Available online at www.sciencedirect.com

SCIENCE DIRECT®

Environmental Pollution 136 (2005) 33–45

ENVIRONMENTAL POLLUTION

www.elsevier.com/locate/envpol

Seasonal trends in reduced leaf gas exchange and ozone-induced foliar injury in three ozone sensitive woody plant species

K. Novak[a,*], M. Schaub[a], J. Fuhrer[b], J.M. Skelly[c], C. Hug[a], W. Landolt[a], P. Bleuler[a], N. Kräuchi[a]

[a] *Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zuercherstrasse 111, 8903 Birmensdorf, Switzerland*
[b] *Swiss Federal Research Station for Agroecology and Agriculture FAL, 8046 Zürich, Switzerland*
[c] *Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA*

Received 2 April 2004; accepted 13 December 2004

Reductions in leaf gas exchange corresponded to the onset of ozone-induced visible foliar injury for seedlings exposed to ambient ozone exposures.

Abstract

Seasonal trends in leaf gas exchange and ozone-induced visible foliar injury were investigated for three ozone sensitive woody plant species. Seedlings of *Populus nigra* L., *Viburnum lantana* L., and *Fraxinus excelsior* L. were grown in charcoal-filtered chambers, non-filtered chambers and open plots. Injury assessments and leaf gas exchange measurements were conducted from June to October during 2002. All species developed typical ozone-induced foliar injury. For plants exposed to non-filtered air as compared to the charcoal-filtered air, mean net photosynthesis was reduced by 25%, 21%, and 18% and mean stomatal conductance was reduced by 25%, 16%, and 8% for *P. nigra*, *V. lantana*, and *F. excelsior*, respectively. The timing and severity of the reductions in leaf gas exchange were species specific and corresponded to the onset of visible foliar injury.

Keywords: Tropospheric ozone; Leaf gas exchange; Visible foliar injury; Open-top chambers; Southern Switzerland

---

1. Introduction

Areas of southern Switzerland and northern Italy are exposed to some of the highest ozone concentrations throughout much of Europe (Staffelbach et al., 1997; NABEL, 1995; de Leeuw and de Paus, 2001; Gerosa et al., 1999; VanderHeyden et al., 2001; Novak et al., 2003). As a result, ozone-induced foliar injury has been observed on a variety of tree, shrub, and herbaceous species native to Switzerland (Innes et al., 2001; Innes and Skelly, 1996; Novak et al., 2003; Skelly et al., 1998, 1999; VanderHeyden et al., 2001). Recent studies in southern Switzerland at the Lattecaldo Cantonal Forest Nursery in the Valle di Muggio, Canton Ticino have confirmed ambient tropospheric ozone exposures as the cause of visible foliar injury on 22 different woody plant species (VanderHeyden et al., 2001; Novak et al., 2003); *Populus nigra* L., *Viburnum lantana* L., and *Fraxinus excelsior* L. were among the most sensitive of the species investigated.

Many experiments have demonstrated the relationships between ozone exposure and reductions in both growth and physiological gas exchange (Reich, 1987; Pye, 1988; Matyssek et al., 1995; Sandermann et al., 1997; Schaub et al., 2003). Studies investigating physiological sensitivity to ambient ozone concentrations indicate that ozone stress may reduce carbon fixation,

---

* Corresponding author. Tel.: +41 1 739 2483; fax: +41 1 739 2215.
E-mail address: kristopher.novak@wsl.ch (K. Novak).

0269-7491/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2004.12.018

increase foliar and root respiration, shift the partitioning of carbon into different chemical forms, and disrupt carbon and nutrient allocation patterns (Friend and Tomlinson, 1992; Friend et al., 1992; Kelly et al., 1993; Baker et al., 1994; Laurence et al., 1994; Samuelson and Kelly, 1996; Scagel and Andersen, 1997). There is general agreement that ozone enters the leaf interior through the stomata to cause leaf tissue injury and it is widely accepted that leaf conductance is modified by environmental and phenological parameters: soil factors such as soil moisture deficit, plant development factors such as phenological stages, and factors influencing the instantaneous uptake of ozone by plants, including air temperature, leaf-to-air vapor pressure deficit, global radiation, and wind speed. Therefore, stomatal regulation must be an important factor in controlling ozone sensitivity of plants (Reich, 1987; Taylor and Hanson, 1992; Zhang et al., 2000; Wieser et al., 2000; Schaub et al., 2003). In addition, stomatal function may itself be affected by ozone injury (Keller and Häsler, 1984; Mansfield and Pearson, 1996).

The stomata also control carbon uptake, a crucial process for plant growth. When plants close their stomata, thereby avoiding further ozone uptake, CO_2 is also reduced, affecting for both plant growth and photosynthate allocation towards the repair of cells injured during ozone exposures (Zhang et al., 2000). It has also been suggested that ozone may directly inhibit stomatal opening, leading to reduced carbon assimilation (Torsethaugen et al., 1999). Therefore, it is important to consider the relationships between net photosynthesis and stomatal conductance when assessing plant sensitivity to ozone (Tjoelker et al., 1993; Volin et al., 1993; Fredericksen et al., 1996).

Given the current knowledge about the physiological and morphological responses of plants to ozone, there is general consensus that ozone exposures can induce a variety of responses in sensitive woody plant species. The relationships, timing, and implications of these responses and how they are influenced by various ozone exposures remain an important question. The objective of the current study was to assess the seasonal trends and relationships between the onset and development of ozone-induced visible foliar injury and leaf gas exchange responses for three ozone sensitive woody plant species exposed to ambient tropospheric ozone.

2. Materials and methods

2.1. Study site and plot design

The study took place from May to October of 2002 in the sub-alpine region of southern Switzerland at the Lattecaldo Cantonal Forest Nursery in the Valle di Muggio, Canton Ticino (09°03′E, 45°51′N, elev. 600 m asl) and was a continuation of a previous open-top chamber study conducted by Novak et al. (2003). The current study focused on 4-year-old seedlings of three ozone-sensitive species, *Viburnum lantana* L., *Populus nigra* L., and *Fraxinus excelsior* L., grown in open-top chambers and open plots. Each plot contained three individuals of each species. The experimental setup consisted of three ozone treatments with four replications (12 plots). The treatments included approximately 34% of the ambient ozone concentration for the plants grown within charcoal-filtered (CF) open-top chambers, 93% of the ambient ozone concentration for the plants grown within non-filtered (NF) open-top chambers, and 100% ambient (Amb) ozone exposures for plants grown within the open plots. Details pertaining to planting design and species composition were previously described by Novak et al. (2003).

2.2. Ozone and meteorological monitoring

Ozone concentrations (in parts-per-billion, ppb) were continuously monitored throughout the 2002 growing season from 11 May through 21 October using a Monitor Labs Model ML 8810 ozone monitor that

Table 1
Monthly and seasonal peaks and 24-h and 12-h (07:00–18:59) average ozone concentrations (ppb) for ambient (Amb), non-filtered (NF), and charcoal-filtered (CF) air treatments from 11 May–2 October 2002

2002	Amb	NF	CF
May[a]			
24-h avg.	46.7	42.7	19.6
12-h avg.	51	47.2	26.1
1-h peak	100.8	92.4	56.6
June			
24-h avg.	56	50.5	21
12-h avg.	60.2	54.6	26.3
1-h peak	152.5	134.9	68.3
July			
24-h avg.	46	42.4	16.2
12-h avg.	48.8	45.1	19.5
1-h peak	104.8	89.7	50.5
August			
24-h avg.	41.3	38.7	14.1
12-h avg.	44.1	41.7	16.7
1-h peak	83.1	77.2	36.7
September[b]			
24-h avg.	31.4	29.2	10
12-h avg.	32.8	30.7	11.3
1-h peak	73.4	70.6	31.8
Season			
24-h avg.	43.9	40.4	15.9
12-h avg.	46.9	43.5	19.5
1-h peak	152.5[c]	134.9[c]	68.3[d]

[a] Beginning on 11 May 2002.
[b] Finishing on 2 October.
[c] 22 June 2002.
[d] 17 June 2002.

was calibrated monthly. Two-minute air samples drawn at 1 m height were taken from one open plot (100% ambient air) and each of the open-top chambers; a repeating 20-min sampling interval was used 24 h/day. Meteorological data were provided by the Institute for Applied Plant Biology (IAP) (Switzerland) from a measurement station located next to the OTC facility within the Lattecaldo nursery. Hourly measurements included air temperature (°C), relative humidity (%), global radiation (W/m^2), and precipitation (mm).

