

Contrasting effects of ozone under different water supplies in two Mediterranean tree species

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Abstract

The effects of ozone (O_3) exposure under different water availabilities were studied in two Mediterranean tree species: *Quercus ilex* and *Ceratonia siliqua*. Plants were exposed to different O_3 concentrations in open top chambers (charcoal-filtered air (CF), non-filtered air (NF)) and non-filtered air plus 40 ppb_v of O_3 ((7:00–17:00 solar time) (NF +)) during 2 years, and to different water regimes (IR, sample irrigation, and WS, reduced water dose to 50%) through the last of those 2 years. AOT40 in the NF + treatment was 59265 ppb_vh (from March 1999 to August 1999) while in the NF treatment, the AOT40 was 6727 ppb_vh for the same period. AOT40 was always 0 in the CF treatment. WS plants presented lower stomatal conductances and net photosynthetic rates, and higher foliar N concentrations than IR plants in both species. The irrigation treatment did not change the response trends to ozone in *Q. ilex*, the most sensitive species to O_3 ambient concentrations, but it changed those of *C. siliqua*, the least sensitive species, since its ozone-fumigated WS plants did not decrease their net photosynthetic rates nor their biomass accumulation as it happened to its ozone-fumigated IR plants. These results show interspecific variations in O_3 sensitivity under different water availabilities.

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1. Introduction

Tropospheric ozone (O_3) is an air pollutant of major concern at the regional and continental scales. For instance, exceedances of the former critical level for forest tree species (Kärenlampi and Skärby, 1996), an AOT40 (sum of 1-h mean ozone concentrations above a threshold of 40 ppb_v) value of 10 ppm_v calculated during the 6-month growing season, have been reported every

year over USA and Europe (Stockwell et al., 1997). As a result, potential phytotoxic effects on tree species of those areas can be expected since O_3 exposure can negatively impact physiological characteristics associated with carbon acquisition such as foliar pigmentation, stomatal function and photosynthesis (Miller et al., 1963; Anderson et al., 1997; Zheng et al., 2002). Ozone effects on plant growth are usually related to an acceleration of leaf senescence, involving chlorophyll degradation, reductions in CO_2 assimilation (Elvira et al., 1998; Zheng et al., 2002), alterations in N metabolism and even changes in anatomical traits (Ribas et al., 2005).

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However, the phytotoxicity of this pollutant will depend on the flux within the leaf cells, which in turn will depend on stomatal opening, a process linked to various climatic factors. Other factors that determine plant sensitivity or tolerance are not clearly understood but it is thought to be related to many underlying physiological, anatomical and biochemical factors (Alonso et al., 2001).

The Mediterranean climate favours the generation of high O_3 concentrations but soil water shortage during summer causes partial stomatal closure for lengthy periods (Peñuelas et al., 1998), which could be translated into an avoidance or a lower uptake if this pollutant by Mediterranean vegetation when concentrations are at their highest levels (Bussotti and Gerosa, 2002). On the other hand, moderately elevated ozone concentrations have been shown to interfere with stomatal closure during drought (Pearson and Mansfield, 1993; Broadmeadow and Jackson, 2000) which might alter O_3 -induced physiological responses.

However, the interactions of O_3 and water availability on stomatal aperture are very complex since contrasting results have been found (see review by Darrall, 1989). The pollution-induced changes in stomatal aperture (Mansfield and Majernik, 1970; LeThiec et al., 1994; Broadmeadow et al., 1999) have been explained in terms of pollutant concentration, duration of exposure or external conditions during the measurements. However, the precise mechanisms underlying these stomatal responses have not been identified.

We aimed to study the effects of these two stressors, O_3 and drought, and whether additive, synergistic or antagonist interactions occur when plants are exposed simultaneously to both stresses. We tested the effects of different O_3 concentrations and irrigation regimes on the physiology and growth of two Mediterranean tree species: *Quercus ilex* and *Ceratonia siliqua*. We hypothesized that the interaction would be stronger in the less O_3 sensitive species, *C. siliqua* (Orendovici et al., 2003; Ribas et al., 2005), since in that species a moderate drought would revert the weaker ozone effects on stomatal conductance, O_3 uptake senescence enhancement and O_3 damage. In order to test this hypothesis, we measured the seasonal responses of their gas exchange rates, fluorescence, foliar $\delta^{15}N$ and $\delta^{13}C$, leaf morphology, foliar N concentration, and growth in response to ozone fumigation (ambient plus 40 ppb_v) and reduced water supply.

