Short Communication
Proceedings: Impacts of Air Pollution and Climate Change on Forest Ecosystems
TheScientificWorldJOURNAL (2007) 7(S1), 90–97
ISSN 1537-744X; DOI 10.1100/tsw.2007.10

TheScientificWorldJOURNAL
www.thescientificworld.com

Visible Foliar Injury and Physiological Responses to Ozone in Italian Provenances of *Fraxinus excelsior* and *F. ornus*

Nicla Contran[1] and Elena Paoletti[2,*]

[1]*Department of Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza 1, Milan, Italy;* [2]*IPP-CNR, Via Madonna del Piano 10, I-50019 Sesto Fiorentino, Florence, Italy*

E-mail: nicla.contran@unimib.it, e.paoletti@ipp.cnr.it

Received September 29, 2006; Revised November 30, 2006; Accepted December 1, 2006; Published March 21, 2007

We compared leaf visible injury and physiological responses (gas exchange and chlorophyll *a* fluorescence) to high O_3 exposure (150 nmol mol^{-1} h, 8 h day^{-1}, 35–40 days) of two woody species of the same genus with different ecological features: the mesophilic green ash (*Fraxinus excelsior*) and the xerotolerant manna ash (*F. ornus*). We also studied how provenances from northern (Piedmont) and central (Tuscany) Italy, within the two species, responded to O_3 exposure. Onset and extent of visible foliar injury suggested that *F. excelsior* was more O_3 sensitive than *F. ornus*. The higher stomatal conductance in *F. ornus* than in *F. excelsior* suggested a larger potential O_3 uptake, in disagreement to lower visible foliar injury. The higher carbon assimilation in *F. ornus* suggested a higher potential of O_3 detoxification and/or repair. Contrasting geographical variations of ash sensitivity to O_3 were recorded, as Piedmont provenances reduced gas exchange less than Tuscan provenances in *F. excelsior* and more in *F. ornus.* Visible injury was earlier and more severe in *F. excelsior* from Piedmont than from Tuscany, while the provenance did not affect visible injury onset and extent in *F. ornus.*

KEYWORDS: ash trees, foliar injury, fluorescence, gas exchange, ozone

INTRODUCTION

Tropospheric ozone (O_3) is regarded as one of the most widespread air pollutants[1]. Current O_3 levels in Europe are potentially high enough to adversely affect forests[1]. Tree species exhibit a wide range of sensitivity, even at the intraspecific level[2,3,4,5,6,7]. Ozone exposure can modify important physiological processes, such as photosynthesis and stomatal function[2]. Species-specific chlorotic flecking, necrosis, or bronzing may appear on the upper leaf surface[7]. Degree and type of invisible and visible O_3 foliar injuries depend on several plant factors: stomatal conductance, leaf morphological features, apoplastic detoxification, and the response that plants are able to activate[8,9]. These factors are strongly dependent on genotype and on the ecological strategies that plants adopt to avoid or tolerate O_3 stress.

A first objective of this investigation was to perform a preliminary screening of leaf visible injury and physiological responses (leaf gas exchange, chlorophyll *a* fluorescence) to high O_3 concentrations in

*Corresponding author.

Published by TheScientificWorld; www.thescientificworld.com

seedlings of two woody species of the same genus with different ecological features: the mesophilic green ash (*Fraxinus excelsior* L.) and the xerotolerant manna ash (*F. ornus* L.). *F. excelsior* is considered an O_3-sensitive plant and activates a hypersensitive response to O_3[10,11]. O_3 responses of *F. ornus* have never been investigated. We hypothesize that this species is oxidative-stress tolerant, since it is drought tolerant[6] and thus should have a good pool of constitutive enzymatic and nonenzymatic antioxidants or should be able to increase antioxidant defense on oxidative stress[12].