2.3. Leaf gas exchange measurements

Leaf gas exchange, including net photosynthesis (P_n) and stomatal conductance to water vapor (g_{wv}), was measured from 17 June to 2 October in 2002 using a LiCor 6200 portable photosynthesis system equipped with a 0.25 l cuvette (LiCor Inc., Lincoln, NE). The area within the cuvette was marked at 11.88 cm^2 and only that area was covered by the inserted leaf. The LiCor 6200 system parameters were checked and calibrated using known CO_2 concentrations (0 and 400 ppm) at the beginning of each measurement period. Twenty-one measurement periods were conducted between 09:00 and 14:00 every 2–15 days depending on suitable weather conditions and elevated ozone episodes (during elevated ozone episodes, measurements were made every 2–3 days). Within each plot, one plant per species was selected and one lower and one upper fully sun-exposed leaf was tagged and measured throughout the experiment, resulting in a total of four lower and four upper leaves measured per treatment. The measurement of upper leaves began in mid-July. In the event that leaves were shed or damaged, the next measurable leaf upwards along the stem was selected.

Leaf gas exchange measurements were made only during optimal environmental conditions to avoid outliers and values measured under extreme ambient conditions. Measurements taken below in situ photosynthetically saturated light intensity levels ($PAR < 600$ $\mu mol\ m^{-2}\ s^{-1}$) and high ambient CO_2 concentrations

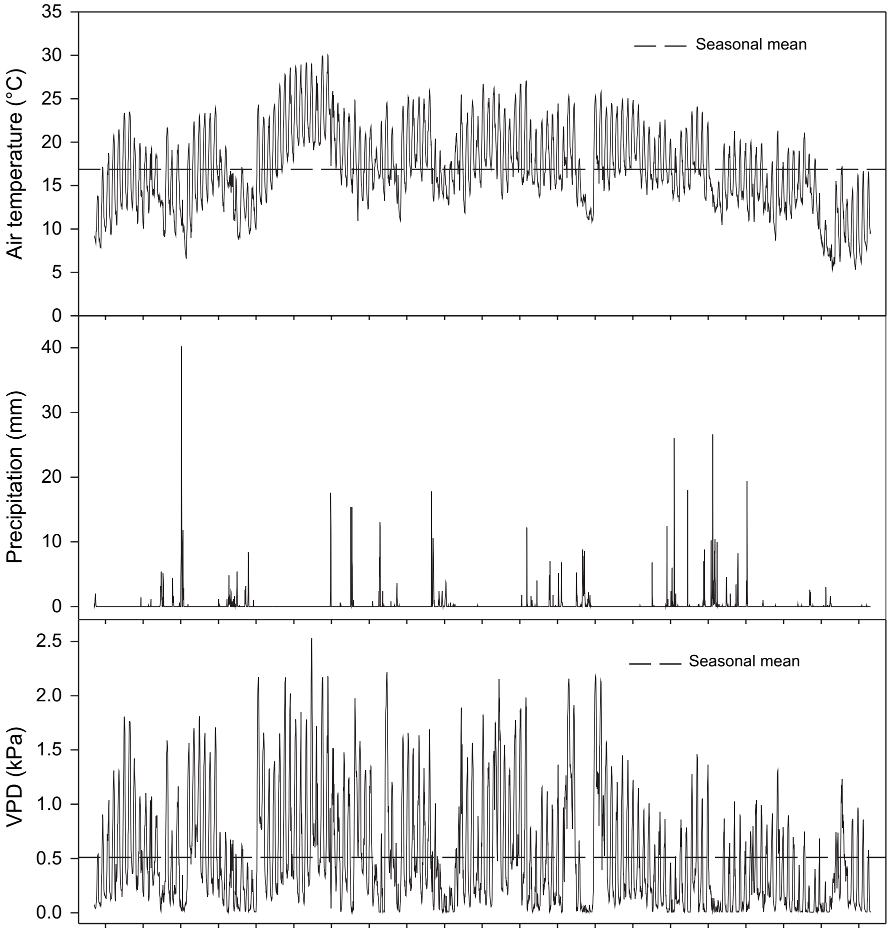

Fig. 1. Average and hourly values for air temperature (°C), precipitation (mm), and vapor pressure deficit (VPD) (kPa) from 11 May to 2 October 2002.

(>400 ppm) were omitted from the data analysis (Schaub et al., 2003). The average environmental conditions inside the cuvette throughout the measurement period were 28 °C for air temperature, 29 °C for leaf temperature, 51% for relative humidity and 1572 μmol m^{-2} s^{-1} for photosynthetically active radiation (PAR).

2.4. Visible foliar injury assessment

Visible foliar injury evaluations were made on the very same leaves used for leaf gas exchange measurements to determine the percentage of leaf area affected per leaf (%AA). Observations took place simultaneously to leaf gas exchange measurements; potential ozone-induced symptoms were further examined using a 10× hand lens. Symptoms on plants growing in non-filtered and open plots were compared to plants growing in the charcoal-filtered air chambers to further confirm ozone as the cause of foliar symptoms. A modified Horsfall–Barratt scale (0, 1, 3, 6, 12, 25, 50, 75, 88, 94, 97, 99, and 100%) was used to evaluate the percentage of area injured on symptomatic leaves (Horsfall and Barratt, 1945). A Forest Health Expert Advisory System (Nash et al., 1992a,b) was used for eye calibration to assure accuracy and consistency. Fully expanded leaf maturity dates following bud break were estimated to be 15 April for *V. lantana* and *P. nigra*; leaves of *F. excelsior* reached maturity approximately 2 weeks later, towards the end of April.

2.5. Data analysis

Data were checked for normality and homogeneous variance prior to analysis of variance. Leaf gas exchange data were checked for outliers and values outside the determined physiological limits were omitted, resulting in an unbalanced data set. A split plot design was employed for leaf gas exchange and foliar injury data using analysis of variance (ANOVA) in the General Linear Model procedure of the Statistical Analysis System (SAS) (SAS Institute, Inc, 1999), with ozone