2. Material and methods

2.1. Growth conditions: ozone and water treatments

Plants of *Q. ilex* ssp. *ilex* and *C. siliqua* were raised from seeds. In July 1998, 1-year old homogeneous

seedlings of each species were transplanted into 6 dm³ containers filled with Universal substrate (peat and bark pine) with a 34% of organic material. Soil pH was 6.6, and 9 g of slow-release fertilizer (NPK 15:8:11; Osmocote plus) were added to each pot.

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 ambient O_3 levels, and non-filtered air supplemented with 40 ppb_v O_3 from 7:00 to 17:00 GMT 5 days every week (NF+). Three OTC replicates were used for each O_3 treatment. An automatic system provided a continuous monitoring of O_3 concentrations in the different treatments along with meteorological parameters such as wind speed and direction, air temperature and relative humidity, and photosynthetically active radiation (PAR). Complete descriptions of the chambers and the operation of the system are provided in Alonso et al. (2001). We introduced eight individual plants per treatment level and species.

Plants were irrigated with a droplet system to ensure adequate and homogeneous water availability to plant material. Well-watered plants (irrigated plants, IR from now on) were irrigated twice a week. Plants under low water supply treatment (water-stressed, WS from now on) were irrigated once a week. The ozone experiment lasted 2 years (July 1998–August 2000), while the restrictions in the water availability started in plants after 1 year of ozone exposure (since spring 1999–summer 2000). Shoot water potential was determined using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA) in fall 1999, winter and spring 2000. On each sampling date, shoots of three plants (one shoot per plant, chamber and water treatment) of *Q. ilex* and *C. siliqua* were measured at predawn (02:30–04:30 h solar time in spring and 04:30–06:30 h solar time in autumn and winter) and mid-day (11:00–13:00 h, solar time).

2.2. Chlorophyll fluorescence, gas exchange, and chlorophyll measurements

Chlorophyll fluorescence and gas exchange measurements were conducted during 2–4 consecutive days in spring 2000 (after 2 years of ozone treatment). Well-developed leaves were measured under clear-sky conditions. The maximum photochemical efficiency of PSII (F_v/F_m), yield ($\Delta F_v/F'_m$) and the apparent photosynthetic electron transport rate (ETR) were measured with a PAM-2000 fluorometer (Walz, Effeltrich, Germany). The maximum photochemical efficiency of PSII (F_v/F_m) was measured on leaves kept in the dark for 20 min (dark adaptation) prior to fluorescence measurements.

We previously checked that this fluorescence coefficient reached constant values after this 20 min dark period. Chlorophyll fluorescence was measured on three well-developed leaves of three plants per treatment level from 07:00 to 11:00 h (solar time). ETR was estimated as $ETR = \Delta F_v/F'_m \times PPFD \times 0.84 \times 0.5$, where $\Delta F_v/F'_m$ (fluorescence yield or actual photochemical efficiency of PSII) was calculated according to Genty et al. (1989), 0.84 is the coefficient of absorption of the leaves, and 0.5 is the fraction of electron involved in the photoexcitation produced by one quanta, since two photosystems are involved.

Net photosynthetic rates (A) and stomatal conductances (g_s) were measured with a portable gas exchange system ADC LCA4, with a PLC4B chamber (ADC Inc., Hoddesdon, Hertfordshire, UK) inside the open-top growth chambers. Two well-developed leaves of two different plants per treatment level and species were measured from 07:00 to 11:00 h (solar time). A and g_s values were expressed on a projected leaf area basis. The leaf area was measured with an Li-Cor 3100 Area Meter (Li-Cor Inc., Lincoln, Nebraska, USA).

Chlorophyll content was determined non-destructively using an SPAD-502 meter (Minolta Co, LTD, Osaka, Japan). This instrument uses measurements of transmitted radiation in the red and near-infrared wavelengths to provide numerical values related to leaf chlorophyll content. Close linear correlations between SPAD values and extractable chlorophyll concentration have been reported for a wide range of species, including plants exposed to elevated O_3 (Tenga and Ormrod, 1990). SPAD measurements were conducted in March 2000. We measured four plants per treatment level and species (each plant measure as a mean of four leaves per plant).

