.4%</td>
<td>17.2%</td>
<td>27.4%</td>
<td>23.7%</td>
<td>30.3%</td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.03</td>
<td>1.24 ± 0.41</td>
<td>1.98 ± 0.66</td>
<td>1.71 ± 0.57</td>
<td>2.19 ± 0.73</td>
</tr>
<tr>
<td>+Phy</td>
<td>1.3%</td>
<td>21.5%</td>
<td>25.9%</td>
<td>23.2%</td>
<td>28.1%</td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.03</td>
<td>1.64 ± 0.42</td>
<td>1.97 ± 0.51</td>
<td>1.77 ± 0.46</td>
<td>2.14 ± 0.55</td>
</tr>
<tr>
<td>+O3/+Phy</td>
<td>1.4%</td>
<td>28.1%</td>
<td>26.6%</td>
<td>16.7%</td>
<td>27.2%</td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.03</td>
<td>2.02 ± 0.52</td>
<td>1.91 ± 0.49</td>
<td>1.20 ± 0.31</td>
<td>1.95 ± 0.50</td>
</tr>
</tbody>
</table>
Hypothesis 1 was tested through belowground competition for $^{15}$N double-labelled NH$_4$NO$_3$. In the case of beech, data support this hypothesis, because total $^{15}$N uptake under both enhanced ozone and *P. citricola* infection was less than half of the uptake in the control (Fig. 4). This reduction was not merely the result of a reduced root biomass, since belowground efficiency of $^{15}$N uptake was also reduced by enhanced ozone (Fig. 5). Conversely, $^{15}$N uptake and its efficiency were higher in spruce under enhanced ozone and/or *P. citricola*. This appears to be consistent with spruce’s lower susceptibility to O$_3$ (at least under controlled conditions; Lippert et al., 1996; Grams et al., 2002) and to *P. citricola* (Nechwatal and Oßwald, 2001). In addition, the high $^{15}$N uptake in spruce may also result from the low belowground competitiveness of beech, which may have been the case, in particular, under the +O$_3$ +Phy treatment. Thus, hypothesis 1 was not supported in spruce.

The contrasting responses in belowground competitiveness of beech and spruce to the applied treatments may be a consequence of competition. The less O$_3$ and pathogen-sensitive competitor (i.e., spruce) may profit from the increased resource availability caused by the lowered resource uptake of the more sensitive competitor (i.e., beech, cf. Schwinning, 1996). Direct competition effects may dominate plant response, in particular, to changing environmental conditions such as elevated O$_3$ and CO$_2$ concentrations (Kozovits et al., 2005a).

Fig. 5 Daily $^{15}$N uptake per unit of fine root biomass (a, b) and per unit of total root biomass (c, d) of beech and spruce, during the 56 days of label application (means ± SE, n = 9 for control and +O$_3$ each, and n = 15 for +Phy and +O$_3$/+Phy each). Significant ozone and Phytophthora effects and their interaction are given by *, **, and ***, correspond to $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Interactions between pathogens and abiotic factors in plant response demand increased awareness, as they affect competition, species composition, and ecosystem functioning (Laurance and Andersen, 2003). This is in line with the present study that demonstrated such interactions in trees are species-specific, although phytotron experiments cannot directly be extrapolated to field conditions. As beech responded to ozone by decreasing N acquisition and biomass production, these responses were not exacerbated by additional pathogen infection. Acclimation to ozone may have facilitated pathogen defence. Conversely, these two parameters were insensitive in spruce to O$_3$ stress but constrained by additional pathogen infection, although the whole plant N concentration was enhanced. The N not used in biomass production perhaps reflects demand and physiological costs of stress defence. Sensitivity to O$_3$ appears to pre-determine the differential response to pathogen infection under O$_3$ stress.

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References