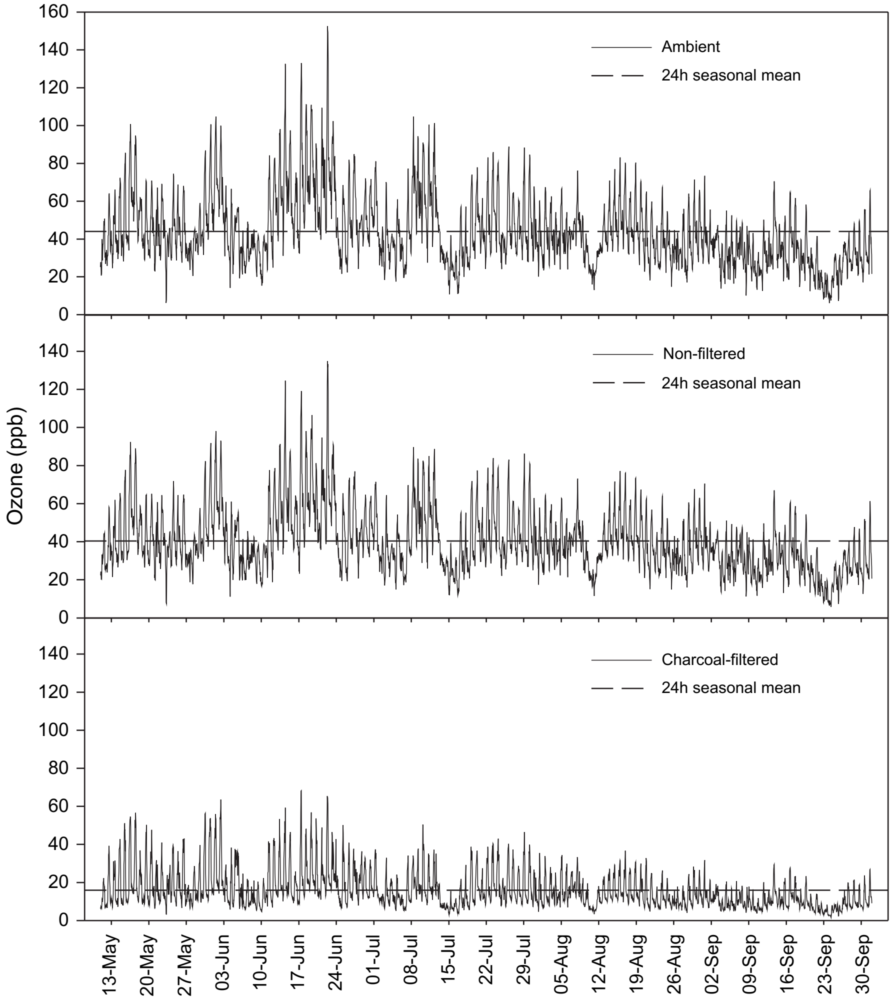

Fig. 2. Hourly (24-h) ozone concentrations (ppb) and 24-h seasonal averages (ppb) in ambient, non-filtered, and charcoal-filtered air treatments from 11 May to 2 October, 2002. (*Note: leaf gas exchange measurements began on 17 June.)

treatment as the main plot and species as the subplot. Foliar injury data were transformed using the square root of (injury + 0.5) prior to the analysis (Zhang et al., 2000). Correlations were made between visible foliar injury and net photosynthesis over the 21 measurement periods from June to October and correlation coefficients were calculated. Treatment effect values for net photosynthesis (P_{effect}) and stomatal conductance to water vapor (g_{effect}) were calculated by subtracting the values measured under charcoal-filtered air conditions from values measured under ambient and non-filtered air conditions (Amb-CF and NF-CF). Repeated measures analysis of the gas exchange data resulted in a loss of data due to unbalanced observations and was not used. For ozone concentrations, the AOT40 statistics (Accumulated exposure Over a Threshold of 40 ppb for daylight hours 07:00–18:59) were employed.

3. Results

3.1. Ozone exposures and climatic conditions

The monthly and seasonal peak and mean ozone concentrations (ppb) from 11 May to 2 October 2002 are summarized in Table 1. Seasonal 12-h (07:00–18:59) mean ozone concentrations in the ambient, non-filtered, and charcoal-filtered air treatments were 47 ppb, 44 ppb, and 20 ppb, respectively. Extended periods of elevated ozone exposures were not frequent in 2002 with only one major ozone episode occurring in June. Following a 6-day rain period, the elevated ozone episode began on 10 June and lasted until the following rainfall on 24 June (Figs. 1 and 2). During this period, ozone concentrations exceeded 100 ppb on 7 out of 14 days with the highest peak of 152.5 ppb occurring on 22 June. The AOT40 value (07:00–18:59) for this 2-week period was 5.6 ppm h ozone for ambient air (Fig. 3). AOT40 ozone exposures from 11 May to 2 October reached 20.6 ppm h in the ambient air treatment, 16.4 ppm h in the non-filtered air treatment, and 1.0 ppm h in the charcoal-filtered air treatment (Fig. 3). The seasonal trends (May to October) for air temperature (°C), precipitation (mm), and vapor pressure deficit (VPD) (kPa) are shown in Fig. 1; mean ambient temperature was 17 °C, sum precipitation reached 919 mm, and mean VPD was 0.51 kPa.

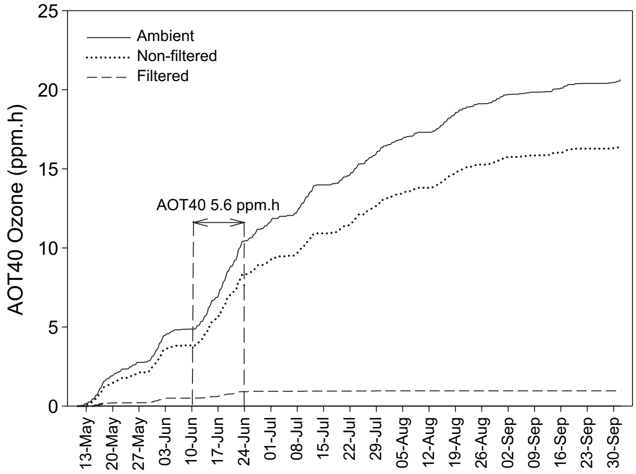

Fig. 3. Cumulative AOT40 ozone exposures (ppm h) for ambient air and, non-filtered and charcoal-filtered air open-top chamber treatments from 11 May to 2 October 2002 for daylight hours (07:00–18:59). Vertical dashed lines mark a 14-day elevated ozone episode from 10 June to 24 June during which AOT40 5.6 ppm h of ozone were accumulated.

3.2. Visible foliar ozone injury

Based on observations of the leaves used for leaf gas exchange measurements, ozone-induced visible foliar injury began in late June on *V. lantana* and *P. nigra*, and in early July on *F. excelsior* in the ambient and non-filtered air treatments (Figs. 4–6). Significant differences among ozone treatments and species were observed (Table 2). Also, the type and severity of symptoms were species specific.

Foliar injury on the lower leaves of *P. nigra* began on 25 June in the ambient and non-filtered air treatments (Fig. 4A). The upper leaves showed injury starting on 12 and 18 August for the non-filtered and ambient air treatments, respectively (Fig. 4B). Injury began as chlorotic mottling followed by dark upper surface, interveinal stipple. Stippling increased until most of the interveinal leaf surface appeared dark brown or black. Leaf yellowing often occurred simultaneously to stippling and the most severely injured leaves were shed. Leaf abscission in the non-filtered and ambient air treatments began on 18 July and 5 August, respectively (Fig. 4A). On the other hand, plants grown in the charcoal-filtered air treatment retained green leaves throughout much of the growing season and only exhibited injury later in the season starting on 5 August.

Foliar injury on the lower leaves of *V. lantana* began on 17 June in the ambient and non-filtered air treatments (Fig. 5A). Foliar injury on the upper leaves of *V. lantana* was only observed in the ambient air treatment starting on 2 August (Fig. 5B). The injury was characterized as fine reddish upper surface, interveinal stipple. As the injury progressed, the reddish stipple covered much of the upper leaf surfaces.

Injury onset was 11 July in the ambient and non-filtered air treatments for *F. excelsior* and began as fine upper surface, interveinal brown stipple. An increase in symptom severity was observed in the non-filtered air treatment during the latter part of the season as the upper surface of some leaves became entirely brown,