2.3. Isotope and elemental analyses

To analyse C and N isotopic composition, 4–6 well-developed leaves from different plants were collected and pooled for each O_3 treatment, water regime and species in summer 2000 before harvesting. The foliar $\delta^{15}N$ and $\delta^{13}C$ were measured in an SIRA Series II isotope ratio mass spectrometer (VG Isotech, Middlewich, UK) operated in direct inlet continuous flow mode after combustion of the samples in an elemental analyser (NA1500, Series 1, Carlo Erba Instrumentazione, Milan, Italy). The reference CO_2 , calibrated against standard Pee Dee belemnite (PDB) was obtained from Oztech (Dallas, TX, USA). A system check of analysis was achieved with interspersed working standards of cellulose, atropine and urea (Sigma, St. Louis, MO, USA). The accuracy of the measurement was $\pm 0.1\%$ for $\delta^{13}C$ and $\pm 0.2\%$ for $\delta^{15}N$.

Carbon (C) and nitrogen (N) leaf concentrations were analysed with a Carlo Erba NA 1500 Analyser (Milan,

Italy) by using a standard configuration for those determinations. The samples were previously dried at 70 °C to constant weight. They were weighed by using a Mettler UM3 microbalance in tin containers.

2.4. Leaf morphological and anatomical characteristics and final biomass harvest

Trees were visually inspected for foliar symptoms of chronic ozone injury seasonally throughout the 2 years of exposure. Foliar samplings were also collected to measure leaf area and leaf mass area ($n=3$ per treatment level). The leaf area was measured with a LICOR LI-3100 area meter (LICOR, Lincoln, Nebraska). Thereafter, the leaves were dried at 70 °C until constant weight.

At the end of the exposure experiment, after spring 2000 ecophysiological measurements, all aboveground biomass was harvested and placed in a forced-air oven at 70 °C. Final dry weight was determined when dry biomass reached constant weight.

2.5. Statistical analyses

The main design was a randomized complete block with the mentioned three ozone and two irrigation treatments. Data for fluorescence (F_v/F_m , yield and ETR), gas exchange measurements (A , g_s), chlorophyll concentrations, $\delta^{13}C$, N concentrations, $\delta^{15}N$, morphological parameters (leaf area, LMA) and above-ground biomass were analysed as dependent variables using two way factorial ANOVAs with ozone and water regime as independent variables. Analyses were conducted for each individual species. All mentioned analyses were performed with the software package Statistica 6.0 (StatSoft Inc., Tulsa, USA).

3. Results

3.1. Ozone exposure and meteorological data

AOT40 in the NF+ treatment was 59265 ppb_vh (from March 1999 to August 1999) while in the NF treatment, the AOT40 was 6727 ppb_vh for the same periods. AOT40 was always 0 in the CF treatment (see Fig. 1). 24-mean ozone values for the same periods were 57.2 ± 1.66 , 37.2 ± 1.66 and 11.2 ± 0.70 ppb_v for NF+, NF and CF, respectively. Therefore, the suggested daylight AOT40 critical level of 10 ppm_vh in 6 months (Kärenlampi and Skärby, 1996) was exceeded all years in NF+. Typical seasonal coastal Mediterranean trends were observed in temperature and ozone concentrations (Fig. 1), with soft winters and warm and dry summers. Minimum temperatures occurred from