A second objective was to study preliminarily whether diverse provenances exhibited different responses to high O_3 concentrations in terms of leaf visible injury and physiological responses (leaf gas exchanges, chlorophyll *a* fluorescence). We investigated provenances from northern and central Italy within the two species. The aim was to test the hypothesis of a relationship between plants' sensitivity to O_3 and a geographical gradient, thought as an ecological gradient. As a result of the adaptation to the environment, plants of different provenances may exhibit different physiological features, which in turn differently influence O_3 sensitivity at the intraspecific level[3,13].

MATERIAL AND METHODS

Plant Material and O_3 Exposure

Two-year-old potted uniform-size seedlings of *F. excelsior* and *F. ornus* were collected from forest nurseries of Piedmont (northern Italy) and Tuscany (central Italy). Pots were 20 cm in diameter and filled with two-thirds potting medium and one-third vermiculite. The seed sources were of local origin: three provenances from Piedmont and one from Tuscany for *F. excelsior*, and one provenance from Piedmont and one from Tuscany for *F. ornus*. Three months before O_3 exposure, 20 seedlings from each provenance were moved to a greenhouse. Seedlings were fertilized with Osmocote and watered to field capacity once a week. One week before O_3 exposure, six plants from each group were randomly selected (three as controls and three for O_3 exposure per provenance, for a total of 36 seedlings) and allowed to acclimatize in a growth chamber, ventilated with charcoal-filtered air (two air changes per minute) at 20 ± 1°C, 85 ± 5% RH, and 500 μmol m^{-2} sec^{-1} photon flux density (PPFD) at plant height during a 14-h photoperiod. In June 2004, the seedlings were moved to a charcoal-filtered chamber and an O_3-enriched chamber, where their position was rotated once a week. Both chambers were located in the same growth chamber. The environmental conditions were as above. O_3 was generated by a Model 500 O_3 generator (Fisher, Zurich, Switzerland) supplied with pure O_2. Its concentration was continuously monitored with a PC-controlled photometric analyzer (Monitor Labs mod. 8810, San Diego, CA). The exposure regime was a square wave of 150 nmol mol^{-1}, from 10:00–18:00 (GMT), for 40 days. The AOT40 accumulated exposure over a threshold of 40 nmol mol^{-1}[14] yielded 35.2 μmol mol^{-1} h.

Visible Ozone Injury

Leaves were surveyed daily to detect the onset of visible O_3 injury. At the end of O_3 exposure, visible injury was assessed by: (1) counting the number of injured seedlings, expressed as the percentage of injured seedlings of all the seedlings present; (2) counting the number of leaflets showing visible injury, expressed as the percentage of injured leaflets of all leaflets present (%I.L.); and (3) visually assessing the percent surface injury (according to the guide in Innes et al.[15]), expressed as the percentage of injured leaflet surface per symptomatic leaflet surface (%L.A.). The position of each symptomatic leaf and leaflet was recorded, with the apical one as position 1.

Gas Exchange and Chlorophyll *a* Fluorescence

Measurements were carried out at 7-day intervals on the adaxial surface of subapical leaflets of two fully expanded leaves per plant, on three plants per provenance and per exposure. The measured leaves were free of any symptom. Steady-state measurements of light-saturated photosynthesis (P_{net}) and stomatal conductance to water vapor (G_w) were made using O_3-free air by an infrared gas analyzer (CIRAS-1 PP-System, Herts, U.K.) equipped with a Parkinson leaf cuvette, which controlled leaf temperature (26 ± 1°C), leaf-to-air vapor pressure deficit (1 ± 0.2 kPa), light (1300 ± 20 μmol m^{-2} sec^{-1} PPFD), and CO_2 concentration (360 ± 10 μmol mol^{-1}). Chlorophyll *a* transient fluorescence was measured *in vivo* with a direct fluorometer (Handy PEA, Hansatech Instr., Kings Lynn, U.K.). Before measurement, leaves were dark adapted for 40 min with leaf clips. The rising transient was induced by saturating red-actinic light (1300 μmol m^{-2} sec^{-1}, peak at 650 nm, duration 1 sec). Data acquisition was recorded for 1 sec, starting from 10 μsec after the onset of illumination. The values of Fo, i.e., ground fluorescence yield in the dark-adapted state (when all reaction centers of PSII are considered open) and Fm, i.e., the maximal fluorescence yield in the dark (when all reaction centers of PSII are considered closed), were collected. Maximum quantum yield for primary photochemistry (Fv/Fm) was calculated as (Fm-Fo)/Fm[16].

