Ozone air pollution effects on tree-ring growth, δ^{13}C, visible foliar injury and leaf gas exchange in three ozone-sensitive woody plant species

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Summary   We assessed the effects of ambient tropospheric ozone on annual tree-ring growth, δ^{13}C in the rings, leaf gas exchange and visible injury in three ozone-sensitive woody plant species in southern Switzerland. Seedlings of *Populus nigra* L., *Viburnum lantana* L. and *Fraxinus excelsior* L. were exposed to charcoal-filtered air (CF) and non-filtered air (NF) in open-top chambers, and to ambient air (AA) in open plots during the 2001 and 2002 growing seasons. Ambient ozone exposures in the region were sufficient to cause visible foliar injury, early leaf senescence and premature leaf loss in all species. Ozone had significant negative effects on net photosynthesis and stomatal conductance in all species in 2002 and in *V. lantana* and *F. excelsior* in 2001. Water-use efficiency decreased and intercellular CO_2 concentrations increased in all species in response to ozone in 2002 only. The width and δ^{13}C of the 2001 and 2002 growth rings were measured for all species at the end of the 2002 growing season. Compared with CF seedlings, mean ring width in the AA and NF *P. nigra* seedlings was reduced by 52 and 46%, respectively, in 2002, whereas in *V. lantana* and *F. excelsior,* ring width showed no significant reductions in either year. Although δ^{13}C was usually more negative in CF seedlings than in AA and NF seedlings, with the exception of *F. excelsior* in 2001, ozone effects on δ^{13}C were significant only for *V. lantana* and *P. nigra* in 2001. Among species, *P. nigra* exhibited the greatest response to ozone for the measured parameters as well as the most severe foliar injury and was the only species to show a significant reduction in ring width in response to ozone exposure, despite significant negative ozone effects on leaf gas exchange and the development of visible foliar injury in *V. lantana* and *F. excelsior*. Thus, significant ozone-induced effects at the leaf level did not correspond to reduced tree-ring growth or increased δ^{13}C in all species, indicating that the timing of ozone exposure and severity of leaf-level responses may be important in determining the sensitivity of tree productivity to ozone exposure.

Introduction

Plant responses to tropospheric ozone pollution are often species specific and depend on several morphological, biochemical and physiological characteristics as well as environmental factors. A variety of negative forest tree responses to ozone have been documented in open-top chambers (OTCs), growth chamber fumigations and field studies (see review by Matyssek and Sandermann 2003). Of the various tree responses, annual increment growth is one of the most important because it is an integration of many underlying processes (Innes 1993, Ferretti et al. 2002, McLaughlin et al. 2002). Tree growth responses to ozone have frequently been assessed by measurements of height, relative growth rate (RGR), leaf production and biomass partitioning between root and shoot, but fewer studies have examined tree ring growth and stable carbon isotope composition (δ^{13}C) to assess ozone effects on tree growth.

Early reports on the negative impacts of ozone on trees included foliar damage in coniferous species (Miller et al. 1963), reductions in radial growth in *Pinus ponderosa* Laws. (McBride et al. 1975) and reductions in mean annual growth increment of eastern white pine (*Pinus strobus* L.) (Benoit et al. 1982) and two broadleaf species (Somers et al. 1998). More recently, changes in whole-canopy ozone uptake have been related to changes in radial growth in mature *Fagus sylvatica* L. trees as a possible basis for deriving critical threshhold values for ozone effects on forests (Dittmar et al. 2005). Although these studies generally attribute a reduction in radial growth to ozone exposure, some uncertainty remains because

of the varying environmental conditions, such as extended periods of drought or other biotic stressors under which these studies were conducted. In this study, we assessed the usefulness of tree-ring width analysis for assessing accumulated ozone effects on radial growth of plants grown under semi-controlled conditions with known ambient ozone air pollution exposures.

When investigating past climate and air pollution, tree-ring width and density are commonly used proxies (Tognetti et al. 2000, Hughes 2001, Saurer et al. 2004), but other parameters such as the stable carbon isotope signatures ($\delta^{13}C$) of tree rings have proved useful in assessing plant responses to air pollution, because they provides an integration of underlying physiological processes as influenced by local environmental factors (e.g., Saurer et al. 1995). Exposure to ozone consistently results in less negative $\delta^{13}C$ values for a range of plants including trees (Freyer 1979, Martin et al. 1988, Matyssek et al. 1992, Saurer et al. 1995), crop plants (Greitner and Winner 1988, Saurer et al. 1991) and semi-natural vegetation (Jäggi et al. 2005). However, the increase is often unrelated to reduced intercellular CO_2 concentration (c_i) (higher stomatal limitation of photosynthesis), as predicted by the model of Farquhar et al. (1982). Saurer et al. (1995) investigated the influences of ozone and nutrition on $\delta^{13}C$ in *Betula pendula* Roth. and suggested increased phosphoenol pyruvate carboxylase (PEPC) activity in response to elevated ozone exposures as the potential cause of this anomaly. Jäggi et al. (2002) investigated the relationship between the $\delta^{13}C$ signal in current-year and 1-year-old needles, starch extracts, and early- and latewood in mature spruce trees (*Picea abies* L. Karst.) to identify the influence of climate on the $\delta^{13}C$ signatures and found that the $\delta^{13}C$ signature of current-year and 1-year-old needles is to some extent transferred to the earlywood, but reported that some fractionation may occur during post-photosynthetic processes in the plant. Therefore, we made a combined analysis of leaf gas exchange and $\delta^{13}C$ within the cells and tissues of annual tree rings to obtain a better understanding of the physiological links between these parameters as influenced by ozone.