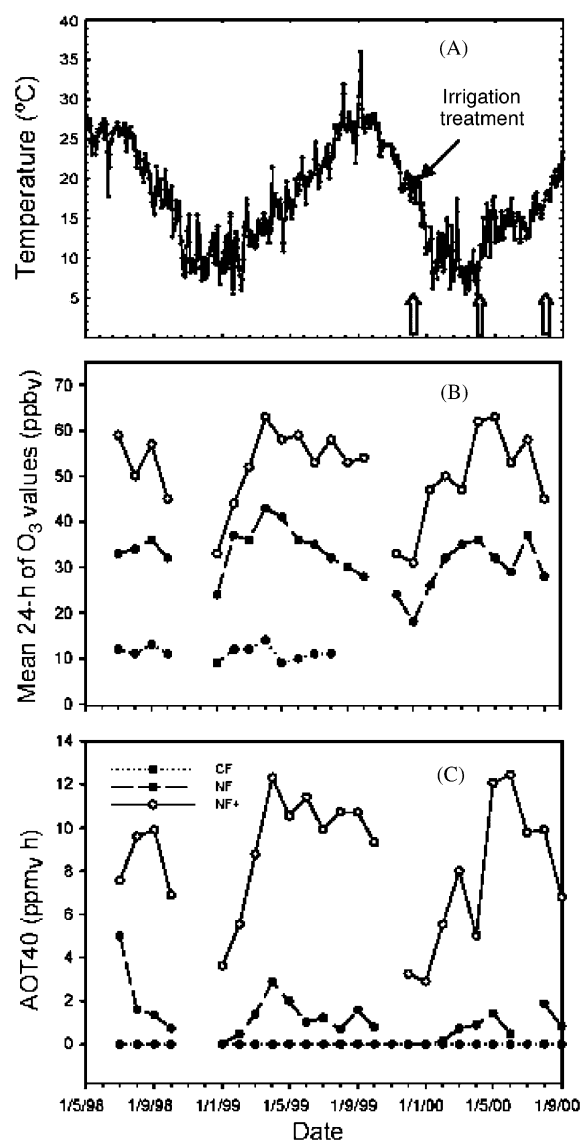


Fig. 1. (A) Field site daily mean Temperature ($^{\circ}\text{C}$), (B) 24 h-mean ozone concentrations (ppbv), and (C) monthly AOT40 during the experimental period, from July 1998 to September 2000, in the different O_3 treatments. CF, charcoal-filtered air; NF, non-filtered air; NF+, non-filtered air plus 40 ppbv ozone. The white arrows show the sampling dates after irrigation treatment started.

January to March, between 5°C and 7°C , and the maximum temperatures occurred in summer, between 25°C and 28°C as monthly mean values (Fig. 1). Humidity values were very high throughout all the year (between 70% and 90% as monthly mean values) (data not shown).

3.2. Effects on water potential, F_v/F_m , stomatal conductance and net photosynthesis

Measurements showed that the “drought” treatment did not affect water potential, stomatal conductance, or net photosynthetic rates neither in fall nor in winter (data not shown) as expected from the low evaporative demands of these seasons that precluded any clear symptom of drought stress. However, in spring 2000, a reduction in the pre-dawn and midday water potential in response to the WS treatment was detected in the two species (Fig. 2). The WS treatment reduced the water potential of the *Q. ilex* plants ca. 0.7 MPa both at predawn and midday (Fig. 2) and those of *C. siliqua* ca. 0.2 MPa at predawn and ca. 0.5 MPa at midday (Fig. 2). There were no significant differences in shoot water potentials among the ozone treatments. There was not either any significant interaction effect of irrigation and ozone treatments in any of the two species.

The dark-adapted maximum photochemical efficiency of photosystem II (F_v/F_m) tended to decrease under higher ozone concentrations, especially in well-watered plants, but no statistically significant differences were found in any of the two species (Table 1). Neither significant interactions (irrigation $\times\text{O}_3$) were detected. Fluorescence yield and ETR did not either show any significant difference among treatments except for a slight decrease of yield in response to reduced irrigation (Table 1).

Reduced water supply produced lower stomatal conductance in both species (Fig. 3). Stomatal conductance tended to be higher under NF+ conditions in both species, but this trend was only significant in *Q. ilex* (Fig. 3). This trend disappeared in WS *C. siliqua* plants.

Lower net photosynthetic rates were found in WS plants, being the decrease statistically significant in *C. siliqua* (Fig. 4). While *Q. ilex* plants did not change their photosynthetic response to ozone fumigation because of reduced irrigation dose, *C. siliqua* ozone-fumigated plants showed a significant reduction in net photosynthetic rates relative to plants growing at O_3 ambient concentrations when well watered but not when half-irrigated (Fig. 4).

3.3. Effects on biomass, N , $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

The two studied species showed different sensitivity to ozone. *Q. ilex* reduced its biomass accumulation already in response to ambient O_3 concentrations while *C. siliqua* only reduced its biomass accumulation in response to O_3 fumigation. The irrigation treatment significantly affected the total biomass accumulation of both studied species (Fig. 5). The reduction in irrigation reduced final above-ground biomass from 7% in NF+ *C. siliqua* plants to 30.1% in NF *C. siliqua* plants (Fig. 5). There was thus a marginally interactive effect

