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Severe drought events increase the sensitivity to ozone on poplar clones

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ABSTRACT

An open-top chamber experiment has been carried out at the facilities of Curno (North Italy), in June–August 2009, to assess the response to ozone in two poplar clones *Populus maximowiczii* Henry × *P. berolinensis* Dippel (Oxford clone, OX), and *Populus nigra* "Jean Pourtet" (JP) in concomitance of severe drought events. Three different water regimes were applied: W – Well Watered Control: field capacity; D1 – Drought Treatment 1: field capacity until begin July, then reduced water availability (plants were then subjected to severe drought events); D2 – Drought Treatment 2: constant water shortage (plants were then subjected to severe drought events). Leaf water potential, gas exchange and chlorophyll fluorescence (JIP-test) were assessed every 2 weeks; growth parameters and stable isotope composition ($\delta^{13}C$ and $\delta^{18}O$) were measured at the end of the experiment. The main results were: (i) drought, but not ozone, reduced photosynthesis and growth and increased $\delta^{13}C$; (ii) the two clones showed different strategies to cope with ozone stress: JP shed the damaged leaves, whereas OX maintained their leaves ozone provoked the loss of leaves in W plants of the JP clone; (iii) in the D1 plants the response to drought provokes an additional effects with the effect of ozone absorbed before the severe drought events; (iv) D2 plants did not respond to ozone until the last event, when a clear synergistic effect between the two stressors was observed. We conclude that ozone had different effects in relation to the way the drought stress was applied. These results are discussed for their ecological consequence on vegetation in field conditions.

1. Introduction

At median latitudes trees are often subjected to drought in the summer months. Water shortage, in terms of both low VPD (*Vapour Pressure Deficit*) and low soil water potential, occurs at the same time as high temperatures and high light. Trees have developed mechanisms to defend themselves against these stressors all together; thus, their resistance to one of them, implies resistance to the others (cross-resistance, Bussotti, 2008). In recent decades these environmental factors have been joined by tropospheric ozone (O_3), a secondary pollutant that is formed in the atmosphere through photochemical reactions. In nature ozone has a role in forest ecosystems, since there is a natural background level of this compound which is generated from the volatile compounds emitted by the vegetation itself (Calfapietra et al., 2009). Drought stress and ozone exert similar effects on photosynthesis, consisting in stomatal closure (stomatal limitation: Torsethaugen et al., 1999; Flexas and Medrano, 2002; Moldau et al., 2011) and deactivation of Rubisco (non stomatal limitation: Parry et al., 2002; Flexas and Medrano, 2002; Dann and Pell, 1989; Fontaine et al., 2003; Galmés et al., 2011). Because of photosynthesis constraints, plants subjected to ozone and/or drought stress reduce their growth (Ainsworth et al., 2012; Eilmann and Rigling, 2012).

Although the photosynthetic apparatus is protected from the direct action of ozone by means of the reduction of stomatal ozone fluxes (Pääkkönen et al., 1998), the combination of ozone and drought stress can exacerbate the overall effect, and many authors (Alonso et al., 2001; Yonekura et al., 2004; Grulke et al., 2002; Ribas et al., 2005; Tausz et al., 2007) claim there is a synergistic effect of these two stressors. Several hypotheses have been formulated, including the high production of Reactive Oxygen Species (ROS) due to their concomitant action (Tausz et al., 2007), and altered stomatal function due to ozone that leads to uncontrolled water loss (Maier-Maercker, 1989; McLaughlin and Downing, 2002; Grulke et al., 2004; Paoletti and Grulke, 2010; Hoshika et al., 2012). Wilkinson and Davies (2009, 2010), Tanaka et al. (2005) and Mills et al. (2009)

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Table 1
Meteorological and ozone parameters during the experimental period (June–August 2009). *T* = temperature; [O_3] = ozone concentration; AOT40 = Above Ozone Threshold 40 ppb (parts per billion); mean = mean of the month; max = maximum hourly in the month; OA = open air; NF = not filtered open-top chambers; CF = charcoal filtered open-top chambers.

	Unit	June	July	August	Total (AOT40)
T (mean)	°C	22.5	24.8	25.3	
T (max)	°C	32	33.2	31.7	
Rainfall	mm	151	158	12	
[O_3] (mean)	ppb	44	53	52	
[O_3] (max)	ppb	112	142	96	
AOT40 – OA	ppb h	4703	8621	2170	15494
AOT40 – NF	ppb h	4153	5884	1675	11713
AOT40 – CF	ppb h	1010	1009	994	3013

showed that drought induced stomatal closure, as a consequence of the accumulation of abscisic acid (ABA) in the leaves, is counteracted by the ethylene production induced by ozone.

In a natural environment the initial exposures to water stress and to ozone may not occur at the same time. The photochemical season begins whit sunny days and when temperature rises in concomitance with leaf unfolding (i.e. April–May, where the present experiment was carried out). When severe drought stress occurs (July–August) the plants may have already absorbed a consistent dose of ozone. Moreover, drought stress may be not a condition continuative and constant through the growing season, but severe drought events are interspersed by recovery due to occasional rainfalls.