After 3 weeks of exposure, A/C_i curves and Performance Index were measured. A/Ci curves were obtained by changing CO_2 concentration entering the cuvette (C_a) from 50 to 1000 μmol mol^{-1}, in light-saturated condition (1300 ± 20 μmol m^{-2} sec^{-1} PPFD), at constant leaf temperature (26 ± 1°C) and leaf-to-air vapor pressure deficit (1 ± 0.2 kPa). Steady-state CO_2 assimilation was first measured by setting the reference CO_2 concentrations near ambient (400 μmol mol^{-1}) and then at 300, 200, 100, 50, 400, 500, 700, and 1000 μmol mol^{-1}[17]. Maximum RuBP-saturated rate of carboxylation (V_{cmax}), mitochondrial respiration rate in the light (R_{day}), and maximum rate of electron transport (J_{max}) were estimated with an iterative procedure according to Farquhar et al.[18] and Harley et al.[19]. Performance Index ($P.I._{abs}$), an indicator for the photosynthetic status of the leaf, was acquired by the JIP-test with Biolyzer 3.06 software (by Ronald Maldonado-Rodriguez, Biogenetics Laboratory, Geneva, Switzerland)[20].

Data and Statistical Analyses

Data were checked for normal distribution (Shapiro-Wilk W test) and homogeneity of variance (Levene's test). Percents were arcsine-square root transformed prior to analysis. A preliminary ANOVA did not show significant differences among the three Piedmont provenances of *F. excelsior*. Therefore, they were treated as only one statistical group. For data collected weekly, effects of O_3 exposure, species, and provenance were tested with a repeated-measure ANOVA, with time as repeated-measure factor. For data collected after 3 weeks of exposure, effects of O_3 exposure, species, and provenance were tested with a three-way ANOVA. A t-test was applied to compare the effects of O_3 exposure at each date of measurement. Tests of significance were made at a 95% confidence level. Analyses were processed using STATISTICA 6.0 Package for Windows (StatSoft 2001, Tulsa, OK).

RESULTS

Both species displayed interveinal reddish stipples on the adaxial leaf surface. Visible injury was earlier and more severe in *F. excelsior* than in *F. ornus* (Table 1). In *F. excelsior*, the Piedmont provenances appeared to be more O_3 sensitive than the Tuscan provenances, in terms of visible injury, while the provenance did not affect visible injury onset and extent in *F. ornus*. However, a strong data variability prevented statistical significance. Both for species and provenances, the position of leaf and leaflet did not affect %I.L and %L.A (data not shown).

TABLE 1

Onset and Extent (Percent of Injured Seedlings, of Injured Leaflets [I.L.], and of Injured Surface per Symptomatic Leaflet [L.A.]) of Visible Ozone Injury in Piedmont and Tuscan Provenances of *F. excelsior* and *F. ornus*, Exposed to 150 nmol O_3 mol^{-1} (8 h day^{-1}, 40 days) and F Values of ANOVA of the Effects of Species (*F. excelsior* and *F. ornus*) and Provenance (Piedmont and Tuscany)

	Onset (day of exposure)	Injured seedlings (%)	I.L. (%)	L.A. (%)
Fraxinus excelsior				
from Piedmont	12	78	54.3	9.03
from Tuscany	21	67	31.2	4.04
Fraxinus ornus				
from Piedmont	37	67	8.05	1.04
from Tuscany	38	33	7.05	1.05
Species	-	-	1.16^{ns}	3.55^{ns}
Provenance	-	-	0.44^{ns}	0.14^{ns}
Species x Provenance	-	-	0.01^{ns}	0.67^{ns}

ns= $p > 0.05$ (not significant).