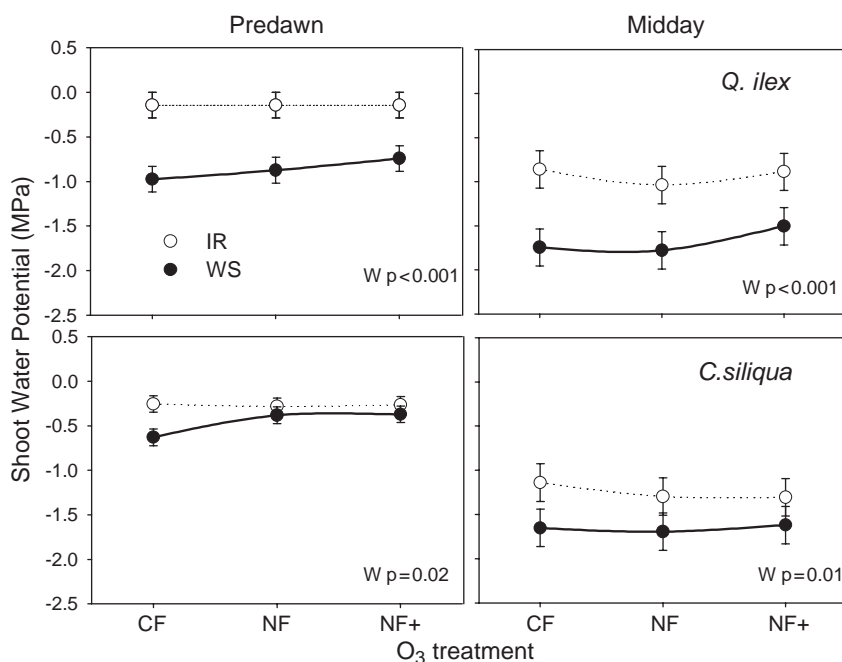


Fig. 2. Shoot water potential (MPa) of *Q. ilex* and *C. siliqua* at predawn and midday points in spring 2000. Error bars indicate the standard error of the mean ($n = 3$ plant measurements, one per chamber). Statistically significant effects are depicted in the panels. CF, charcoal-filtered air; NF, non-filtered air; NF +, non-filtered air plus 40 ppb_v ozone. IR, well-watered and WS, reduced irrigation (50% of IR).

($p = 0.09$) of ozone fumigation and irrigation treatment for this species. In *C. siliqua* the ozone fumigation reduced 28% biomass accumulation relative to plants growing at ambient O₃ concentrations in IR plants and only 4% in WS plants (Fig. 5).

After 2 years of exposure, elevated O₃ caused a significant decrease in the *Q. ilex* foliar concentrations of nitrogen (17%) but not in *C. siliqua* (Table 1). WS plants presented higher N foliar concentrations in both species but there was no interaction with O₃ fumigation. Both species increased their $\delta^{15}\text{N}$ values with increasing ozone exposures after 2 years of fumigation (Table 1). No significant effect of irrigation or the interaction irrigation-ozone fumigation on $\delta^{15}\text{N}$ was found in any of the studied species (Table 1). No changes were either found in $\delta^{13}\text{C}$ either due to ozone or irrigation except for an irrigation effect on filtered *C. siliqua* plants (Table 1).

3.4. Leaf morphology, chlorophyll and visible injury

Increasing O₃ concentrations tended to decrease the leaf area in both species but only in a marginally significant way in *Q. ilex* (Table 1) ($p = 0.09$). This effect occurred in IR plants. No significant effects were found in WS plants. Therefore, there was a significant interaction effect between ozone and irrigation ($p = 0.02$). There were no significant responses of

LMA to increased ozone concentrations or irrigation (Table 1). Significant decreases in response to elevated O₃ concentrations were observed in chlorophyll SPAD values for *Q. ilex* (Table 1). After 2 years of O₃ fumigation no clear visual leaf injuries occurred in any of the studied species. Only in *Q. ilex* a slight mottle stipple bronzing appeared on a few old leaves that had been exposed for 2 years. The bronzing frequency seemed to be related to the irrigation treatment. It was found only in one NF+ chamber for the 12% of WS plants, while it was found in two of NF+ chambers for the 38% and 25% of IR plants.

4. Discussion

Both species presented a different sensitivity to water stress or O₃ exposure. *Q. ilex* was most sensitive to O₃ and *C. siliqua* was relatively most affected by water stress. Ozone exposure induced reductions of the biomass of the seedlings of *Q. ilex* even at ambient concentrations, whereas *C. siliqua* only presented a significant response under the fumigation (NF+) treatment (Fig. 5). This interspecific variation in O₃ sensitivity was also reported by Ribas et al. (2005). Results confirmed our hypothesis that the interaction would be stronger in the less O₃ sensitive species,