Ozone exposures at the Lattecaldo research site regularly exceeds European Critical Levels with hourly peak concentrations reaching 150 ppb during the summer months (Bacci et al. 1990, NABEL 1995, Staffelbach et al. 1997, Gerosa et al. 1999, Wotawa et al. 2000, de Leeuw and de Paus 2001, VanderHeyden et al. 2001, Novak et al. 2003, 2005). Ozone-induced visible foliar injuries have been observed in a variety of tree, shrub and herbaceous plants in the southern Alps region (Innes and Skelly 1996, Skelly et al. 1998, 1999, Innes et al. 2001, VanderHeyden et al. 2001, Novak et al. 2003, 2005). Several leaf-level studies at the Lattecaldo site have described relationships between the onset and development of visible, ozone-induced foliar injury and changes in leaf gas exchange (Zhang et al. 2001, Novak et al. 2005) and between visible foliar injury and chlorophyll fluorescence and ultrastructural characteristics (Gravano et al. 2004). However, to assess the ozone damage risk to forest ecosystems in the southern Alps region, a reliable method of assessing ozone effects on tree growth is needed.

As an alternative to assessing the effects of ozone at the leaf level or on mean annual growth increment, we determined ozone effects on tree-ring growth and $\delta^{13}C$ in three ozone-sensitive woody plant species grown in open plots, and in non-filtered and charcoal-filtered air in open-top chambers (OTCs). Leaf gas exchange and ozone-induced visible foliar injury were also measured to determine if relationships exist between measurable ozone-induced effects at the leaf level and subsequent changes in annual tree-ring growth and $\delta^{13}C$ signatures.

Materials and methods

Seedlings and site characteristics

Seedlings of three ozone-sensitive species, *Populus nigra* L., *Viburnum lantana* L. and *Fraxinus excelsior* were grown in OTCs and open plots in the subalpine region of southern Switzerland at the Lattecaldo Cantonal Forest Nursery in the Valle di Muggio, Canton Ticino (09°03′ E, 45°51′ N, 600 m asl). The experimental treatments consisted of three ozone exposures with four replications. The ozone treatments involved season-long (May–October) exposures of about 36, 92 and 100% of ambient ozone concentrations for seedlings grown in OTCs in charcoal-filtered (CF) and non-filtered (NF) air, and in open plots (AA), respectively. Each plot contained three individuals of each species. Survival was 100% except in *P. nigra*, of which three seedings died soon after planting. Seedlings were three years old at the start of the 2001 growing season. All seedlings were well watered.

Ozone concentrations in parts per billion (ppb) were continuously monitored throughout the 2001 and 2002 growing seasons (May–October; Model ML 8810 ozone monitor, Monitor Labs, Eaglewood, CO). The monitor was calibrated monthly. Meteorological data were collected throughout each growing season from a measurement station located next to the OTC. Hourly measurements included air temperature (°C), relative humidity (%), global radiation (W m^{-2}) and precipitation (mm) (Novak et al. 2003, 2005).

Visible foliar injury assessment

Visible foliar injury assessments were made weekly from May 2 to August 20, 2001 and on a weekly to bi-weekly basis from May 10 to 8 October 8, 2002. Visible leaf symptoms on seedlings growing in NF and open plots were compared with seedlings growing in the CF air chambers to confirm ozone as the cause of foliar symptoms, and potential ozone-induced symptoms were further confirmed with the aid of a 10× hand lens. A scale in 5% increments (0, 5, 10, 15… 100%) was used to evaluate the percentage of symptomatic leaves per seedling and the Horsfall-Barratt scale (0, 1, 3, 6, 12, 25, 50, 75, 88, 94, 97, 99, 100%) was used to evaluate the mean percentage of leaf area injured on symptomatic leaves (Horsfall and Barratt 1945). The estimated values were then multiplied by one another and divided by 100 to obtain the estimated percentage of total leaf area affected (% LAA) by ozone. The onset of ozone-induced visible injury, premature leaf senescence (i.e., yellowing) and premature leaf loss were recorded for each

seedling during both growing seasons. The percentage of premature leaf senescence (i.e., yellowing) was estimated in 2001 and 2002. In 2002, the percentage of premature leaf loss (i.e., abscission) was estimated separately from visible injury (i.e., stippling), but in 2001, premature leaf loss was integrated into the recorded values for visible injury. All evaluations were made by the same observer throughout the 2001 and 2002 growing seasons.