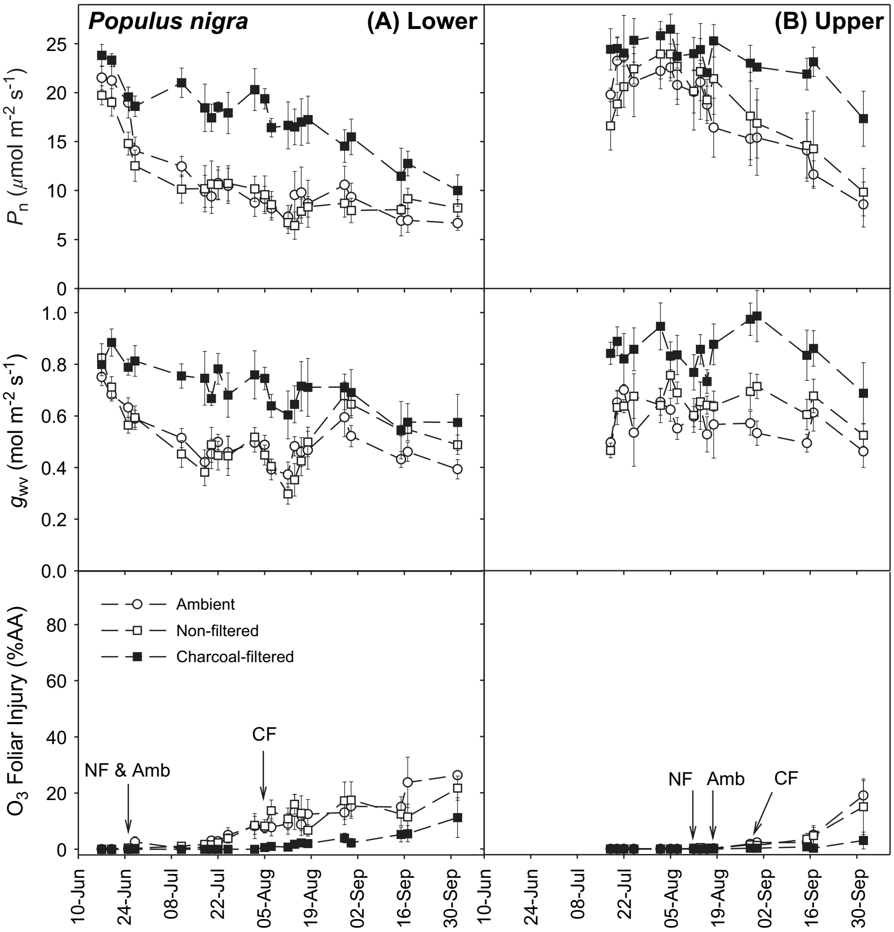

Fig. 4. Mean net photosynthesis (P_n), stomatal conductance (g_{wv}), and ozone-induced foliar injury (%AA) for (A) lower leaves and (B) upper leaves of *P. nigra* grown in ambient air, and non-filtered and charcoal-filtered open-top chamber treatments. Arrows indicate the dates of injury onset for each treatment. Abscission of the lower leaves used for gas exchange and injury assessments occurred on 5, 12, 16 and 29 August and 15 September in the ambient air treatment, and 18 July, 12 and 16 August and 15 September in the non-filtered air treatment.

followed by leaf abscission starting on 7 August (Fig. 6A). The upper leaves showed injury starting on 18 July and 2 August for the non-filtered and ambient air treatments, respectively (Fig. 6B); leaf abscission in the non-filtered air began on 29 August.

3.3. Leaf gas exchange

Significant differences in net photosynthesis (P_n) and stomatal conductance (g_{wv}) were observed among ozone treatments and species (Table 2). Ozone exposures in the ambient air treatment, as compared to the charcoal-filtered air treatment, reduced seasonal mean (average of all measurement periods from June through October) P_n of the lower and upper leaves combined by 26%, 22%, and 8% for *P. nigra*, *V. lantana*, and *F. excelsior*, respectively. Likewise, seasonal mean g_{wv} was reduced by 28%, 24%, and 20% for *P. nigra*, *V. lantana*, and *F. excelsior*, respectively. Ozone exposures in the non-filtered air treatment, as compared to the charcoal-filtered air treatment, reduced seasonal mean P_n of the lower and upper leaves combined by 25%, 21%, and 18% for *P. nigra*, *F. excelsior*, and *V. lantana*, respectively. Likewise, seasonal mean g_{wv} was reduced by 25%, 16%, and 8% for *P. nigra*, *V. lantana*, and *F. excelsior*, respectively. *Populus nigra* exhibited the highest mean (averaged over all positions, treatments, and measurement dates) leaf gas exchange rates of the three species ($P_n = 16.2$ µmol m^{-2} s^{-1}, $g_{wv} = 0.63$ mol m^{-2} s^{-1}) followed by *V. lantana* ($P_n = 14.1$ µmol m^{-2} s^{-1}, $g_{wv} = 0.43$ mol m^{-2} s^{-1}) and *F. excelsior* ($P_n = 10.1$ µmol m^{-2} s^{-1}, $g_{wv} = 0.42$ mol m^{-2} s^{-1}), which had similar g_{wv} rates.

3.4. Trends in leaf gas exchange and visible foliar injury

Net photosynthesis and visible foliar injury of lower leaves for all three species showed a moderate negative correlation. R^2 values for *P. nigra* were equal to 0.44, 0.49 and 0.70 for ambient, non-filtered and charcoal-filtered treatments, respectively. R^2 values were equal to 0.53 and 0.43 for *V. lantana* and 0.39 and 0.56 for

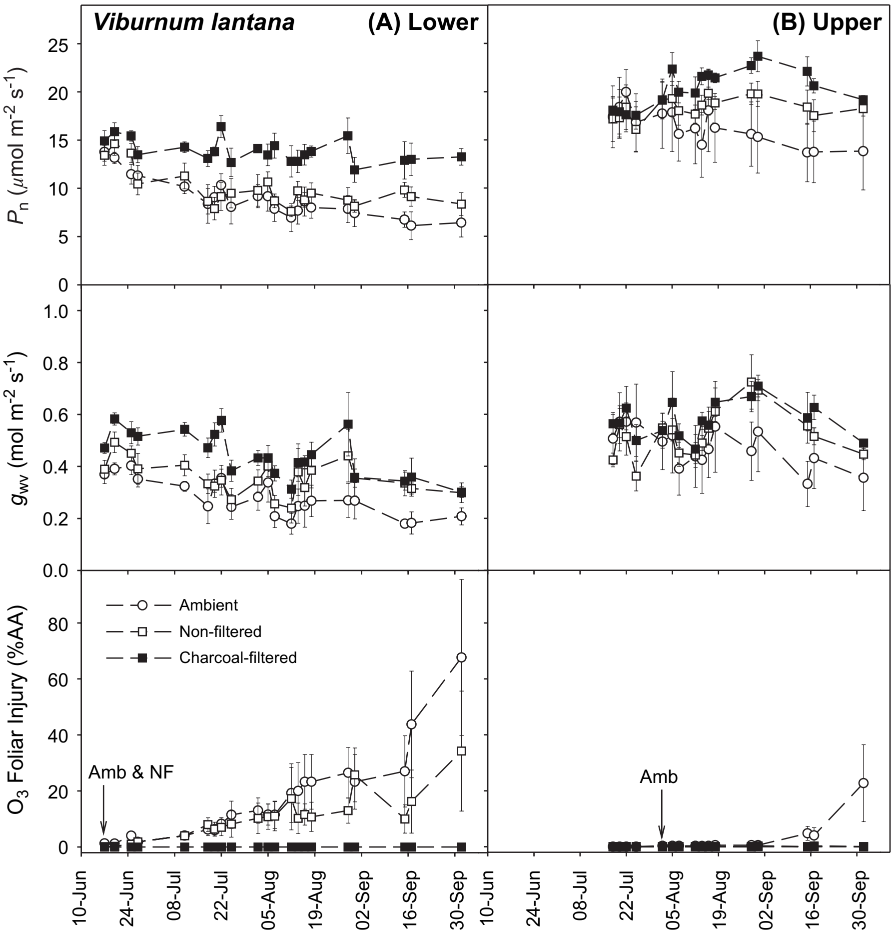

Fig. 5. Mean net photosynthesis (P_n), stomatal conductance (g_{wv}), and ozone-induced foliar injury (%AA) for (A) lower leaves and (B) upper leaves of *V. lantana* grown in ambient air, and non-filtered and charcoal-filtered open-top chamber treatments. Arrows indicate the dates of injury onset for each treatment. Lower leaves used for gas exchange and injury assessments were changed on 18 July and 31 August in the ambient air treatment, and 14 August and 15 September in the non-filtered air treatment.

F. excelsior in the ambient and non-filtered treatments, respectively.

Seasonal curves of leaf gas exchange and visible foliar injury for lower (A) and upper (B) leaves of *P. nigra*, *V. lantana*, and *F. excelsior* are shown in Figs. 4–6. In addition, the seasonal curves for the treatment effect (Amb-CF and NF-CF) for net photosynthesis (P_{effect}) and stomatal conductance (g_{effect}) of lower and upper leaves are shown in Figs. 7–9 along with the AOT40 ozone exposures. A negative P_{effect} and g_{effect} on the lower leaves of *P. nigra* and *V. lantana* was present at the start of gas exchange measurements (Figs. 7 and 8). For *P. nigra*, a distinct reduction in both P_n and g_{wv} and the onset of visible foliar injury occurred between 20 and 27 June (Figs. 4 and 7) when ozone concentrations reached a seasonal peak of 152 ppb (Table 1) and AOT40 values increased sharply (Fig. 3). A slight downward trend was detectable in g_{wv} in the lower leaves of *F. excelsior* at the time of the ozone episode, but differences among treatments in P_n and the onset of visible foliar injury in the non-filtered treatment occurred later, beginning on 11 July (Figs. 6 and 9). The greatest differences among treatments in P_n and g_{wv} occurred on 14 August, corresponding to increases in visible injury.

