Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental Pollution 134 (2005) 291–300
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Ozone exposure induces the activation of leaf senescence-related processes and morphological and growth changes in seedlings of Mediterranean tree species

Àngela Ribas[a,*], Josep Peñuelas[a], Susana Elvira[b], Benjamín S. Gimeno[b]

[a]CSIC-CEAB-CREAF Ecophysiology Unit, CREAF (Center for Ecological Research and Forestry Applications), Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
[b]CIEMAT, Avd. Complutense 22, 28040 Madrid, Spain

Received 12 January 2004; accepted 30 July 2004

Ozone induces species-specific leaf senescence-related processes and morphological and growth changes in seedlings of Mediterranean tree species.

Abstract

Four Mediterranean tree taxa, *Quercus ilex* subsp. *ilex*, *Quercus ilex* subsp. *ballota*, *Olea europaea* cv. *vulgaris* and *Ceratonia siliqua*, were exposed to different ozone (O_3) concentrations in open top chambers (OTCs) during 2 years. Three treatments were applied: charcoal-filtered air (CF), non-filtered air (NF) and non-filtered air plus 40 ppb$_v$ of O_3 (NF+). The photochemical maximal efficiency, Fv/Fm, decreased in NF+ plants during the second year of exposure, especially during the most stressful Mediterranean seasons (winter and summer). An increase of $\delta^{13}C$ was found in three of the four studied species during the first year of exposure. This finding was only maintained in *C. siliqua* during the second year. Decreases in the chlorophyll content were detected during the first year of fumigations in all the species studied, but not during the second year. The NF+ treatment induced changes in foliar anatomical characteristics, especially in leaf mass per area (LMA) and spongy parenchyma thickness, which increased in some species. A reduction in N content and an increase in $\delta^{15}N$ were found in all species during the second year when exposed in the NF+ OTCs, suggesting a change in their retranslocation pattern linked to an acceleration of leaf senescence, as also indicated by the above mentioned biochemical and anatomical foliar changes. The two *Q. ilex* subspecies were the most sensitive species since the changes in N concentration, $\delta^{15}N$, chlorophyll, leaf area, LMA and biomass occurred at ambient O_3 concentrations. However, *C. siliqua* was the most responsive species (29% biomass reduction) when exposed to the NF+ treatment, followed by the two *Q. ilex* subspecies (14–20%) and *O. europaea* (no significant reduction). Ozone resistance of the latter species was linked to some plant traits such as chlorophyll concentrations, or spongy parenchyma thickness.

Keywords: Biomass reduction; *Ceratonia siliqua*; *Olea europaea*; Ozone exposure; *Quercus ilex*

1. Introduction

Ozone (O_3) is a phytotoxic air pollutant widely distributed in the Mediterranean region where adverse effects on different plant tree species have been reported. Probably the most studied species has been Aleppo pine (Alonso et al., 2001; Elvira et al., 1998; Peñuelas et al., 1995; Sanz et al., 2000). Recently, a screening study carried out by Inclán et al. (1999) indicated that ozone exposure induced adverse effects on the biomass of holm oak (*Quercus ilex*) and olive trees (*Olea europaea*). However, some studies have highlighted the great

* Corresponding author. Fax: +34 93 581 4151.
E-mail addresses: a.ribas@creaf.uab.es (À. Ribas), josep.penuelas@uab.es (J. Peñuelas).

0269-7491/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2004.07.026

intraspecific variability of Mediterranean vegetation to ozone (Elvira et al., 2004; Minnocci et al., 1999). As a result, the information regarding the response of Mediterranean tree species is still scarce, increasing the uncertainties derived from the definition of O_3 critical levels. Critical level is defined as the concentration of the pollutant above which adverse effects are likely to occur (UNECE, 1988). The UN-ECE Convention on Long-Range Transboundary Air Pollution has promoted the definition of ozone critical levels for different plant receptors and its activities have largely influenced current European ozone directive. The present critical level for forest tree species is defined as a 6 months AOT40 (ozone accumulated exposure over a threshold of 40 nl l^{-1}) value of 10 $ppm_v \cdot h$. The suitability of this critical level is controversial since many uncertainties still remain to be resolved. Most of them are derived from the lack of experimental or field studies addressing this issue. This is especially the case for the Mediterranean area, making it difficult to establish an adequate regulatory policy for the protection of Mediterranean forests (Bussotti and Gerosa, 2002).