Leaf gas exchange measurements

Leaf gas exchange (net photosynthesis (P_n), stomatal conductance to water vapor (g_w) and intercellular CO_2 concentrations (c_i)) were measured from June to August in 2001 and from June to October in 2002 with an LI-6200 portable photosynthesis system equipped with a 0.25-l cuvette (Li-Cor, Lincoln, NE). In 2001, gas exchange measurements were made once in June, three times in July and twice in August. In 2002, measurements were conducted every 2–15 days depending on the weather, resulting in a total of 21 sets of measurements. Within each plot, one seedling per species was selected, and one mature (mid-canopy), fully sun-exposed leaf was tagged and measured throughout the season. In the event that leaves were shed or damaged, the next measurable leaf upward along the stem was selected. Measurements were made between 0900 and 1400 h in both 2001 and 2002. Because of time constraints, seedlings of *P. nigra* were not routinely measured in 2001 and thus were not included in the 2001 dataset. The area within the gas exchange cuvette was set to 11.88 cm^2, and only that area was covered by the inserted leaf. The Li-Cor LI-6200 system parameters were checked and calibrated with known CO_2 concentrations (0 and 400 ppm) at the beginning of each measurement period. Cuvette environmental conditions throughout the measurement period were 29 and 28 °C for air temperature, and 40 and 49% for relative humidity in 2001 and 2002, respectively. Measurements taken below the in situ saturating photosynthetic photon flux (PPF < 600 mmol m^{-2} s^{-1}) or at high ambient CO_2 concentrations (> 400 ppm) were omitted from the data analysis (Schaub et al. 2003). Intrinsic water-use efficiency (WUE) was calculated by dividing P_n by g_w (Zhang et al. 2001). Gas exchange data for 2002 were recalculated from Novak et al. (2005).

Tree-ring width and stable carbon isotope analysis

A total of 36 individuals of *F. excelsior* and *V. lantana* and 27 individuals of *P. nigra* were harvested at the end of October 2002. Cross sections were cut from the main stem of each harvested seedling at 10 cm above the soil surface. Tree-ring widths (mm × 10^{-2}) of each sample were measured for the years 2001 and 2002 with a stereomicroscope coupled with a Lintab measurement table, and data were handled with the Time Series Analysis Programme (TSAP) software (Rinntech, Heidelberg, Germany). The same samples used for ring-width measurements were dissected and separated by year. The wood samples were finely ground and analyzed for stable carbon isotope composition. An aliquot of the samples was weighed into tin capsules and combusted in an elemental ana-

lyzer (EA 1108, Finnigan, Germany). The evolving CO_2 was passed into the isotope ratio mass spectrometer (Delta S, Finnigan, Germany), where the ^{13}C/^{12}C ratio of the sample was determined relative to the international PeeDee Belemnite (PDB) reference according to the equation:

$$\delta^{13}C_{Sample} = \left[\frac{\left(^{13}C/^{12}C\right)_{Sample}}{\left(^{13}C/^{12}C\right)_{PDB}} - 1 \right] \times 1000 \qquad (1)$$

The standard deviation for the repeated analysis of an internal standard was better than 0.1‰.

Data analysis

Data were checked for normality and homogeneous variance before statistical analysis. Ozone treatment effects on leaf gas exchange (P_n, g_{wv}, c_i, WUE), δ^{13}C and tree-ring width between the NF and CF treatments were tested by one-way ANOVA for each species and year. Because of a possible chamber effect, direct comparison of the AA and OTC treatments was not possible; therefore, the AA treatment was omitted from the ANOVA. To test for general relationships between the measured parameters, data were pooled for all treatments, species and years, and a correlation analysis was conducted. The AOT40 statistic, accumulated exposure over a threshold of 40 ppb for daylight hours (0700–1859 h), was used. Statistical analyses were performed with Statistical Analysis System software (SAS) (SAS Institute, Cary, NC).

Results

Ozone exposures and climatic conditions

Seasonal mean ozone concentrations were similar in 2001 and 2002 (Table 1), but the seasonal distribution of ozone exposures varied between years as shown by the distinctly different AOT40 curves (Figure 1). Elevated ozone episodes were more pronounced in 2001, with a series of major episodes occurring in late June and July as well as early August; the highest peak of 139 ppb was recorded on July 23. Extended periods of elevated ozone exposures were less frequent in 2002 with only one major ozone episode beginning on June 10, following a 6-day rain period, and lasting until another rainfall on June 24. During this period, ozone concentrations often exceeded 100 ppb with a peak of 153 ppb occurring on June 22; AOT40 values also rose sharply during the period June 10–24 (Figure 1). Frequent rains occurred during the rest of the 2002 season and ozone concentrations remained moderate. AOT40 ozone exposures from May to October reached 27, 21 and 2 ppm·h in 2001 and 21, 16 and 1 ppm·h in 2002 in the AA, NF and CF treatments, respectively (Figure 1).