Table 1

Mean \pm standard error of the mean values of Fv/Fm, yield, ETR, $\delta^{13}\text{C}$, LMA, leaf area, SPAD, foliar N concentration and $\delta^{15}\text{N}$ of *Q. ilex* and *C. siliqua* leaves in March 2000 for each one of the ozone and irrigation treatments

<i>Q. ilex</i>	Filtered		Non-filtered		Non-filtered + 40 ppb _v		Significant effects
	IR	WS	IR	WS	IR	WS	
Fv/Fm	0.802 \pm 0.005	0.797 \pm 0.005	0.799 \pm 0.005	0.795 \pm 0.005	0.788 \pm 0.005	0.797 \pm 0.005	—
Yield	0.60 \pm 0.05	0.48 \pm 0.05	0.60 \pm 0.05	0.56 \pm 0.05	0.56 \pm 0.05	0.53 \pm 0.05	—
ETR ($\mu\text{mol electron m}^{-2}\text{s}^{-1}$)	48.09 \pm 8.38	41.02 \pm 8.38	46.04 \pm 8.38	48.47 \pm 8.38	35.38 \pm 8.38	50.16 \pm 8.38	—
$\delta^{13}\text{C}$ (‰)	−26.47 \pm 0.36	−26.52 \pm 0.36	−26.48 \pm 0.44	−25.78 \pm 0.36	−26.41 \pm 0.41	−26.57 \pm 0.36	—
LMA (g cm^{-2})	0.021 \pm 0.001	0.025 \pm 0.001	0.021 \pm 0.001	0.022 \pm 0.001	0.022 \pm 0.002	0.020 \pm 0.001	—
Leaf area (cm^{-2})	6.59 \pm 0.54	5.68 \pm 0.54	4.64 \pm 0.54	6.55 \pm 0.54	4.61 \pm 0.54	5.44 \pm 0.54	O ₃ p = 0.09, int p = 0.02
SPAD (relative units)	53.7 \pm 1.4	56.7 \pm 1.4	55.8 \pm 1.4	51.8 \pm 1.4	52.3 \pm 1.4	53.4 \pm 1.4	O ₃ p = 0.02
N concentration	1.78 \pm 0.08	1.94 \pm 0.08	1.73 \pm 0.08	1.79 \pm 0.08	1.47 \pm 0.08	1.78 \pm 0.08	O ₃ p = 0.04, W p = 0.02
$\delta^{15}\text{N}$ (‰)	−1.26 \pm 0.3	−1.07 \pm 0.3	−0.58 \pm 0.3	0.003 \pm 0.3	−0.11 \pm 0.3	−0.34 \pm 0.3	O ₃ p = 0.03
<i>C. siliqua</i>							
Fv/Fm	0.746 \pm 0.012	0.745 \pm 0.012	0.747 \pm 0.012	0.727 \pm 0.012	0.725 \pm 0.012	0.731 \pm 0.012	—
Yield	0.48 \pm 0.05	0.43 \pm 0.05	0.44 \pm 0.05	0.56 \pm 0.06	0.47 \pm 0.05	0.39 \pm 0.05	W p = 0.08
ETR ($\mu\text{mol electron m}^{-2}\text{s}^{-1}$)	35.69 \pm 7.03	42.84 \pm 7.03	39.11 \pm 7.03	37.77 \pm 7.03	31.31 \pm 7.03	47.37 \pm 7.03	—
$\delta^{13}\text{C}$ (‰)	−27.45 \pm 0.80	−23.93 \pm 0.80	−24.89 \pm 0.80	−25.77 \pm 0.80	−25.56 \pm 0.80	−24.20 \pm 0.80	W p = 0.07, int p = 0.05
LMA (g cm^{-2})	0.019 \pm 0.001	0.022 \pm 0.001	0.021 \pm 0.001	0.020 \pm 0.001	0.019 \pm 0.001	0.019 \pm 0.001	—
Leaf area (cm^{-2})	12.16 \pm 1.22	10.86 \pm 1.22	11.36 \pm 1.22	10.34 \pm 1.22	10.66 \pm 1.22	10.09 \pm 1.22	—
SPAD (relative units)	56.0 \pm 2.1	60.7 \pm 2.1	53.8 \pm 2.1	57.6 \pm 2.1	55.7 \pm 2.1	52.4 \pm 2.1	—
N concentration	1.39 \pm 0.3	3.01 \pm 0.3	1.51 \pm 0.4	1.97 \pm 0.4	1.39 \pm 0.3	3.19 \pm 0.3	W p < 0.001
$\delta^{15}\text{N}$ (‰)	−1.72 \pm 1.3	−2.83 \pm 1.3	−1.34 \pm 1.6	−2.08 \pm 1.3	−1.09 \pm 1.3	−0.17 \pm 1.3	O ₃ p = 0.10