O_3 exposure significantly decreased P_{net}, G_w, and Fv/Fm (Table 2). P_{net} and G_w values were higher in *F. ornus* than in *F. excelsior,* and in the seedlings from Tuscany than in those from Piedmont, both in O_3-exposed and control samples. In both species, P_{net} declined mostly during the first week of O_3 exposure and then it was stable (Fig. 1). P_{net} decline was faster in *F. excelsior* (–59% after 1 week of exposure) than in *F. ornus* (–31% after 1 week and –56% after 2 weeks). While G_w of *F. ornus* showed the same change as P_{net}, *F. excelsior* took 3 weeks of O_3 exposure to show a significant decrease in G_w of O_3-exposed seedlings. In *F. excelsior* control, G_w remained constant while in *F. ornus,* it increased over time. Therefore, G_w decrease in *F. ornus* (O_3-exposed vs. control) was higher than in *F. excelsior*. In O_3-exposed plants of both species, Fv/Fm decreased substantially in a similar way (4% after 1 week of exposure) and then it kept constant over time in *F. excelsior*, while an increase after 28 days of exposure was observed in *F. ornus* when the efficiency of PSII recovered to control values.

In O_3-exposed F. ornus, P_{net} and G_w of Tuscan provenance decreased more slowly than those of Piedmont provenance. In O_3-exposed F. excelsior, P_{net} and G_w of Tuscan provenance decreased at a larger extent than those of Piedmont provenances. The effects of O_3 exposure on Fv/Fm were observed only in the Piedmont provenances of F. excelsior and in the Tuscan provenance of F. ornus.

R_{day} was higher in F. ornus than in F. excelsior, but did not vary with O_3 exposure and provenance (Table 3). On the contrary, V_{cmax}, J_{max}, and $P.I._{abs}$ showed a significant reduction in O_3-exposed seedlings. They were significant lower in Piedmont provenances than in Tuscan provenances of both species. While V_{cmax} and J_{max} showed no differences between the species, $P.I._{abs}$ was significantly higher in *F. ornus* than in *F. excelsior*.

TABLE 2
F Values of Three-Way Repeated ANOVA of the Effects of O_3 Exposure (0 and 150 nmol mol^{-1}, 8 h day^{-1}), Species (*F. excelsior* and *F. ornus*), and Provenance (Piedmont and Tuscany) with Time as Repeated-Measure Factor (0, 7, 14, 21, 28, and 35 Days of Exposure)

Effects	d.f.	P_{net}	G_w	Fv/Fm
O_3 exposure	1	299.2***	289.3***	139.9***
Species	1	91.18***	188.5***	34.66***
Provenance	1	127.9***	8.11*	19.56***
Species x Provenance	1	24.75***	1.59^{ns}	30.52**
O_3 exposure x Provenance	1	51.83***	3.13^{ns}	8.29**
O_3 exposure x Species	1	3.21^{ns}	68.78***	6.98*
O_3 exposure x Species x Provenance	1	4.04^{ns}	18.65***	22.86***
Time	5	4.27***	12.99***	10.87***
Time x O_3 exposure	5	6.75***	15.89***	1.11***
Time x Provenance	5	5.98***	12.19***	4.26***
Time x Species	5	14.41***	12.23***	13.65***
Time x Species x Provenances	5	13.53***	17.01***	4.01***
Time x O_3 exposure x Provenances	5	6.07***	20.73***	0.58^{ns}
Time x O_3 exposure x Species	5	4.18**	23.03***	3.67**
Time x O_3 exposure x Species x Provenances	5	9.89***	23.85***	1.53^{ns}

d.f. represents the degrees of freedom; *=$p \le 0.05$, **=$p \le 0.01$, ***=$p \le 0.001$, ns = $p > 0.05$ (not significant).

For abbreviations see Fig. 1.