Mean air temperature from April through September was slightly higher in 2001 (17 °C) than in 2002 (15 °C), and it was slightly drier in 2001 than in 2002, as reflected by a lower mean relative humidity (74 versus 77%), higher vapor pressure deficit (VPD) (0.58 versus 0.48 kPa) and a lower total precipitation (575 versus 1041 mm).

Table 1. Hourly peak and 24-h and 12-h (0700–1859 h) mean ozone concentrations (ppb) for ambient (AA), non-filtered (NF) and charcoal-filtered (CF) air treatments measured from May to the end of September in 2001 and 2002 at the Lattecaldo open-top chamber facility, Switzerland.

	AA		NF		CF	
	2001	2002	2001	2002	2001	2002
May						
24-h mean	44	47	39	43	18	20
12-h mean	48	51	43	47	22	26
1-h peak	136	101	126	92	82	57
June						
24-h mean	54	56	49	50	23	21
12-h mean	60	60	55	55	30	26
1-h peak	139	153	128	135	79	68
July						
24-h mean	53	46	49	42	19	16
12-h mean	60	49	55	45	24	19
1-h peak	139	105	128	90	67	50
August						
24-h mean	49	41	45	39	17	14
12-h mean	53	44	49	42	20	17
1-h peak	97	83	93	77	56	37
September						
24-h mean	26	31	24	29	9	10
12-h mean	27	32	25	30	10	11
1-h peak	66	73	61	71	28	32
Season						
24-h mean	45	44	41	41	17	16
12-h mean	50	47	45	44	21	20
1-h peak	139	153	128	135	82	68

Figure 1. The AOT40 ozone exposures (ppm·h) (accumulated over threshold of 40 ppb during daylight hours (0700–1859 h)) for ambient (AA), non-filtered (NF) and charcoal-filtered (CF) air treatments measured from May to the end of September at the Lattecaldo open-top chamber facility in 2001 and 2002.

Visible foliar ozone injury

Mean dates of onset of visible injury, early leaf senescence (i.e., yellowing), and premature leaf loss, as well as the total mean percentage of ozone-induced visible foliar injury (% LAA), for each species, year and treatment are summarized in Table 2. Total mean percentages were based on assessments made on August 19, 2001 and August 20, 2002. On average, ozone-induced visible foliar injury in AA and NF treated seedlings of *F. excelsior* began in early to mid-July in both 2001 and 2002 and was characterized as a fine brown stipple on the adaxial leaf surface only. Stippling progressed until the upper surface of some leaves became entirely brown. In 2002, leaf abscission was initially observed at the end of August and beginning of September. No visible injury was observed in CF seedlings of *F. excelsior* in either year.

Ozone-induced visible foliar injury in AA and NF seedlings of *P. nigra* began in late May in 2001 and the beginning of June in 2002; of the three species, *P. nigra* was the most severely injured. Typical injury began as chlorotic mottling followed by dark stipple on the upper leaf surface. Stippling increased until most of the interveinal leaf surface appeared dark brown or
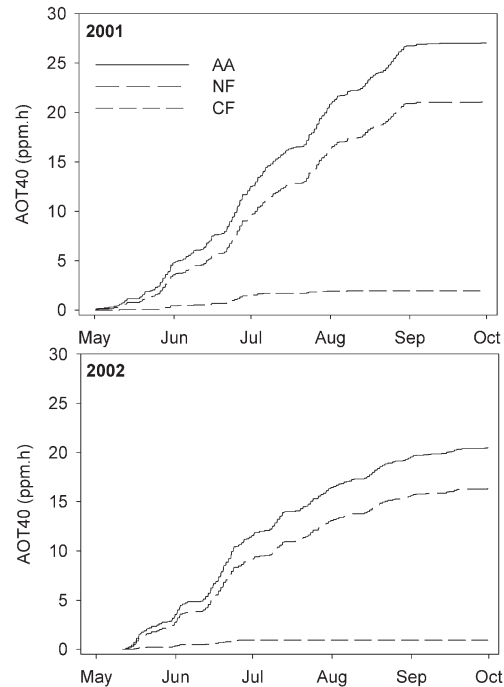
black. Early leaf senescence also occurred in combination with ozone-induced stipple beginning in mid- to late-June in 2001 and 2002. Premature leaf loss in AA and NF seedlings started in mid-June and mid-July in 2001 and 2002, respectively. In comparison, CF seedlings retained green leaves throughout much of the growing season and only exhibited minor injury, yellowing and leaf loss beginning in late-July and August.