Stomatal conductance was significantly lower in the ambient and non-filtered air treatments as compared to the charcoal-filtered air treatment throughout much of the season for *P. nigra* and *V. lantana* (Figs. 4, 5, 7, 8), whereas noticeable reductions in g_{wv} in the non-filtered air treatment began later in the season for *F. excelsior* (Figs. 6 and 9). On the contrary, differences in g_{wv} between the ambient and charcoal-filtered air treatments were present at the start of measurements.

Reductions in P_n and the development of foliar injury for *P. nigra* in the charcoal-filtered air were observed starting on 5 August. Stomatal conductance to water vapor also decreased during the same period, but to a lesser extent than P_n (Fig. 4).

The seasonal curves in gas exchange and treatment effect for the upper leaves of *P. nigra*, *V. lantana*, and *F. excelsior* are shown in Figs. 4–9B. For *P. nigra*, P_n was generally lower in the ambient and non-filtered air

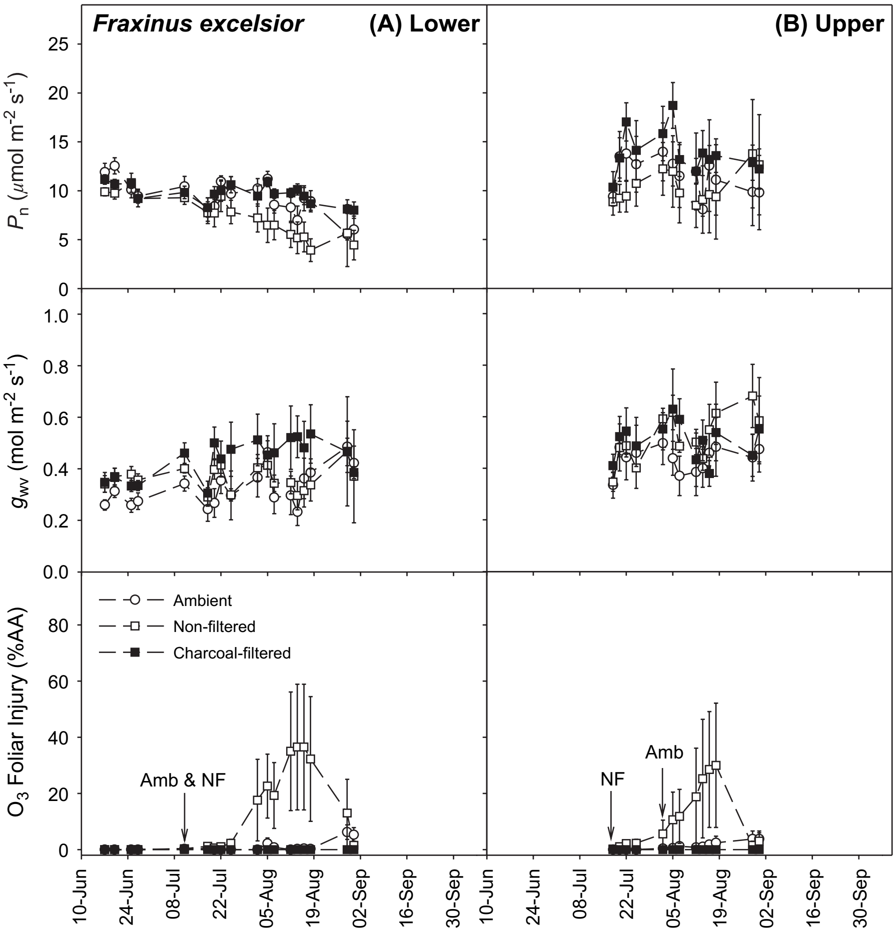

Fig. 6. Mean net photosynthesis (P_n), stomatal conductance (g_{wv}), and ozone-induced foliar injury (%AA) for (A) lower leaves and (B) upper leaves of *F. excelsior* grown in ambient air, and non-filtered and charcoal-filtered open-top chamber treatments. Arrows indicate the dates of injury onset for each treatment. Abscission of the lower leaves used for gas exchange and injury assessments occurred on 7 and 31 August in the ambient air treatment, and 7, 18, and 31 August in the non-filtered air treatment. Leaf abscission of the upper leaves in the non-filtered air treatment occurred on 29 August.

treatment as compared to the charcoal-filtered treatment and distinct decreases began in mid-August, accompanied by the development of visible foliar injury on 12 and 18 August in the non-filtered and charcoal-filtered air, respectively (Figs. 4 and 7). Stomatal conductance to water vapor was also lower throughout the measurement period for the ambient and non-filtered air treatments as compared to the charcoal-filtered air treatment. For *V. lantana*, reductions in P_n for ambient and non-filtered air treatments with respect to the charcoal-filtered air became significant on 18 August; the decrease was greatest in the ambient air treatment on 15 September, when visible foliar injury was also observed (Figs. 5 and 8). Stomatal conductance to water vapor was reduced in the ambient air treatment, whereas it remained similar in the charcoal-filtered and non-filtered air treatments. Although no clear trends were observed in leaf gas exchange values for the upper leaves of *F. excelsior*, P_n values were generally lower in the ambient and non-filtered air as compared to the charcoal-filtered air treatment; no clear treatment effect was observed for g_{wv}.

Table 2
Degrees of freedom (df), mean squares (MS), and F values (F) for a split-plot design of analysis of variance for net photosynthesis (P_n), stomatal conductance to water vapor (g_{wv}), and visible foliar injury among three species, grown in non-filtered and charcoal-filtered air treatments

Source of variation	df	P_n (µmol m^{-2} s^{-1})		g_{wv} (mol m^{-2}s^{-1})		Foliar injury	
		MS	*F*	MS	*F*	MS	*F*
Block (A)	3	184.85		0.12		24.9	
O$_3$ treatment (B)	2	1689.67	10.53*	2.06	5.67*	248.08	17.89**
AB	6	160.42		0.36		13.87	
Species (C)	2	1560.61	19.87***	4.45	38.54***	37.46	2.21
BC	4	263.16	3.35*	0.11	0.99	38.88	2.29
ABC	18	78.54		0.12		16.96	
Error	706	34.08		0.05		3.73	

* $P<0.05$;
** $P<0.01$;
*** $P<0.001$.