Due to the great diversity of tree species and populations occurring in the Mediterranean area, it would be useful to define those plant traits that could be used as indicators of their potential sensitivity to ozone exposure. Such an approach has been recently adopted by Paoletti et al. (2003) for Mediterranean vegetation, using antioxidant levels as biomarkers of this sensitivity. Although their results are controversial, since the combination of ozone exposure, drought stress and low VPD values could induce an activation of these molecules; the conceptual scheme may be still valid. But what is the key parameter is still an open issue.

The factors that determine plant sensitivity or tolerance are not clearly understood but it is thought to be related to many underlying physiological, anatomical, biochemical and environmental factors (Alonso et al., 2001; Pääkkönen et al., 1998). Ozone effects on tree biomass are the result of several processes occurring at the cellular and physiological levels. Acceleration of leaf senescence has been widely reported as one of the most characteristic processes derived from ozone exposure. These processes involve chlorophyll degradation and reductions in CO_2 assimilation (Elvira et al., 1998; Zheng et al., 2002). Leaf senescence can also be characterized by reductions in N foliar levels. Therefore, carbon and nitrogen isotopic discrimination may be useful tools to describe the integrative responses of tree species to O_3 exposure. The fractionation of ^{13}C and ^{12}C is caused by diffusion of CO_2 through the leaf, boundary layer and stomata, dissolution of CO_2 in the apoplastic fluid and enzymatic reactions involved in carbon fixation (Farquhar et al., 1989). In general, the processes of N loss (NH_4 volatilization, nitrification, denitrification and leaching) enrich the system with the heavy isotope ^{15}N (Peñuelas and Estiarte, 1997).

Ozone effects on leaf morphology have also been reported. For instance, the early exposure of birch to O_3 resulted in a reduction in leaf size, with an increase in the density of stomata, hairs and veins (Gunthardt-Goerg et al., 1993). Similarly, the pectinaceous layer of spongy parenchyma cells was found to swell and protrude (Gunthardt-Goerg et al., 1997) following the exposure of birch to ozone. Many of these defence mechanisms are similar to those found in the schlerophyllous Mediterranean vegetation to prevent damage derived from drought, low VPD values and excessive solar radiation intensity.

This paper focuses on the response of four Mediterranean taxa presenting different physiological and anatomical traits: *Quercus ilex* subsp. *ilex*, *Quercus ilex* subsp. *ballota*, *Ceratonia siliqua* and *Olea europaea* cv. *sylvestris*, to assess interspecific differences in sensitivity to ozone. The response of two subspecies of *Q. ilex* was also studied to assess the intraspecific sensitivity to O_3. The following hypotheses were tested: (1) ozone exposure enhances plant senescence-related processes; (2) plant responses in biomass are related to alterations in integrated carbon assimilation; and (3) plant traits can be associated with a differential sensitivity to ozone exposure.

2. Materials and methods

2.1. Growth conditions and ozone treatments

Plants of *Quercus ilex* subsp. *ilex, Quercus ilex* subsp. *ballota, Olea europaea* cv. *sylvestris*, and *Ceratonia siliqua* were raised from seeds. One-year old uniform seedlings (approx. same height and diameter) of each species were transplanted in July 1998 into 6 dm^3 containers filled with Universal substrate (peat and pine bark) with a 34% of organic material. Soil pH was 6.6, and 9 g per pot of a slow-release fertilizer (NPK 15:8:11; Osmocote plus) were supplied.

This experiment was conducted in an experimental field of slightly modified NCLAN-type open top chambers (OTCs) (Gimeno et al., 1999), located at the Ebro Delta (NE Spain, 40° 41.5′ North, 0° 48′ East), 10 m above sea level. Three O_3 treatments were established: charcoal filtered air (CF), non-filtered air (NF) with close to ambient O_3 levels, and non-filtered air supplemented with 40 nl l^{-1} O_3 from 7:00 to 17:00 GMT 5 days per week (NF+). Ozone concentrations in the NF+ treatment were in the range of those reported by Millán et al. (2000). Three OTC replicates were used for each O_3 treatment. An automatic system provided a continuous monitoring of O_3, sulphur dioxide and nitrogen oxides concentrations in the different