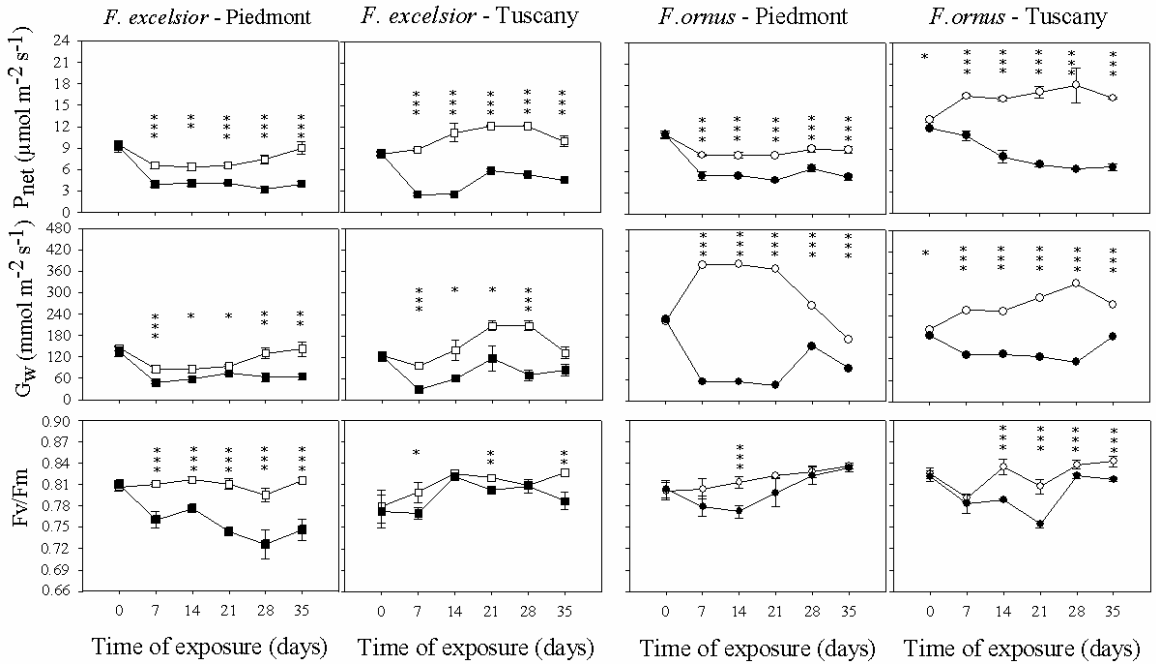

FIGURE 1. Effects of ozone exposure (150 nmol mol^{-1}, 8 h day^{-1}, 35 days) on net photosynthesis (P_{net}), stomatal conductance to water vapor (G_w), and maximum quantum yield for primary photochemistry (Fv/Fm) in Piedmont and Tuscan provenances of *F. excelsior* and *F. ornus*. Values represent means ±S.E. Symbols represent the results of t-test of O_3 exposure. *, $p \le 0.05$; **, $p \le 0.01$; ***, $p \le 0.001$; ns (not significant), $p > 0.05$; □, *F. excelsior* control; ■, *F. excelsior* O_3 exposed; ○, *F. ornus* control; ●, *F. ornus* O_3 exposed.

TABLE 3
Mean Values (±S.E.) of Mitochondrial Respiration Rate in the Light (R_{day}[µmol m^{-2} sec^{-1}]), Maximum RuBP-Saturated Rate of Carboxylation (V_{cmax}[µmol m^{-2} sec^{-1}]), Maximum Rate of Electron Transport (J_{max}[µmol m^{-2} sec^{-1}]), and Performance Index ($P.I._{abs}$) and F Values of Three-Way ANOVA of the Effects of O_3 Exposure (0 and 150 nmol mol^{-1} h, 8 h day^{-1}, 21 days), Species (*F. excelsior* and *F. ornus*) and Provenances (Piedmont and Tuscany)

