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

Nicla Contran¹ and Elena Paoletti²,*

¹Department of Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza 1, Milan, Italy; ²IPP-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 O₃ 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 O₃ exposure. Onset and extent of visible foliar injury suggested that *F. excelsior* was more O₃ sensitive than *F. ornus*. The higher stomatal conductance in *F. ornus* than in *F. excelsior* suggested a larger potential O₃ uptake, in disagreement to lower visible foliar injury. The higher carbon assimilation in *F. ornus* suggested a higher potential of O₃ detoxification and/or repair. Contrasting geographical variations of ash sensitivity to O₃ 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 (O₃) is regarded as one of the most widespread air pollutants[1]. Current O₃ 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 O₃ 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 O₃ 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 O₃ concentrations in
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 O₃-sensitive plant and activates a hypersensitive response to O₃[10,11]. O₃ 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 O₃ 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 O₃ 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 O₃ sensitivity at the intraspecific level[3,13].

**MATERIAL AND METHODS**

**Plant Material and O₃ 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 O₃ 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 O₃ exposure, six plants from each group were randomly selected (three as controls and three for O₃ 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 O₃-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. O₃ was generated by a Model 500 O₃ generator (Fisher, Zurich, Switzerland) supplied with pure O₂. 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 O₃ injury. At the end of O₃ 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 F_o, i.e., ground fluorescence yield in the dark-adapted state (when all reaction centers of PSII are considered open) and F_m, 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 (F_{v/Fm}) was calculated as (F_m-F_o)/F_m[16].

After 3 weeks of exposure, A/C_i curves and Performance Index were measured. A/C_i 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 (I_{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 stipple 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 O3 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 (-) | Provenance (-) | Species x Provenance (-) |
|------------------|-------------|----------------|--------------------------|
|                  | 1.16<sup>ns</sup> | 0.44<sup>ns</sup> | 0.01<sup>ns</sup> |
|                  | 3.55<sup>ns</sup> | 0.14<sup>ns</sup> | 0.67<sup>ns</sup> |

<sup>ns</sup> = p > 0.05 (not significant).

O₃ exposure significantly decreased P<sub>net</sub>, G<sub>w</sub>, and F<sub>v/Fm</sub> (Table 2). P<sub>net</sub> and G<sub>w</sub> values were higher in *F. ornus* than in *F. excelsior*, and in the seedlings from Tuscany than in those from Piedmont, both in O₃-exposed and control samples. In both species, P<sub>net</sub> declined mostly during the first week of O₃ exposure and then it was stable (Fig. 1). P<sub>net</sub> 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<sub>w</sub> of *F. ornus* showed the same change as P<sub>net</sub>, *F. excelsior* took 3 weeks of O₃ exposure to show a significant decrease in G<sub>w</sub> of O₃-exposed seedlings. In *F. excelsior* control, G<sub>w</sub> remained constant while in *F. ornus*, it increased over time. Therefore, G<sub>w</sub> decrease in *F. ornus* (O₃-exposed vs. control) was higher than in *F. excelsior*. In O₃-exposed plants of both species, F<sub>v/Fm</sub> 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 O₃-exposed *F. ornus*, P<sub>net</sub> and G<sub>w</sub> of Tuscan provenance decreased more slowly than those of Piedmont provenance. In O₃-exposed *F. excelsior*, P<sub>net</sub> and G<sub>w</sub> of Tuscan provenance decreased at a larger extent than those of Piedmont provenances. The effects of O₃ exposure on F<sub>v/Fm</sub> were observed only in the Piedmont provenances of *F. excelsior* and in the Tuscan provenance of *F. ornus*.

R<sub>day</sub> was higher in *F. ornus* than in *F. excelsior*, but did not vary with O₃ exposure and provenance (Table 3). On the contrary, V<sub>cmax</sub>, J<sub>max</sub>, and P.I.ₐₖₛ showed a significant reduction in O₃-exposed seedlings. They were significant lower in Piedmont provenances than in Tuscan provenances of both species. While V<sub>cmax</sub> and J<sub>max</sub> showed no differences between the species, P.I.ₐₖₛ was significantly higher in *F. ornus* than in *F. excelsior*. 
TABLE 2
F Values of Three-Way Repeated ANOVA of the Effects of O3 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  | Gw   | Fv/Fm |
|------------------------------------------------------|------|--------|------|-------|
| O3 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*** | 1.59ns | 30.52** |
| O3 exposure x Provenance                             | 1    | 51.83*** | 3.13ns | 8.29**  |
| O3 exposure x Species                                | 1    | 3.21ns  | 68.78*** | 6.98*  |
| O3 exposure x Species x Provenance                   | 1    | 4.04ns  | 18.65*** | 22.86*** |
| Time                                                  | 5    | 4.27*** | 12.99*** | 10.87*** |
| Time x O3 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 O3 exposure x Provenances                     | 5    | 6.07*** | 20.73*** | 0.58ns |
| Time x O3 exposure x Species                         | 5    | 4.18**  | 23.03*** | 3.67**  |
| Time x O3 exposure x Species x Provenances           | 5    | 9.89*** | 23.85*** | 1.53ns |

d.f. represents the degrees of freedom; *=p ≤ 0.05, **=p ≤ 0.01, ***=p ≤ 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 (Gw), 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 O3 exposure. *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001; ns (not significant), p > 0.05; □, F. excelsior control; ■, F. excelsior O3 exposed; ○, F. ornus control; ●, F. ornus O3 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 |
|----------|-----------|------------|-----------|---------|
|          | (μmol m$^{-2}$ sec$^{-1}$) | (μmol m$^{-2}$ sec$^{-1}$) | (μmol m$^{-2}$ sec$^{-1}$) |         |
| 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 | O$_3$ exposure x Species | Species x Provenance | O$_3$ exposure x Provenance |
|--------------|-----------------|--------------------------|----------------------|-----------------------------|
| d.f.         | 1               | 1                        | 1                    | 1                           |
|              | 0.034ns         | 1.144ns                  | 0.009ns              | 0.019ns                     |
|              | 31.90***        | 0.009ns                  | 13.93***             | 3.624ns                     |
|              | 27.08***        | 0.131ns                  | 9.405**              | 2.378**                     |
|              | 36.75***        | 0.329ns                  | 34.604***            | 0.012ns                     |
|              | 8.799*          | 1.068**                  |                      |                             |
|              | 34.604***       |                          |                      |                             |
|              |                  |                          |                      |                             |

**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 O₃ uptake and reduce foliar injury[26]. In agreement to this view, F. ornus had higher Pnet and Rday values. The repair capacity of F. ornus is supported by the recovery of efficiency of PSII (Fv/Fm) after 4 weeks of O₃ exposure. Plants adapted to high oxidative stress levels, like the xerotolerant F. ornus, may be less sensitive to O₃ 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 O₃ sensitivity was observed between the provenances. Based on gas exchange, the provenances of the two species differed in their response to O₃ 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 O₃ sensitivity has been demonstrated in other studies[3,13], it is possible that our provenances were not too far away to show different O₃ 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) Ecophysiologica l 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 americana and Acer rubrum canopy trees to ambient ozone under forest conditions. Environ. Pollut. 133, 553–567.
8. Pell, E.J., Schlanghaufner, 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 characterist ics. 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 CO₂ assimilation in leaves of C₃ 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 CO₂. 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.