



A flux-based assessment of above and below ground biomass of Holm oak (*Quercus ilex* L.) seedlings after one season of exposure to high ozone concentrations



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HIGHLIGHTS

- Ozone dose–response relationships for biomass losses of young Holm oak trees are presented.
- Stem growth of Holm oak under high levels of O₃ is less reduced than roots.
- Holm oak showed an O₃ sensitivity between deciduous broadleaved and evergreen coniferous species.

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ABSTRACT

Young plants of Holm oak (*Quercus ilex*) were exposed in non-limiting water conditions to four different levels of ozone (O₃) concentrations in Open-Top Chambers during one growing season to evaluate biomass losses on roots, stems and leaves in relation to O₃ exposure (AOT40) and phytotoxic ozone dose (POD₁) absorbed. The exposure-effect and dose-effect relationships for the total biomass were statistically significant and indicated a reduction of 4% and 5.2% of the total biomass for each increase step of 10000 ppb h of AOT40 and 10 mmol m⁻² of POD₁, respectively. The results indicate a critical level for Holm oak protection of 7 mmol m⁻² of POD₁, which corresponds to 4% of total biomass reduction. The linear regressions based on the POD₁ were significant for roots and stem biomass losses, but not significant for leaf biomass. The biomass loss rate at increasing POD₁ was higher for roots than for stems and leaves, suggesting that stem growth under high levels of O₃ is less affected than root growth. Because of the scarcity of data from the Mediterranean area, these results can be relevant for the O₃ risk assessment models and for the definition of new O₃ critical levels for forests in Europe.

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1. Introduction

Tropospheric ozone (O₃) is considered an important phytotoxic pollutant, threatening forest vegetation, crops and seminatural vegetation (Ashmore and Marshall, 1998; Sitch et al., 2007; Matyssek et al., 2014; Feng et al., 2011; Davison and Barnes, 1998; Mills et al., 2011a) in many areas of developed and developing countries with rapidly increasing industrialization (Emberson et al., 2001, 2009).

This secondary atmospheric pollutant in fact, is a strong photo-oxidant able to cause damage to vegetation at the biochemical, cellular, physiological level (Sandermann et al., 1998; Faoro and Iriti, 2005; Zheng et al., 2002), and also with potential consequences at ecological levels (Fuhrer and Booker, 2003; Cape, 2008).

The Mediterranean area is characterized by very high levels of tropospheric O₃ during the summer, with frequent exceedances of the critical levels established for the protection of natural vegetation and crops (EEA, 2013). Furthermore, climate change projections foresee a rise in temperatures that likely will be responsible for increasing O₃ background concentrations in the future (Vingarzan, 2004; Dentener et al., 2006).

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Holm oak (*Quercus ilex*) is a broadleaf evergreen species widely distributed in Mediterranean forests (Spain, North Africa, coasts of Italy and the East Mediterranean basin) and considered particularly tolerant to hot and dry summer climatic conditions, typical of this geographical area. Moreover, on the basis of several published studies and reviews *Q. ilex* is considered quite tolerant to high levels of O₃ exposure (Paoletti, 2006).

In the last ten years considerable evidence has shown that O₃ damage to vegetation is more strictly linked to the O₃ dose absorbed by the leaves through the stomata than to simple exposure of the plants to the pollutant (Mills et al., 2011b; CLRTAP, 2010). As a consequence, stomatal conductance has an important role in determining the amount of O₃ entering the leaves and the manifestation of negative effects at the plant level.

Mediterranean forests experience dry conditions due to extremely low levels of rain precipitation during Summer, and many tree species (including *Q. ilex*) tend to reduce stomatal conductance to avoid water losses. Therefore, it is plausible that *Q. ilex* tolerance to O₃ is a consequence of low O₃ fluxes entering the stomata of leaves during the driest periods characterized by high levels of O₃ concentrations (Butković et al., 1990; Paoletti, 2006). This avoidance strategy implies that a minor amount of ozone enters inside the leaf thanks to stomatal closure (Taylor, 1978), as reported in many species in Mediterranean ecosystems (Ferretti et al., 2007; Gratani and Varone, 2004).

The United Nations Economic Commission for Europe (UNECE) established ozone critical levels for forest trees protection based on the diurnal accumulated exposure over a phytotoxic threshold of ozone concentration (AOT40; CLRTAP, 2010) calculated during six months (from April to September) of the year. With the intent to achieve more realistic estimates of ozone damage to plants, the scientific community agree to define and test new critical levels based on relationships between ozone stomatal fluxes (i.e., the phytotoxic ozone dose absorbed by the plants, POD) and significant growth reductions in plants, measured as relative biomass losses with respect to hypothetical conditions of null POD.