		R_{day}	V_{cmax}	J_{max}	$P.I._{ABS}$
F. excelsior					
Piedmont	Control	2.56 (±0.56)	93.93 (±9.41)	128.03 (±16.81)	58.19 (±7.01)
	Treated	1.90 (±0.23)	41.37(±5.03)	60.90 (±6.72)	35.58 (±4.42)
Tuscany	Control	1.56 (±0.68)	124.00 (±7.49)	171.61 (±14.51)	96.21 (±4.31)
	Treated	3.16 (±0.85)	90.82 (±10.00)	111.12 (±11.19)	64.34 (±6.89)
F. ornus					
Piedmont	Control	4.746 (±1.21)	88.26 (±17.34)	111.53 (±23.69)	79.42 (±8.03)
	Treated	3.69 (±1.55)	63.59 (±10.14)	88.05 (±15.76)	52.12 (±8.42)
Tuscany	Control	3.56 (±2.11)	125.91 (±1.69)	167.00 (±18.18)	110.28 (±3.74)
	Treated	4.29 (±1.03)	61.83 (±16.67)	79.46 (±17.26)	71.59 (±4.80)
Effects	**d.f.**				
O_3 exposure	1	0.034^{ns}	31.90***	27.08***	36.75***
Species	1	4.570*	0.117^{ns}	0.312^{ns}	8.799*
Provenance	1	0.009^{ns}	13.93**	9.405**	34.604***
O_3 exposure x Species	1	1.144^{ns}	0.009^{ns}	0.131^{ns}	0.329^{ns}
O_3 exposure x Provenance	1	1.146^{ns}	0.317^{ns}	1.566^{ns}	1.068^{ns}
Species x Provenance	1	0.066^{ns}	1.987^{ns}	1.045^{ns}	0.675^{ns}
O_3 exposure x Species x Provenance	1	0.019^{ns}	3.624^{ns}	2.378^{ns}	0.012^{ns}

d.f. represents the degrees of freedom; *=$p \leq 0.05$, **=$p \leq 0.01$, ***=$p \leq 0.001$, ns= $p > 0.05$ (not significant).

DISCUSSION

Onset and extent of visible foliar injury as well as faster decline in photosynthesis suggested that *F. excelsior* was more sensitive to O_3 than *F. ornus*. In both species, gas exchange and chlorophyll *a* fluorescence measurements showed that O_3 affected photosynthetic performances. After 1 week of exposure, O_3 significantly reduced P_{net} and G_w. Even if the experiment lasted only 40 days, the plants adapted to O_3 since gas exchange kept constant during the following weeks. The decrease in carbon fixation was associated with a reduction in G_w as well as in the quantity of active Rubisco (V_{cmax}) and the capacity for whole-chain electron transport (J_{max}). The decrease of $P.I._{abs}$ confirmed that the energy transduction process around PSII lost performance. Thus, in these ash species, O_3 effects on photosynthesis resulted from effects on stomata and on the photosynthetic apparatus[8].

Avoidance by stomatal regulation, limiting the access of O_3 to sensitive targets, is the first mechanism of plant O_3 sensitivity[21,22]. Accordingly, the more O_3-tolerant species (*F. ornus*) had the stronger reduction of G_w (O_3-exposed seedlings vs. controls). However, G_w values were lower in *F. excelsior* than in *F. ornus*. Probably, in a controlled environment without water stress, the xerotolerant *F. ornus* was allowed to maximize its stomatal capacity. The values of G_w suggest that the potential O_3 uptake in *F. excelsior* plants was lower than in *F. ornus*, even if the former species was more sensitive to O_3. Species-specific G_w is not necessarily correlated with O_3 sensitivity based on the severity of foliar injury[23]. Two other factors control plant O_3 sensitivity: (1) plant resources available for repair of damaged tissues and (2) plant enzymatic and nonenzymatic antioxidant levels[8,9]. Therefore, in condition of stomatal conductance equality, net photosynthesis has been suggested as a better indicator of plant sensitivity to O_3 because the