The AA and NF seedlings of *V. lantana* developed ozone-induced visible foliar injury early in the season, beginning in late-May in 2001 and in early-June in 2002. The injury was characterized by a fine, dark red or violet stipple on the upper leaf surface. As the injury progressed, the red or violet stipple covered much of the adaxial leaf surfaces. No ozone-induced visible injury was observed in CF seedlings in either year, and only minor leaf loss was observed on several individuals in late-September and October 2002.

Leaf gas exchange

In 2002, *P. nigra* had the highest leaf gas exchange rates, followed by *V. lantana* and *F. excelsior*, and showed the greatest ozone-induced change in leaf gas exchange (Figure 2). Mean net photosynthesis and g_w were lower in NF and AA seedlings of all species compared with CF seedlings with the exception of P_n in AA seedlings of *F. excelsior*, which was similar to that of CF seedlings (Table 3, Figures 2C and 2D). In all species,

Table 2. Mean dates (μ) and standard errors (SE) of onset for visible foliar injury, yellowing and premature leaf loss, as well as the total mean percentage of ozone-induced visible foliar injury, leaf yellowing and premature leaf loss for each species, year and treatment. Total mean percentages were based on late-season assessments on August 19, 2001 and August 20, 2002. Abbreviations: n.o. = no observed response; n.a. = not assessed; AA = ambient air; NF = non-filtered air; CF = charcoal-filtered air.

Species	Year	O$_3$	Visible injury				Yellowing				Leaf loss			
			Onset		Total		Onset		Total		Onset		Total	
			μ	SE	μ	SE	μ	SE	μ	SE	μ	SE	μ	SE
Fraxinus excelsior	2001	AA	16 Jul	5.3	6.6	1.5	n.o.		0.0	0.0	n.a.	n.a.	n.a.	n.a.
		NF	15 Jul	6.0	13.7	6.6	n.o.		0.0	0.0	n.a.	n.a.	n.a.	n.a.
		CF	n.o.		0.0	0.0	n.o.		0.0	0.0	n.a.	n.a.	n.a.	n.a.
	2002	AA	14 Jul	3.0	5.9	1.4	n.o.		0.0	0.0	3 Sep	3.9	5.0	3.4
		NF	6 Jul	2.6	22.0	10.1	n.o.		0.0	0.0	29 Aug	1.9	5.0	3.5
		CF	n.o.		0.0	0.0	n.o.		0.0	0.0	13 Sep	4.5	0.0	0.0
Populus nigra	2001	AA	25 May	0.1	28.7	3.6	14 Jun	0.9	18.1	3.3	16 Jun	0.4	n.a.	n.a.
		NF	28 May	2.3	22.8	3.1	14 Jun	0.9	15.0	2.0	19 Jun	3.2	n.a.	n.a.
		CF	21 Jul	1.5	0.2	0.1	17 Jul	6.8	2.0	1.3	3 Aug	1.3	n.a.	n.a.
	2002	AA	4 Jun	5.2	25.2	0.6	26 Jun	2.0	24.4	1.5	10 Jul	1.4	18.8	2.6
		NF	5 Jun	4.6	28.5	1.5	22 Jun	1.5	15.6	2.6	4 Jul	1.6	19.4	4.9
		CF	17 Jul	5.0	0.5	0.03	20 Jul	5.8	8.0	0.8	18 Aug	8	0.4	0.2
Viburnum lantana	2001	AA	31 May	2.5	13.6	3.7	n.o.		0.0	0.0	n.a.	n.a.	n.a.	n.a.
		NF	6 Jun	4.0	10.4	3.2	n.o.		0.0	0.0	n.a.	n.a.	n.a.	n.a.
		CF	n.o.		0.0	0.0	n.o.		0.0	0.0	n.a.	n.a.	n.a.	n.a.
	2002	AA	10 Jun	3.4	14.5	4.2	n.o.		0.0	0.0	28 Sep	5.8	0.0	0.0
		NF	2 Jun	4.8	11.5	2.6	n.o.		0.0	0.0	4 Oct	1.5	0.0	0.0
		CF	n.o.		0.0	0.0	n.o.		0.0	0.0	n.o.		0.0	0.0

WUE was reduced and c_i was increased in NF seedlings compared with CF seedlings; a similar trend was observed in AA seedlings of *P. nigra*, whereas AA seedlings of *V. lantana* and *F. excelsior* showed a variable response. In 2001, P_n and g_w were significantly lower in NF seedlings of *V. lantana* and *F. excelsior* than in CF seedlings, whereas no ozone-induced effects were observed between seedlings in the AA and CF treatments (Figures 2C and 2D). In 2001, no significant ozone effects on WUE and c_i were detected in seedlings of *V. lantana* or *F. excelsior* (Table 3, Figures 2E and 2F) (no 2001 gas exchange data were available for *P. nigra*; see Methods section). In general, data pooled across species, treatment and year showed a positive correlation between P_n and g_w ($R = 0.72$, $P < 0.01$) and a negative correlation between WUE and c_i ($R = -0.91$, $P < 0.001$) (Table 4).