For fluorescence variables (Fv/Fm, yield and ETR) standard error corresponds to $n = 3$ chamber means of $n = 6–9$ plant measurements. For $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and N concentration standard error corresponds to $n = 3$ chamber measurements of $n = 6–9$ pooled leaves. For LMA and leaf area, standard error corresponds to $n = 3$ chamber means of $n = 6–9$ leaves. For SPAD, standard error corresponds to $n = 3$ chamber means of $n = 4$ plants (mean of 4 leaves for plant). Statistically significant effects are depicted in the last column.

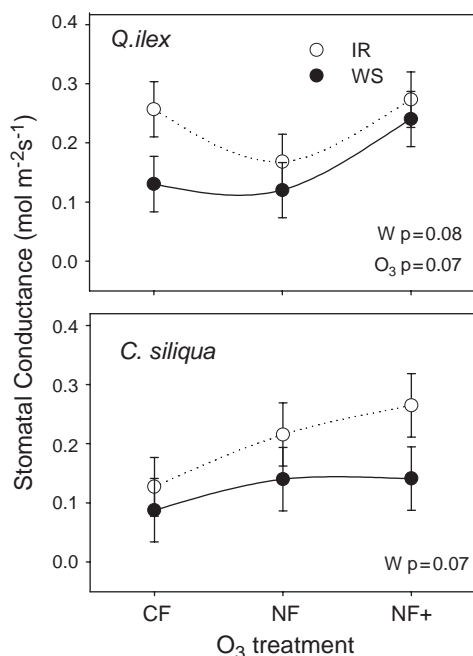


Fig. 3. Stomatal conductances ($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of *Q. ilex* and *C. siliqua* in spring 2000 (from 07:00 to 11:00 h, solar time). Error bars indicate the standard error of the mean ($n = 3$ chamber means of $n = 2$ plant measurements). CF, charcoal-filtered air; NF, non-filtered air; NF+, non-filtered air plus 40 ppb_v ozone. IR, well-watered and WS, reduced irrigation (50% of IR). Statistically significant effects are depicted in the panels.

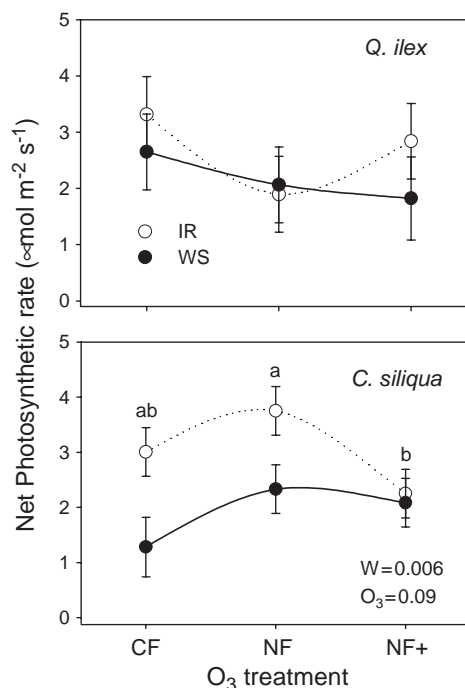


Fig. 4. Net photosynthetic rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of *Q. ilex* and *C. siliqua* exposed to ozone in spring 2000 (from 07:00 to 11:00 h, solar time). Error bars indicate the standard error of the mean ($n = 3$ chambers means of $n = 2$ plant measurements per chamber). CF, charcoal-filtered air; NF, non-filtered air; NF+, non-filtered air plus 40 ppb_v ozone. IR, well-watered and WS, reduced irrigation (50% of IR). Statistically significant effects are depicted in the panels.

C. siliqua (Orendovici et al., 2003; Ribas et al., 2005), since in that species a moderate drought was able to revert the weak effects of ozone on its stomatal conductance. They also seem to confirm that O₃ uptake enhances senescence. The different ozone sensitivity of these two species to O₃ was probably associated to an enhancement of senescence-related processes, as suggested by the observed reductions of N and chlorophyll, or the increases of $\delta^{15}\text{N}$. This is in agreement with the findings reported by several authors (Peñuelas et al., 1994; Gratani et al., 2000; Dizengremel, 2001; Kolb, 2001; Bielenberg et al., 2002; Fuhrer and Booker, 2003).