Our hypothesis is that the combination ozone + severe drought episodes is especially harmful when water shortage is delayed with respect to ozone exposure. On the other hands, plants are better protected against ozone if acclimation to water stress occurs from the very beginning of the growing season.

2. Materials and methods

2.1. Experimental set-up

The experiment was conducted at the open-top chambers (OTC) facility at Curno, Lombardy, North Italy. Ecological features of the site, and technical details of OTCs are reported in previous papers (Bussotti et al., 2007; Gerosa et al., 2008, 2009). Open field ozone concentration was reduced to 50% in three charcoal filtered (CF) OTCs, and only by 5% in three non filtered (NF) OTCs. Mean meteorological features and ozone concentration for the experimental period are shown in Table 1, whereas Table 1Supp. reports the meteorological conditions of the three days before the physiological measurements.

The experiment was carried out on *Populus maximowiczii* Henry × *P. berolinensis* Dippel (Oxford clone) and on *Populus nigra* clone Jean Pourtet. The plants used were reproduced through cuttings. In early April, cuttings about 20 cm long were placed in 5-l pots containing a commercial soil substrate, expanded clay and peat, in the ratio 3:1:0.5 (v/v). The plants were placed in the OTCs in early June, when they were 8 weeks old. In each OTC were placed 24 plants (12 per clone): 12 plants (6 per clone) were used for repeated measurements, and the additional 12 plants were used for destructive analysis. The plants inside each OTC were subjected to 3 different water regimes by means of a drip system irrigation. Well watered (W, control) were provided of 1.3 l daily for the entire duration of the experiment starting in early April. With Drought Treatment 1 (D1), plants were subjected to the same water regime as W until the beginning of observations (30 June); thereafter the plants received 60% of water respect to W. With Drought Treatment 2 (D2), plants received 20% of water respect to W throughout the whole experimental period. Plants in groups D1 and D2 were thus subjected to drought stress events: irrigation was discontinued over the two days prior to measurements, made at the following dates in the summer of 2009: 8 July; 21 July, 4 August. During the experiment the soil water content was monitored by means of EC-5 soil moisture sensors (Decagon Device, Campbell Scientific, Shepshed, UK). The pots of D1 and D2 plants were protected by rain with a plastic cover during the periods of water withdraw. The physiological conditions of the plants were assessed also the 30 June before beginning with drought events treatments.

2.2. Plant water status measurements

Leaf water content was determined by measuring pre-dawn (4:00 am) water potential with a portable pressure chamber (SKPM 1400, Sky Instruments Ltd, Powys, UK). At each sampling date the water potential of 6 plants per clone and per water treatment was measured. Relative Water Content (RWC) was measured on an additional sample of leaves as:

$$RWC = [(FW - DW)/(SW - DW)]100$$

where FW is the sample's fresh weight; DW is the dry weight, obtained after 72 h drying in a 70 °C oven; SW is the saturated fresh weight, obtained by keeping the leaves in the dark for 48 h, with their stem in distilled water.

2.3. Gas exchange measurements

The effect of the water treatment on stomatal closure was measured between 10 am and 1 pm on each sampling date, on those plants subjected to non destructive sampling, using a dynamic diffusion porometer AP-4 (Delta-T Devices, Cambridge, UK). Individual leaf measurements were carried out in ambient light with PAR values ranging between 1100 and 1600 $\mu mol\,m^{-2}\,s^{-1}$ and environmental concentration of CO_2 at 370–380 $\mu mol\,mol$. At the same times, net photosynthesis (P_N) was measured with a portable photosynthesis system (gas analyzer) LCi (ADC BioScientific Ltd Hoddesdon, UK). All gas exchange measurements were done on leaves from the median portion of the crown.

2.4. Biomass and stable isotope ratios measurements

At the end of the experiment (August 2009) all plants were harvested and biomass parameters (height, diameter at the collar, dry mass of stem, roots and leaves) were defined. Dry weight of the different organs was determined after drying at 70 °C until constant weight.

For isotope analysis, stem (collected above the collar), root and leaf samples were collected. These samples were air-dried and, then, finely ground with a ball-mill grinder (Pulverisette 14, Fritsch GmbH, Oberstein, Germany). An aliquot of the samples was weighed (0.800 ± 0.05 mg) out into tin capsules and then combusted in an Elemental Analyser (Flash EA^{TM} 1112, Thermo Scientific, Bremen, Germany) for carbon isotope ratio analysis ($^{13}C/^{12}C$). The CO_2 produced from a flash combustion of the sample (in presence of O_2, at a temperature of 900 °C) flushed into the isotope mass spectrometer (Finnigan Delta Plus XP^{TM}, Thermo Scientific, Bremen, Germany). For oxygen isotope analysis ($^{18}O/^{16}O$), the aliquot of the sample was weighed out into silver capsules, dropped into the pyrolyser (Finnigan TC/EA^{TM}, Thermo Scientific, Bremen, Germany) where at the temperature of 1450 °C without oxygen, the produced CO and H_2 were sent into a gas chromatography column and then into the mass spectrometer. The isotope composition of the stem, root and leaves was reported in the standard delta notation (δ, ‰) against international standards