availability of photosynthate is particularly important in antioxidant defense and repair mechanisms[23,24,25]. This hypothesis implies that high rates of net photosynthesis may balance O_3 uptake and reduce foliar injury[26]. In agreement to this view, *F. ornus* had higher P_{net} and R_{day} values. The repair capacity of *F. ornus* is supported by the recovery of efficiency of PSII (Fv/Fm) after 4 weeks of O_3 exposure. Plants adapted to high oxidative stress levels, like the xerotolerant *F. ornus,* may be less sensitive to O_3 exposure[27] because of a good pool of constitutive enzymatic and nonenzymatic antioxidant levels and/or the ability to increase antioxidant defenses.

Based on visible foliar injury, no significant difference in O_3 sensitivity was observed between the provenances. Based on gas exchange, the provenances of the two species differed in their response to O_3 in that the Piedmont provenances reduced gas exchange less than the Tuscan provenances in *F. excelsior* and more in *F. ornus*. As a correlation between provenances and O_3 sensitivity has been demonstrated in other studies[3,13], it is possible that our provenances were not too far away to show different O_3 responses, and that the results were affected by the small replication and short term of the experiment.

ACKNOWLEDGMENTS

The study was funded by the Regione Piemonte (project FORMEDOZON, Interreg III B). The authors wish to thank Francesco Tagliaferro for providing the plant material from Piedmont, Giacomo Lorenzini and Cristina Nali for performing the ozone exposure, and Francesco Fava, Raffaella Cerana, and Gianni Della Rocca for their helpful comments and practical support.

REFERENCES

1. Ashmore, M.R. (2005) Assessing the future global impacts of ozone on vegetation. *Plant Cell Environ.* **28**, 949–964.
2. Chappelka, A.H. and Samuelson, L.J. (1998) Ambient ozone effects on forest trees of the eastern United States: a review. *New Phytol.* **139**, 91–108.
3. Paludan-Muller, G., Saxe, H., and Leverenz, J.W. (1999) Responses to ozone in 12 provenances of European beech (*Fagus sylvatica*): genotypic variation and chamber effects on photosynthesis and dry-matter partitioning. *New Phytol.* **144**, 261–273.
4. Paoletti, E., Nali, C., and Lorenzini, G. (2002) Photosynthetic behaviour of two Italian clones of European beech (*Fagus sylvatica* Mill.) exposed to ozone. *Phyton* **42**, 149–155.
5. Oksanen, E. (2003) Responses of selected birch (*Betula pendula* Roth) clones to ozone change over time. *Plant Cell Environ.* **26**, 875–886.
6. Nali, C., Paoletti, E., Marabottini, R., Della Rocca, G., Lorenzini, G., Paolacci, A.R., Ciuffi, M., and Badiani, M. (2004) Ecophysiological and biochemical strategies of response to ozone in Mediterranean evergreen broadleaf species. *Atmos. Environ.* **38**, 2247–2257.
7. Schaub, M., Skelly, J.M., Zhang, J.W., Ferdinand, J.A., Savage, J.E., Stevenson, R.E., Davis, D.D., and Steiner, K.C. (2005) Physiological and foliar symptom response in the crowns of *Prunus serotina*, *Fraxinus amer*icana and *Acer rubrum* canopy trees to ambient ozone under forest conditions. *Environ. Pollut.* **133**, 553–567.
8. Pell, E.J., Schlangnhaufer, C.D., and Arteca, R.N. (1997) Ozone-induced oxidative stress: mechanisms of action and reaction. *Physiol. Plant.* **100**, 264–273.
9. Kangasjarvi, J., Jaspers, P., and Kollist, H. (2005) Signalling and cell death in ozone-exposed plants. *Plant Cell Environ.* **28**, 1021–1036.
10. Gravano, E., Bussotti, F., Strasser, R.J., Schaub, M., Novak, K., Skelly, J., and Tani, C. (2004) Ozone symptoms in leaves of woody plants in open-top chambers: ultrastructural and physiological characteristics. *Physiol. Plant.* **121**, 620–633.
11. Bussotti, F., Agati, G., Desotgiu, R., Matteini, P., and Tani, C. (2005) Ozone foliar symptoms in woody plant species assessed with ultra-structural and fluorescence analysis. *New Phytol.* **166**, 941–955.
12. Paoletti, E., Manning, W.J., Spaziani F., and Tagliaferro, F. (2006) Gravitational infusion of ethylenediurea (EDU) into trunks protected adult European ash trees (*Fraxinus excelsior* L.) from foliar ozone injury. *Environ. Pollut.*, in press, doi:10.1016/j.envpol.2006.05.005
13. Larsen, J.B., Yang, W., and Tiedemann, A.V. (1990) Effects of ozone on gas exchange, frost resistance, flushing and growth of different provenances of European silver fir (*Abies alba* Mill.). *Eur. J. For. Pathol.* **20**, 211–218.
14. de Leeuw, F.A.A.M. and van Zantvoort, E.D.G. (1997) Mapping of exceedances of ozone critical levels for crops and forest trees in the Netherlands: preliminary results. *Environ. Pollut.* **96**, 89–98.