An additive effect from the combined exposure of *Q. ilex* to both stresses was found since they determined a biomass reduction of 34% when comparing NF+-reduced water supply plants to CF-irrigated plants, which was the sum of the effects induced by each stress. On the other hand, the less O₃-sensitive *C. siliqua* did not change its response to water stress when simultaneously exposed to the pollutant. Contrasting results have been reported regarding the response of plant biomass to both stresses. Some authors have found an increased sensitivity to O₃ when

plants are properly irrigated when compared with water-stressed plants (Temple et al., 1993; Karlsson et al., 1997; Broadmeadow and Jackson, 2000). No interactions between water stress and O₃ on plant performance have also been reported by several authors (Pearson and Mansfield, 1994; Karlsson et al., 2002; Khan and Soja, 2003) as was the case for *Q. ilex*. These studies also found that the combination of the two stresses caused an additive effect in plant growth. However, we did not find synergic reductions in carbon gain as has been found for other species, such as white fir (Retzlaff et al., 2000).

Biomass reductions were not related with height and diameter responses (data not shown); therefore, they were likely associated with lower leaf retention. In fact, several studies have reported decreased leaf retention of carob trees and *Q. ilex* in polluted areas (Correia and Martins-Loução, 1997; Gratani et al., 2000).

Water stress induced an increase in the N levels of *Q. ilex* and *C. siliqua*, 10% and 50%, respectively. This response was not related with alterations in other parameters. Several authors have associated an increase in N levels in water-stressed plants with retranslocation processes (Correia and Martins-Loução, 1997).

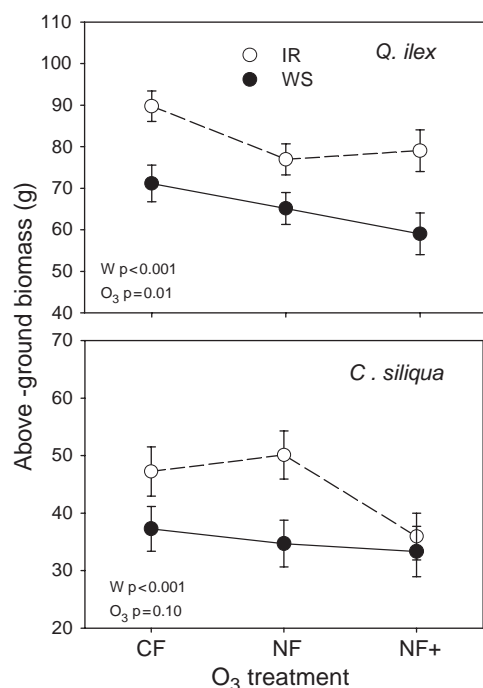


Fig. 5. Final above ground biomass (g dw) of *Q. ilex* and *C. siliqua* exposed to both ozone in open-top chambers from summer 1998 to summer 2000 and to irrigation treatment from fall 1999. Error bars indicate the standard errors of the mean ($n = 3$ chamber mean of 6–8 plants per chamber). CF, charcoal-filtered air; NF, non-filtered air; NF+, non-filtered air plus 40 ppb_v ozone. IR, well watered and WS, reduced irrigation (50% of IR). Statistically significant effects are depicted in the panels.

Both species presented different strategies to cope with daily and seasonal variance in soil water availability that could be related with their differential xerophytic configuration. *Q. ilex* presented a lower area and a greater LMA than *C. siliqua*, traits that have been positively associated with a greater drought tolerance (Salleo and Lo Gullo, 1990; Mediavilla et al., 2001). Water stress determined a reduction of the leaf area of *Q. ilex*, O_3 exposure induced a similar response; the adverse effects of O_3 were greater in the irrigated plants when compared with the water stressed treatment. A similar pattern was found for carob trees although no significant effects were recorded.

Interactive effects of O_3 and WS treatment were also found in C^{13} water use efficiency of carob tree.

So, in summary, the most O_3 sensitive species to O_3 ambient concentrations, *Q. ilex*, presented an additive response to both stressors. The less sensitive species, *C. siliqua*, did not respond to O_3 fumigation when water available was low. We hypothesize that a more severe water stress is needed to affect the ozone responses in the most O_3 -sensitive species or that higher O_3 concentra-

tions would be needed to affect the WS plants of the least O_3 -sensitive species.

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