However, high uncertainties remain in the estimation of POD in Southern Europe (Mills et al., 2011b) due to the particular above-mentioned climatic conditions that can strongly affect the stomatal conductance of plants.

Many studies are available in the literature that address the physiological and biochemical responses of *Q. ilex* to O₃ alone and in combination with other stresses (Vitale et al., 2008; Alonso et al., 2008; Ribas et al., 2005), but few studies were conducted to assess the biomass response of this species after long-term exposure to high levels of O₃ (Calatayud et al., 2011; Alonso et al., 2014).

The main objectives of this study were:

- i) to test the effect of high levels of O₃ on the growth of *Q. ilex* assessing the biomass production at different levels (roots, stems and leaves);
- ii) to define dose–response relationships for *Q. ilex*, based on POD calculation and biomass losses and to propose O₃ critical levels for Mediterranean forests protection;
- iii) to gather information about relative O₃ sensitivity/tolerance of *Q. ilex*.

2. Methods

2.1. Plant material and experimental design

Two years old plantlets of *Q. ilex* originated from the plant nursery of Castelporziano (Roma, Italy) were transported to the Open-Top Chambers (OTC) facility of CRINES (Curno, Northern

Italy) on 3 April 2013, inside the 3 L pots, where they were grown for the last two years. The following day, plants were transferred into 13.5 L cylindrical pots, 28 cm in diameter, by keeping their own clod and by filling the remaining pot volume with standard soil Koro Excell (80% peat, 20% green compost, 25% organic C, 0.8% organic N, C/N ratio of 31, and pH of 5.5–6.5). Plants were left about three weeks in open air to adapt to the new conditions. Then, on 24 April 2013, 48 individuals homogeneous in size and vitality were transferred in 12 OTCs (four pots per chamber), 3 m in diameter and 2.4 m in height, equipped with a high transmissivity outer shell.

Details on the OTC model and structure can be found in Heagle et al. (1973). A fan system supplied each OTC with 70 m³/min of air, producing an average wind speed of 1 m/s inside each OTC (Gerosa et al., 2008).

To avoid unnatural soil heating, the pots were placed into ground holes dug in the OTCs soil so that the soil level of the pots equated the ground level of the OTCs.

The plants were left to acclimate inside the OTCs for two weeks before the O₃ treatments began on 6 May 2013.

For the O₃ treatments the chambers were arranged in three randomized blocks of four OTCs. Every OTC of each block received air with one of the following four different O₃ concentrations: –40% of the ambient O₃ concentration (charcoal-filtered air, CF), ambient air concentration (non-filtered air, NF), ambient air with O₃ concentration increased by 30% (OZ+) and ambient air with O₃ concentration increased by 74% (OZ++).

Ozone for the enriched treatments was produced by an O₃ generator (OGF500, Pilodist, Germany) fed by oxygen obtained by an oxygen generator (OG20, OGSi, New York, USA) and distributed to the different OTC by means of PTFE pipes and valves.

Ozone fumigation was active for 8 h per day, between 9 AM and 5 PM, while the OTC fans were working from 6 AM. to 10 PM.

On 13 September 2013 the O₃ fumigation was switched off and the plants were left in the OTCs until the end of the experiment on 30 September 2013.

From June to September, the plants were automatically irrigated every night, except for the rainy days, with the addition of an average of 0.30 L of water per pot to maintain soil water content at field capacity and avoid any limitation of the stomatal conductance.

2.2. Ozone and meteorological measurements

The O₃ concentrations within the OTCs of each block were monitored by means of three O₃ analyzers (Environnement, 0342M, France) that received air samples cyclically drawn from each chamber of the block through a solenoid valve system driven by a PC with a dedicated Lab View program. A fourth O₃ analyzer (DASIBI Italia, 1308, Italy) was used to assess the performance of the solenoid valve systems by continuously measuring the O₃ concentration within cyclically selected chambers for some days. The O₃ analyzers were calibrated and cross compared before and after the experiment.

Ancillary measurements of air temperature and humidity (50Y, Campbell Scientific Inc., Logan, Utah, USA), global radiation (200SZ pyranometer, LICOR), PPFD (LI-190 quantum sensor, LI-COR Inc., Nebraska, USA), soil water content (EC5, Decagon Devices Inc., Washington, USA) and soil temperature (PT100, GMR Instruments, Florence, Italy) and rain (52202 rain gauge, R.M. Young Company, Michigan, USA) were taken within the OTCs and additional rain (ARG100 Environmental Measurements Limited, UK) and wind measurements (WSU1, Environmental Measurements Limited, UK) were obtained from a meteorological station collocated beside the experimental field.

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