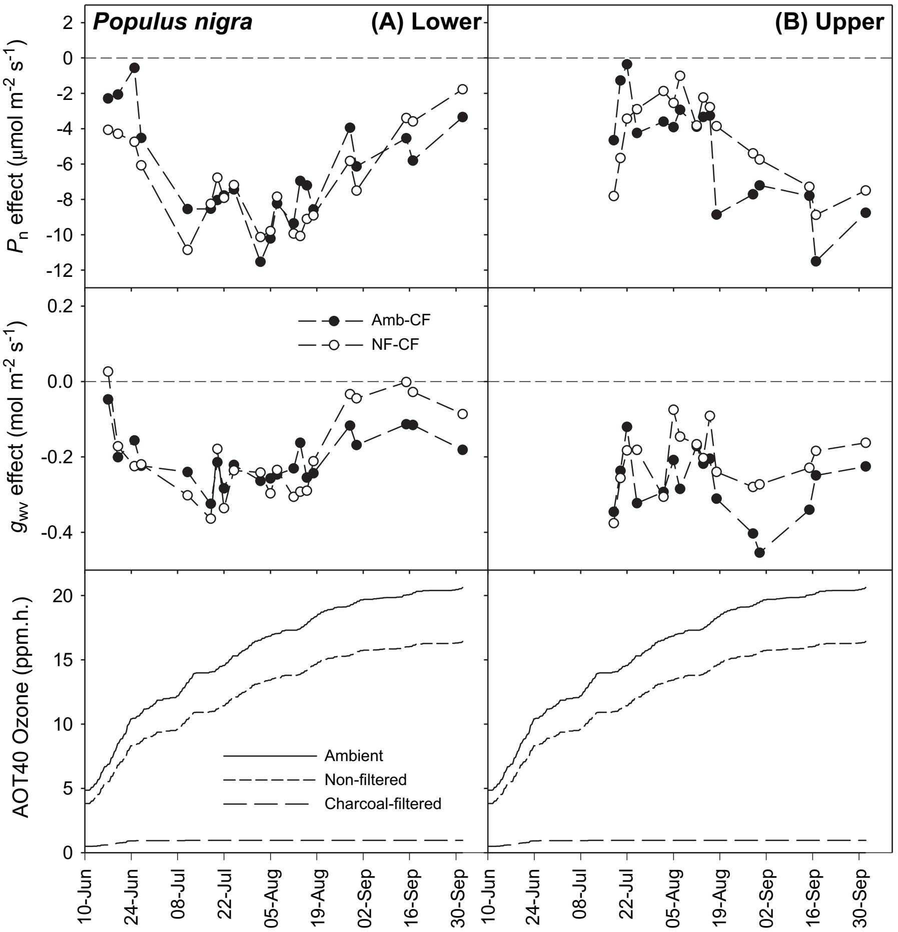

Fig. 7. Treatment effect for net photosynthesis (P_{effect}) and stomatal conductance (g_{effect}) for ambient (Amb) and non-filtered (NF) treatments as compared to the charcoal-filtered (CF) treatment (Amb-CF and NF-CF) and AOT40 ozone exposures for (A) lower leaves and (B) upper leaves of *P. nigra*.

4. Discussion

Ambient ozone exposures at the Lattecaldo open-top chamber research facility were sufficient to induce typical visible foliar injury and to reduce gas exchange in *P. nigra*, *V. lantana*, and *F. excelsior*. Visible injury observed on these species throughout the 2002 observation period were similar to those described previously by Novak et al. (2003); VanderHeyden et al. (2001). Although visible foliar injury generally increased over the course of the season for all three species, there were several decreases in the average percentage of area affected (%AA) in the latter portion of the observation period due to abscission of the leaves used for gas exchange measurements (Figs. 4–6). The next highest leaf along the stem was selected, which was often less injured than the previously measured leaf.

Reductions in P_n and g_{wv} corresponding to the development of visible foliar injury due to ambient ozone exposures were previously reported by Zhang et al. (2000); Gravano et al. (2004) on *V. lantana*, and *F. excelsior*. In addition, gas exchange values reported for *F. excelsior* are consistent with recent findings by Gerosa et al. (2003) in Curno, northern Italy. Although significant reductions occurred across all species, the timing and severity of these responses were species specific. *Populus nigra* showed the greatest reductions of P_n (26%) in the ambient as compared to the charcoal-filtered air treatment. Volin et al. (1993) reported a similar 25% reduction in P_n for hybrid *Populus* spp. grown in fumigation chambers. Among all species, *P. nigra* also showed the highest gas exchange and the greatest reduction in leaf gas exchange in response to ozone exposures (Reich, 1987). This supports findings by Bortier et al. (2000), which suggest that faster growing species tend to be more sensitive than slower growing species. Likewise, Bortier et al. (2000) found significant reductions in net photosynthesis for *P. nigra* grown in open-top chambers.

For *P. nigra*, slight reductions in P_n were detectable at the start of the measurements in mid-June (Figs. 7–9) prior to the onset of visible foliar injury, as was observed by Gravano et al. (2004) for *F. excelsior* and *Prunus avium*. This negative treatment effect was also observed

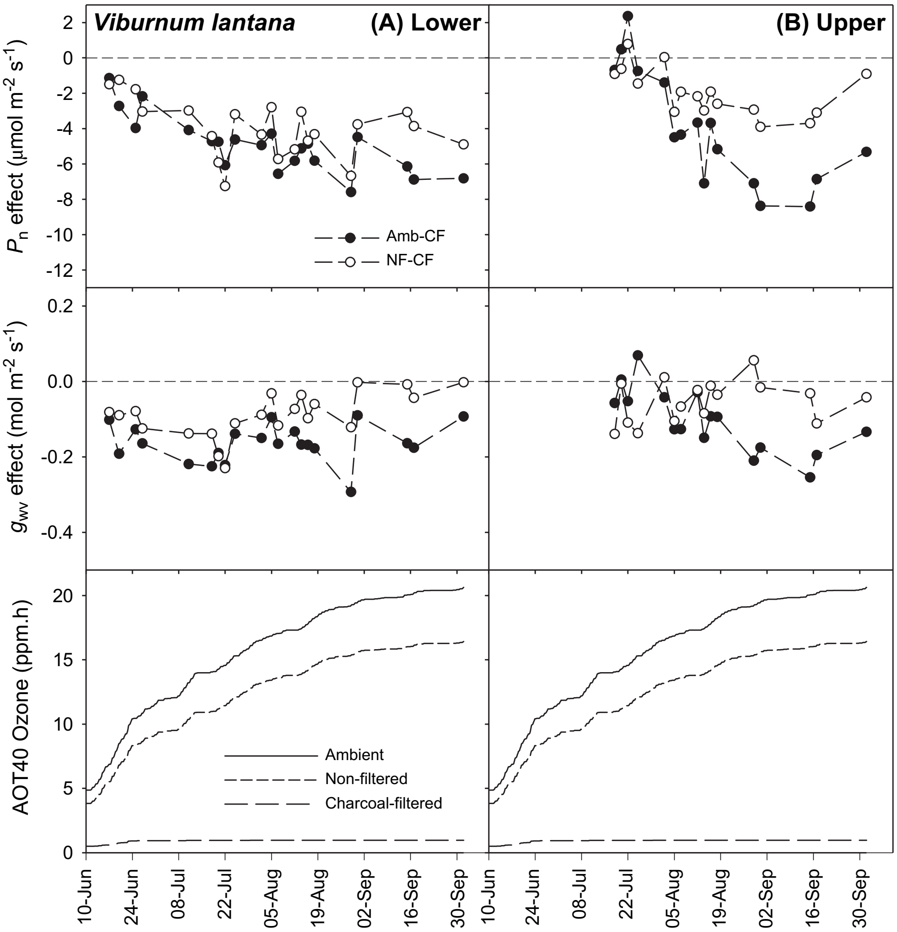

Fig. 8. Treatment effect for net photosynthesis (P_{effect}) and stomatal conductance (g_{effect}) for ambient (Amb) and non-filtered (NF) treatments as compared to the charcoal-filtered (CF) treatment (Amb-CF and NF-CF) and AOT40 ozone exposures for (A) lower leaves and (B) upper leaves of *V. lantana*.

in the upper leaves prior to the detection of visible symptoms, especially for *P. nigra* where values in the ambient and non-filtered air were always lower than in the charcoal-filtered air (Fig. 7).

Despite these slight reductions, the greatest negative effects on gas exchange, especially for P_n, occurred during the same period as the onset of visible injury on those leaves that gas exchange was measured (Figs. 7–9). This can be expected since stippling is a result of necrotic cells, which in return results in less intact leaf area and the degradation of the photosystem (Pell et al., 1992). *Populus nigra* and *V. lantana* showed similar trends of decreased gas exchange and increased visible foliar injury (Figs. 4, 5, 7, 8), but *F. excelsior* exhibited visible foliar injury onset on 11 July, after which, smaller decreases in P_n were observed as compared to the other species (Figs. 6 and 9).

For all species, P_n and visible foliar injury were negatively correlated, but an upward trend in P_{effect} and g_{effect} was observed in the latter part of the season especially for *P. nigra* and *V. lantana*. This could be due to the abscission of leaves (especially in *P. nigra*), which resulted in the selection of the next upper and younger, less injured leaves and thus, higher gas exchange rates. During this same period (late August/early September), a chamber effect, especially in terms of g_{wv}, was detectable, most likely because plants in the non-filtered air were exposed to approximately 10% less ozone than plants in the ambient air; thus, towards the end of the season, plants in the charcoal and non-filtered air treatments responded similarly in terms of g_{wv}. In addition, the onset of foliar injury in the charcoal-filtered air began during the same period, which, in addition to leaf age and cumulative ozone exposures, may reduce gas exchange.