Tree-ring widths

In general, *P. nigra* had the largest ring width, followed by *V. lantana* and *F. excelsior*, and it was the only species to show a significant ozone effect on annual ring width (Table 3). In 2002, mean ring widths of *P. nigra* seedlings grown in the NF and AA treatments were reduced by 46 and 52%, respectively, compared with CF seedlings (Figure 2B). No significant ozone effects on mean ring width were observed in *P. nigra* in 2001 or in *F. excelsior* and *V. lantana* in either year. Ring-width data pooled across species, treatment and year

were positively correlated with P_n ($R = 0.73$, $P < 0.01$) and g_w ($R = 0.84$, $P < 0.001$) (Table 4).

Stable carbon isotope signatures

Values of δ^{13}C were usually more negative in the annual ring tissues in CF seedlings than in NF and AA seedlings (with the exception of *F. excelsior* in 2001); however, ozone effects on δ^{13}C were only significant for *V. lantana* and *P. nigra* in 2001, and no significant treatment effects were observed in any species in 2002. Of the three species, *F. excelsior* showed the least negative δ^{13}C values, and *P. nigra* showed the most negative δ^{13}C values in the 2001 and 2002 annual rings. When comparing differences between years, δ^{13}C values were more negative in 2002 than in 2001, with *F. excelsior* showing the greatest difference between years. The stable carbon isotope ratio data pooled for species, treatments and years were negatively correlated with g_{wv}, ($R = -0.75$, $P < 0.01$), c_i ($R = -0.55$, $P < 0.05$) and ring width ($R = -0.48$, $P < 0.05$), and positively correlated with WUE ($R = 0.62$, $P < 0.05$) (Figure 2, Table 4).

Discussion

During the two-year study, leaf-level responses to ozone exposures were observed for both visible foliar injury and leaf gas exchange, whereas the effects of ozone on ring width and δ^{13}C varied among species and year. Among the study species, an-
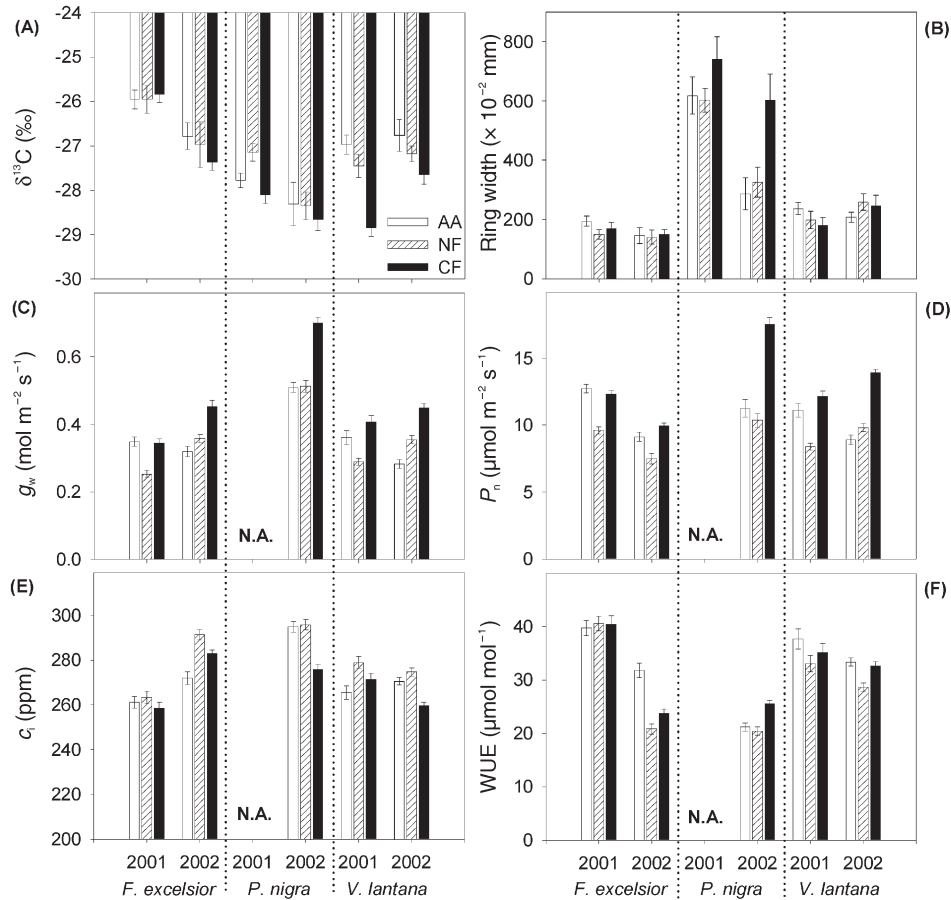
Figure 2. Mean and standard error for stable carbon isotope ratio ($\delta^{13}C$) (A), ring width (B), stomatal conductance to water vapor (g_w) (C), net photosynthesis (P_n) (D), intercellular CO_2 concentrations (c_i) (E) and water-use efficiency (WUE) (F) for *Fraxinus excelsior*, *Populus nigra*, and *Viburnum lantana* grown at the Lattecaldo OTC facility in 2001 and 2002. Gas exchange data for 2002 were recalculated from Novak et al. (2005). Abbreviation: N.A. = not assessed.