15. Innes, J.L., Skelly, J.M., and Schaub, M. (2001) *Ozone Broadleaved Species: A Guide to the Identification of Ozone-Induced Foliar Injury.* Haupt, Berne. p. 136.
16. Maxwell, K. and Johnson, G.N. (2000) Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.* **51**, 659–668.
17. Tognetti, R., Sebastiani, L., and Nocci, A. (2004) Gas exchange and foliage characteristic of two poplar clones grown in soil amended with industrial waste. *Tree Physiol.* **24**, 75–82.
18. Farquhar, G.D., von Caemmerer, S., and Berry, J.A. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149**, 78–90.
19. Harley, P.C., Thomas, R.B., Reynolds, J.F., and Strain, B.R. (1992) Modelling photosynthesis of cotton grown in elevated CO_2. *Plant Cell Environ.* **21**, 659–669.
20. Strasser, B.J. and Strasser, R.J. (1995) Measuring fast fluorescence transient to address environmental questions: the JIP test. In *Photosynthesis: From Light to Biosphere*. Vol. V. Mathis, P., Ed. Kluwer Academic, The Netherlands. pp. 977–980.
21. Kolb, T.E., Fredericksen, T.S., Steiner, K.C., and Skelly, J.M. (1997) Issues in scaling tree size and age responses to ozone: a review. *Environ. Pollut.* **98**, 195–202.
22. Reich, P.B. (1987) Quantifying plant response to ozone: a unifying theory. *Tree Physiol.* **3**, 63–91.
23. Zhang, J., Ferdinand, J.A., Vanderheyden, D.J., Skelly, J.M., and Innes, J.L. (2001) Variation of gas exchange within native plants species of Switzerland and relationships with ozone injury: an open-top experiment. *Environ. Pollut.* **113**, 177–185.
24. Volin, J.C., Tjoelker, M.G., and Oleksyn, J. (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 Phytol.* **124**, 637–647.
25. Tjoelker, M.G., Volin, J.C., Oleksyn, J., and Reich, P.B. (1995) Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment. *Plant Cell Environ.* **18**, 895–905.
26. Fredericksen, T.S., Kolb, T.E., Skelly, J.M., Steiner, K.C., Joyce, B.J., and Savage, J.E. (1996) Light environment alters ozone uptake per net photosynthetic rate in black cherry trees. *Tree Physiol.* **16**, 485–490.
27. Paoletti, E. (2006) Impact of ozone on Mediterranean forests: a review. *Environ. Pollut.* **144**, 463–474.

This article should be cited as follows:

Contran, N. and Paoletti, E. (2007) Visible foliar injury and physiological responses to ozone in Italian provenances of *Fraxinus excelsior* and *F. ornus*. *TheScientificWorldJOURNAL* **7(S1)**, 90–97. DOI 10.1100/tsw.2007.10.