Stomatal conductance was not as negatively correlated with visible foliar injury as P_n even though there were significant differences in g_{wv} among treatments. As observed with P_n, there were significant differences in g_{wv} at the start of measurements for *P. nigra* and *V. lantana* (Figs. 7–9), indicating the onset of a negative ozone effect on the stomatal functioning of the plants (Keller and Häsler, 1984); on the other hand, for *F. excelsior*, differences among treatments in g_{wv} began

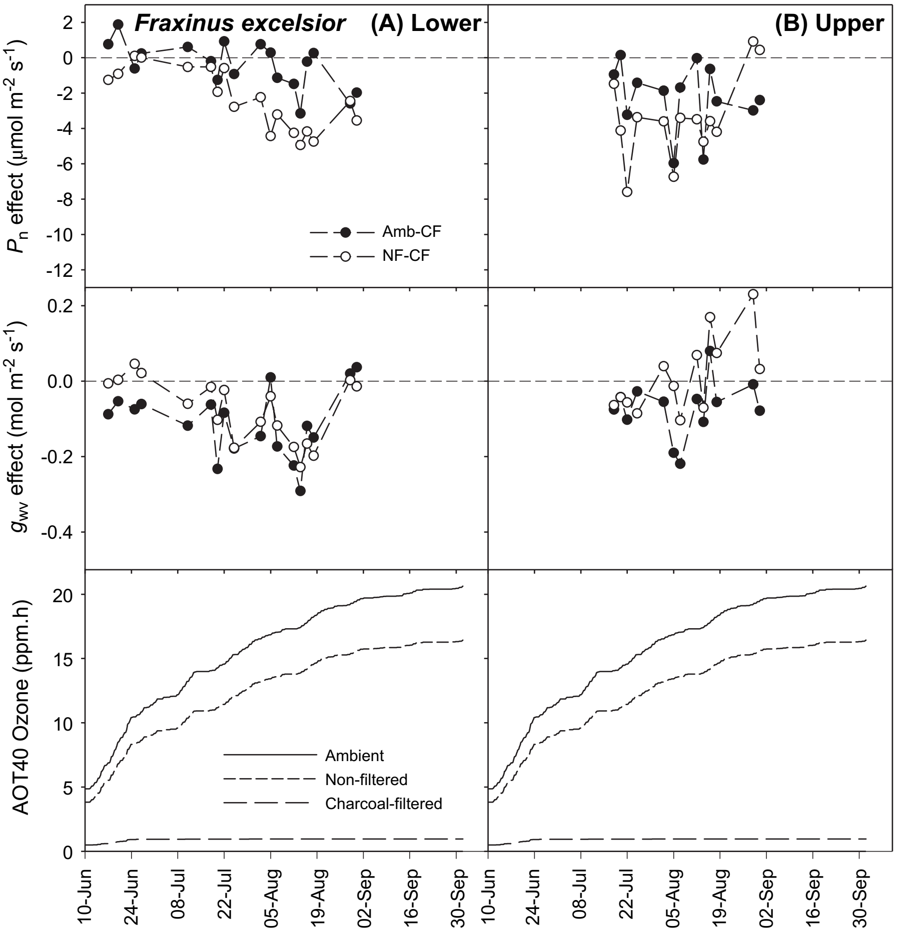

Fig. 9. Treatment effect for net photosynthesis (P_{effect}) and stomatal conductance (g_{effect}) for ambient (Amb) and non-filtered (NF) treatments as compared to the charcoal-filtered (CF) treatment (Amb-CF and NF-CF) ozone and AOT40 ozone exposures for (A) lower leaves and (B) upper leaves of *F. excelsior*.

after 22 July (Figs. 6 and 9) and these differences were less than those observed for the other species.

The difference between upper and lower leaves in the charcoal-filtered air treatment showed a clear age effect on gas exchange (Pearson and Brooks, 1995; Whitehead and Singh, 1995) with the older leaves having lower P_n and g_{wv} values as compared to young, upper leaves. When comparing gas exchange responses between the ozone treatments for upper, uninjured leaves, an ozone-induced reduction of gas exchange was observed (Figs. 7–9). As the season progressed the upper leaves of all species also began to show visible symptoms as they themselves became exposed to higher cumulative values of ozone.

The question remains as to why *F. excelsior* responded later and with more variability than the other two species in terms of both visible injury and leaf gas exchange. One possible explanation may be the difference in the phenological development of the different species and the varying rates of g_{wv}. Leaves of *F. excelsior* seedlings reached maturity approximately 2 weeks later than the other species and exhibited inherently lower g_{wv} rates. Thus, the amount of time available for ozone uptake by the leaves and the rate of uptake would result in less ozone uptake and therefore a slightly later response than the other species. This corresponds to the findings of Reich (1987), which suggest that differences in responses can be predicted from inherent differences in g_{wv}. Another possible explanation, especially for the foliar injury response, could be that several individuals in the non-filtered air were clearly more injured than the others, which is apparent in the high variability in the injury values (Figs. 6).

In conclusion, the greatest reductions in leaf gas exchange for plants exposed to non-filtered and ambient air with respect to plants exposed to charcoal-filtered air corresponded to the onset of visible foliar injury. Both the onset of visible foliar injury and reduced leaf gas exchange appeared to be triggered by a major early season ozone episode. Following the sharp decrease in leaf gas exchange in correspondence with the early season ozone episode and onset of visible injury, leaf gas exchange levels remained fairly constant or slightly decreased even as strong increases in foliar injury occurred late in the season. This suggests that early season peak ozone episodes may have lasting negative effects on leaf gas exchange. This seems to be particularly true for ozone sensitive plant species such as *P. nigra*. There was indeed a correlation between net photosynthesis and foliar injury, but many factors such as leaf age and increases in phenolics, ethylene production, and other secondary compounds may also influence plant response to ozone exposures. Thus, further investigations are needed to better explain the role of these factors and mechanisms such as detoxification, compensation, and avoidance in determining the seasonal trends in plant responses to ozone.

Acknowledgements

Funding for this research project was provided by the Swiss Agency for the Environment, Forests, and Landscape (SAEFL). We would like to thank Dr. Sabine Braun from the Institute for Applied Plant Biology (IAP) (Switzerland) for providing the meteorological data for the study. The project would not have been possible without the exceptional support provided by Mr. Giuseppe Tettamanti and his nursery staff at the Lattecaldo research site. We also would like to thank the Canton Ticino for the continuous support of the Lattecaldo research facility.

References

Baker, T.R., Allen, H.L., Schoeneberger, M.M., Kress, L.W., 1994. Nutritional response of loblolly pine exposed to ozone and simulated acid rain. Canadian Journal of Forest Research 24, 453–461.

Bortier, K., De Temmerman, L., Ceulemans, R., 2000. Effects of ozone in open-top chambers on poplar (*Populus nigra*) and beech (*Fagus sylvatica*): a comparison. Environmental Pollution 109, 509–516.

de Leeuw, F.A., de Paus, T.A., 2001. Exceedance of EC ozone threshold values in Europe in 1997. Water, Air, and Soil Pollution 128, 255–281.

Fredericksen, T.S., Steiner, K.C., Skelly, J.M., Joyce, B.J., Kolb, T.E., Kouterick, K., Ferdinand, J.A., 1996. Dial and seasonal patterns of leaf gas exchange and xylem water potentials of different-sized *Prunus serotina* Ehrh. trees. Forest Science 42, 359–365.

Friend, A.L., Tomlinson, P.T., 1992. Mild ozone exposure alters ^{14}C dynamics in foliage of *Pinus taeda* L. Tree Physiology 11, 215–227.

Friend, A.L., Tomlinson, P.T., Dickson, R.E., O'Neill, E.G., Edwards, N.T., Taylor, G.E., 1992. Biochemical composition of loblolly pine reflects pollutant exposure. Tree Physiology 11, 35–47.

Gerosa, G., Marzuoli, R., Bussotti, F., Pancrazi, M., Ballarin-Denti, A., 2003. Ozone sensitivity of *Fagus sylvatica* and *Fraxinus excelsior* young trees in relation to leaf structure and foliar ozone uptake. Environmental Pollution 125, 91–98.

Gerosa, G., Spinazzi, F., Ballarin-Denti, A.B., 1999. Tropospheric ozone in alpine forest sites: air quality monitoring and statistical data analysis. Water, Air, and Soil Pollution 116, 345–350.