nual ring width was greatest in *P. nigra*, which generally showed the greatest sensitivity to ozone for all response parameters as well as the greatest annual growth. This is consistent with the findings of Bortier et al. (2000), who found fast-growing *Populus nigra* seedlings were more sensitive to ozone than slow-growing *Fagus sylvatica* seedlings. The high g_w of *P. nigra* compared with *V. lantana* and *F. excelsior* (Figure 2C) must have resulted in greater ozone uptake, which may account for this species' high ozone sensitivity (Reich 1987). *Populus nigra* was the only species to show significant reduc-

tions in annual ring growth in the AA and NF treatments compared with the CF treatment, and these reductions occurred only in 2002 (Figure 2B). In concert with ring-width reductions, premature leaf senescence and leaf loss were observed as early as July and reached as much as 50% toward the end of the growing season in *P. nigra* seedlings in the AA and NF treatments (Novak et al. 2005). The observed reductions in annual ring width and the significant premature leaf loss support the findings of Matyssek et al. (1991), who concluded that changes in foliage area through premature leaf loss more

Table 3. Observed probabilities for a one-way analysis of variance to test for ozone treatment effects (non-filtered versus charcoal-filtered) on ring width (RW), stable carbon isotope ratio ($\delta^{13}C$), net photosynthesis (P_n), stomatal conductance to water vapor (g_w), intrinsic water-use efficiency (WUE) and intercellular CO_2 concentrations (c_i) in *Fraxinus excelsior*, *Populus nigra*, *Viburnum lantana* in 2001 and 2002. For all analyses, there were two degrees of freedom. Signifiance: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Abbreviation: n.a. = not assessed.

Species	Year	RW ($mm \times 10^{-2}$)	$\delta^{13}C$ (‰)	P_n ($\mu mol\ m^{-2}\ s^{-1}$)	g_w ($mol\ m^{-2}\ s^{-1}$)	WUE ($\mu mol\ mol^{-1}$)	c_i (ppm)
F. excelsior	2001	0.53	0.12	54.85***	29.05***	0.01	1.66
	2002	0.12	0.47	30.84***	16.85***	5.86*	8.19**
P. nigra	2001	2.45	10.51*	n.a.	n.a.	n.a.	n.a.
	2002	7.14*	0.57	97.81***	57.23***	24.83***	40.72***
V. lantana	2001	0.19	19.38**	60.87***	30.50***	0.81	3.83
	2002	0.09	2.95	110.53***	27.14***	12.04***	37.35***

Table 4. Linear correlation coefficients for pooled data (all species, treatments and years) of ring width (RW), stable carbon isotope ratio (δ^{13}C), net photosynthesis (P_n), stomatal conductance to water vapor (g_w), ozone-induced visible foliar injury, intrinsic water-use efficiency (WUE) and intercellular CO$_2$ concentrations (c_i) for *Fraxinus excelsior*, *Populus nigra*, and *Viburnum lantana* in 2001 and 2002. Significance: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

	RW	δ^{13}C	P_n	g_{wv}	O$_3$ Injury	WUE	c_i
RW	1						
δ^{13}C	−0.48*	1					
P_n	0.73**	−0.35	1				
g_{wv}	0.84***	−0.75**	0.72**	1			
O$_3$ injury	0.16	0.21	−0.72**	−0.50	1		
WUE	−0.35	0.62*	0.11	−0.59*	−0.12	1	
c_i	0.20	−0.55*	−0.33	0.39	0.29	−0.91***	1

strongly limit growth than reductions in the photosynthetic capacity of attached foliage. Although the continuous growth pattern of *Populus* spp. may compensate for ozone stress by producing new shoots and leaves (Pell et al. 1992), this may not be enough to overcome the progressive leaf losses incurred during sustained exposure to high ozone concentrations. Although *P. nigra* is not a large component of most European forests, its high ozone sensitivity may be of concern in regions such as the Po Plain in northern Italy or areas of southern Spain where this fast-growing species is extensively planted for pulpwood production and where ozone exposures are high (Bacci et al. 1990, Millán et al. 1996, Staffelbach et al. 1997, Skelly et al. 1999).

When considering all species, treatments and years, ring width was positively correlated with P_n ($R = 0.73$, $P < 0.01$) and g_w ($R = 0.84$, $P < 0.001$) (Table 4). We predicted that ozone would cause a reduction in leaf gas exchange that would be accompanied by a reduction in ring width, and this was observed in *P. nigra* in 2002 (Figure 2). However, reductions in P_n and g_w and the expression of visible injury in response to ozone do not result in significant reductions in increment growth in either *V. lantana* or *F. excelsior*. Seedlings of *V. lantana* grown in the NF treatment showed a significant decline in leaf gas exchange with no significant reduction in ring width; however, in contrast to *P. nigra*, *V. lantana* retained injured leaves throughout the growing season and avoided the costs of new leaf production, thus allowing more resources for defense against ozone stress. Gravano et al. (2004) suggested that leaf reddening in *V. lantana*, a typical response of this species to ozone (Skelly et al. 1998, 1999, Innes et al. 2001, VanderHeyden et al. 2001, Novak et al. 2003), is a defense mechanism whereby the accumulated anthocyanins protect the chloroplasts from excess excitation by activating a controlled energy dissipation process (Steyn et al. 2002), which may reduce photosynthetic efficiency, but maintain the longevity of the leaves. Furthermore, *V. lantana* has a different architecture than the other species investigated, often producing multiple stems, which may confound the detection of an ozone-induced effect on radial growth.

In response to ozone, seedlings of *F. excelsior* showed severe visible leaf injury as well as a significant decline in leaf gas exchange but no significant reduction in ring width. This

may be because the leaf-level ozone response of *F. excelsior* occured later in the growing season (Novak et al. 2005) by which time radial growth had been largely completed. Hence, significant leaf-level ozone effects beginning in the latter part of the growing season may have little influence on radial growth, unlike earlier and more severe leaf-level responses found for *P. nigra* (Table 2).

In all species, ozone treatments and years, lower δ^{13}C was related to higher g_w, higher c_i and lower WUE (Table 4, Figure 2), consistent with the theory proposed by Farquhar et al. (1982). These general trends suggest that changes in δ^{13}C were representative of the inherent physiological differences among the species. However, the effects of the ozone treatments on δ^{13}C and P_n differed. In general, treatment effects on δ^{13}C values matched well with previous findings of δ^{13}C responses to ozone-induced stress (Freyer 1979, Greitner and Winner 1988, Martin et al. 1988, Saurer et al. 1991, Matyssek et al. 1992, Jäggi et al. 2005); however, the higher δ^{13}C values induced by ozone exposure were unaccompanied by higher WUE or lower c_i, as predicted by the model of Farquhar et al. (1982). This may indicate enhanced phosphoenol pyruvate carboxylase (PEPC) activity as suggested by Saurer et al. (1995). Only *P. nigra* and *V. lantana* showed significant differences in δ^{13}C between treatments in 2001 and, for *V. lantana*, the change in δ^{13}C did not coincide with significant treatment effects on WUE or c_i, indicating that increased PEPC activity may not fully explain the observed effects of ozone on δ^{13}C in our experiment.

The lack of a clear relationship between ozone effects on δ^{13}C content in the tree rings and leaf gas exchange may be related to the times at which the parameters were measured. The δ^{13}C values were determined in wood samples derived from annual tree rings that represented growth throughout an entire growing season and were thus an integration of the various factors affecting gas exchange over the growing season, whereas the gas exchange measurements were based on individual leaves measured instantaneously at intervals during the growing season, and may not fully integrate seasonal gas exchange at the plant level (Fredericksen et al. 1995). In addition, not all photosynthates are translocated from the leaves to the rings (Jäggi et al. 2002).

Differences in microclimate inside the OTCs compared

with ambient conditions may have accounted for differences in response between the AA treatment and the NF treatment (Figures 2A–2F). For this reason, when comparing treatments, the AA treatment was omitted from the statistical analysis of ozone effects. However, the AA data were included in the correlation analysis to assess general trends in the relationships of the selected response parameters across all species and years.

In conclusion, we compared the effects of ozone of annual tree-ring growth and $\delta^{13}C$ in rings with leaf-level responses to ozone, such as visible foliar injury and leaf gas exchange, to assess seedling productivity of ozone-sensitive woody plant species exposed to ozone in OTCs. Significant ozone-induced effects that occurred at the leaf level did not correspond to reduced tree-ring growth or increased $\delta^{13}C$ in all species. Hence, the timing of ozone exposure and the severity of leaf-level responses in relation to the limited period of the growing season during which maximal increment growth and photosynthate translocation occur may be important in determining the sensitivity of tree productivity to ozone exposure. The results demonstrate the importance of the time-dependency of response parameters such as visible injury, tree-ring width, $\delta^{13}C$ and leaf gas exchange when determining the ozone risk of different tree species growing at differing phenological stages across Europe.

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