Gravano, E., Bussotti, F., Strasser, R.J., Schaub, M., Novak, K., Skelly, J.M., Tani, C., 2004. Ozone symptoms in leaves of woody plants in open-top chambers: ultrastructural and physiological characteristics. Physologia Plantarum 121, 620–633.

Horsfall, J.G., Barratt, R.W., 1945. An improved grading system for measuring plant disease. Phytopathology 35, 655.

Innes, J.L., Skelly, J.M., 1996. Effects of ambient concentrations of ozone on visible symptom development of *Prunus serotina* in southern Switzerland. Research Report to the Swiss Federal Institute for Forest, Snow, and Landscape Research, Birmensdorf, Switzerland, 52 pp.

Innes, J.L., Skelly, J.M., Schaub, M., 2001. Ozone and broadleaved species: a guide to the identification of ozone-induced foliar injury/ Ozon, Laubholz- und Krautpflanzen: Ein Führer zum Bestimmen von Ozonsymptomen. Birmensdorf, Eidgenössische Forschungsanstalt WSL Haupt, Bern, Stuttgart, Wien, 136 pp.

Keller, T., Häsler, R., 1984. The influence of a fall fumigation with ozone on the stomatal behavior of spruce and fir. Oecologia 64, 284–286.

Kelly, J.M., Taylor, G.E., Edwards, N.T., Adams, M.B., Edwards, G.S., Friend, A.L., 1993. Growth, physiology, and nutrition of loblolly pine seedlings stressed by ozone and acidic precipitation – a summary of the ROPIS-south project. Journal of Water, Air, Soil Pollution 69, 363–391.

Laurence, J.A., Amundson, R.G., Friend, A.L., Pell, E.J., Temple, P.J., 1994. Allocation of carbon in plants under stress: an analysis of the ROPIS experiments. Journal of Environmental Quality 23, 412–417.

Mansfield, T.A., Pearson, M., 1996. Disturbances in stomatal behavior in plants exposed to air pollution. In: Yunus, M., Iqbal, M. (Eds.), Plant Response to Air Pollution. John Wiley, New York.

Matyssek, R., Reich, P.B., Oren, R., Winner, W.E., 1995. Response mechanisms of conifers to air pollution. In: Smith, W.K., Hinckley, T.M. (Eds.), Ecophysiology of Coniferous Forests. Academic Press, New York, pp. 255–308.

NABEL, 1995. Luftbelastung 1994. In: Schriftenreihe Umwelt 244. Bundesamt für Umwelt. Wald und Landschaft, Bern.

Nash, B.L., Saunders, M.C., Miller, B.J., Bloom, C.A., Davis, D.D., Skelly, J.M., 1992a. ForestHealth, an expert advisory system for assessing foliar and crown health of selected northern hardwoods. Canadian Journal of Forest Research 22, 1770–1775.

Nash, B.L., Saunders, M.C., Miller, B.J., Bloom, C.A., Davis, D.D., Skelly, J.M., 1992b. The ForestHealth expert advisory system as a diagnostic and training tool for assessing tree health. In: Watson, D.G., Zazueta, F.S., Bottcher, A.B. (Eds.), Computers in Agricultural Extension Programs. American Society of Agricultural Engineers, St Joseph, Michigan, pp. 417–442.

Novak, K., Skelly, J.M., Schaub, M., Kräuchi, N., Hug, C., Landolt, W., Bleuler, P., 2003. Ozone air pollution and foliar injury development on native plants of Switzerland. Environmental Pollution 125, 41–52.

Pearson, M., Brooks, G.L., 1995. The influence of elevated CO_2 on growth and age-related changes in leaf gas exchange. Journal of Experimental Botany 46, 1651–1659.

Pell, E.J., Eckardt, N., Enyedi, A.J., 1992. Timing and ozone stress and resulting status of ribulose bisphosphate carboxylase/oxygenase and associated net photosynthesis. New Phytologist 120, 397–405.

Pye, J.M., 1988. Impact of ozone on the growth and yield of trees. Journal of Environmental Quality 17, 347–360.

Reich, P.B., 1987. Quantifying plant response to ozone: a unifying theory. Tree Physiology 3, 347–360.

Samuelson, L.J., Kelly, J.M., 1996. Carbon partitioning and allocation in northern red oak seedlings and mature trees in response to ozone. Tree Physiology 16, 853–858.

Sandermann, H., Wellburn, A.R., Heath, R.L. (Eds.), 1997. Forest Decline and Ozone: A Comparison of Controlled Chamber and Field Experiments. Ecological Studies, 127. Springer, Berlin.

SAS Institute, Inc, 1999. SAS Software Version 8. SAS Institute, Inc, Cary, NC.

Scagel, C.F., Andersen, C.P., 1997. Seasonal changes in root and soil respiration of ozone-exposed ponderosa pine (*Pinus ponderosa*) grown in different substrates. New Phytologist 136, 627–643.

Schaub, M., Skelly, J.M., Steiner, K.C., Davis, D.D., Pennypacker, S.P., Zhang, J., Ferdinand, J.A., Savage, J.E., Stevenson, R.E., 2003. Physiological and symptom responses of *Prunus serotina*, *Fraxinus americana*, and *Acer rubrum* seedlings to varying soil moisture and ozone. Environmental Pollution 124, 307–320.

Skelly, J.M., Innes, J.L., Snyder, K.R., Savage, J.E., Hug, C., Landolt, W., Bleuler, P., 1998. Investigations of ozone induced injury in forests of southern Switzerland: field surveys and open-top chamber experiments. Chemosphere 36, 995–1000.

Skelly, J.M., Innes, J.L., Savage, J.E., Snyder, K.R., VanderHeyden, D.J., Zhang, J., Sanz, M.J., 1999. Observation and confirmation of foliar symptoms of native plant species of southern Switzerland and southern Spain. Water, Air, and Soil Pollution 116, 227–234.

Staffelbach, T., Neftel, A., Blatter, A., Gut, A., Fahrni, M., Stähelin, J., Prevot, A., Hering, A., Lehning, M., Neininger, B., Baumle, M., Kok, G.L., Dommen, J., Hutterli, M., Anklin, M., 1997. Photochemical oxidant formation over southern Switzerland 1. Results from summer. Journal of Geophysical Research 102 (D19), 23345–23362.

Taylor Jr., G.E., Hanson, P.J., 1992. Forest trees and tropospheric ozone: role of canopy deposition and leaf uptake in developing exposure-response relationships. Agriculture, Ecosystems and Environment 42, 255–273.

Tjoelker, M.G., Volin, J.C., Oleksyn, J., Reich, P.B., 1993. Light environment alters response to ozone stress in seedlings of *Acer saccharum* Marsh. and hybrid *Populus* L. New Phytologist 124, 627–636.

Torsethaugen, G., Pell, E.J., Assmann, S.M., 1999. Ozone inhibits guard cell K+ channels implicated in stomatal opening. Plant Biology 96, 13577–13582.

VanderHeyden, D.J., Skelly, J.M., Innes, J.L., Hug, C., Zhang, J., Landolt, W., Bleuler, P., 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. Environmental Pollution 111, 321–331.

Volin, J.C., Tjoelker, M.G., Oleksyn, J., Reich, P.B., 1993. Light environment alters response to ozone stress in seedlings of *Acer saccharum* Marsh. and hybrid *Populus* L. II. Diagnostic gas exchange and leaf chemistry. New Phytologist 124, 637–646.

Whitehead, W.F., Singh, B.P., 1995. Leaf age affects gas exchange in orca. HortScience 30, 1017–1019.

Wieser, G., Häsler, R., Gotz, B., Koch, W., Havranek, W.M., 2000. Role of climate, crown position, tree age, and altitude in calculated ozone flux into needles of *Picea abies* and *Pinus cembra*: a synthesis. Environmental Pollution 109, 415–422.

Zhang, J., Ferdinand, J.A., VanderHeyden, D.J., Skelly, J.M., Innes, J.L., 2000. Variation in gas exchange within native plant species of Switzerland and relationships with ozone injury: an open-top experiment. Environmental Pollution 113, 117–185.