Visible ozone-like injury, defoliation, and mortality in two *Pinus uncinata* stands in the Catalan Pyrenees (NE Spain)

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Abstract

Ozone concentrations in the Pyrenees have exceeded the thresholds for forest protection since 1994. We surveyed the severity of visible O_3 injuries, crown defoliation, and tree mortality of *Pinus uncinata*, the dominant species in subalpine forests in this mountain range, along two altitudinal and O_3 gradients in the central Catalan Pyrenees and analysed their relationships with the local environmental conditions. The severity of visible O_3 injuries increased with increasing mean annual $[O_3]$ when summer water availability was high (summer Precipitation/Potential evapotranspiration above 0.96) whereas higher $[O_3]$ did not produce more visible injuries during drier conditions. Mean crown defoliation and tree mortality ranged between 20.4-66.4 and 0.6-29.6%, respectively, depending on the site. Both were positively correlated with the accumulated O_3 exposure during the last five years and with variables associated with soil-water availability, which favours greater O_3 uptake by increasing stomatal conductance. The results indicate that O_3 contributed to the crown defoliation and tree mortality, although further research is clearly warranted to determine the contributions of the multiple stress factors to crown defoliation and mortality in *P. uncinata* stands in the Catalan Pyrenees.

Keywords

Ozone, Pyrenees, *Pinus uncinata*, visible ozone injury, defoliation, mortality.

Acknowledgements

This research was supported by the European Research Council Synergy grant ERC-2013-SyG-610028 IMBALANCE-P, the Spanish Government grant CGL2013-48074-P, and the Catalan Government grant SGR 2014-274. The first author was funded by a grant from the CSIC JAE programme.

1. Introduction

Ozone (O_3) concentrations since 1994 in the Catalan Pyrenees have consistently exceeded the critical level (5000 ppb·h), target value (9000 ppb·h), and long-term objective (3000 ppb·h) for the protection of forest and semi-natural vegetation set by the CLRTAP/UNECE and the European Directive 2008/50/EC (Díaz-de-Quijano et al., 2012). An increase of the O_3 concentrations by a factor of five ($1.6\%\cdot y^{-1}$) has been observed in the Pyrenees from the end of the 19th century to the early 1990s (Marenco et al., 1994). O_3 concentrations increased significantly along an altitudinal gradient in the central Catalan Pyrenees, from annual averages of 35 ppb_v at 1040 m a.s.l. to 56 ppb_v at 2300 m a.s.l. for 2004-2007, but reaching 38 and 74 ppb_v, respectively, during the warm period (April-September) (Diaz-de-Quijano et al., 2009).

O_3 pollution in the Pyrenees is potentially detrimental to the natural vegetation and forests (Díaz-de-Quijano et al., 2012). High levels of O_3 pollution have caused typical O_3-induced injuries in studies in other European countries under controlled conditions (Gimeno et al., 2004; Manninen et al., 2003; Marzuoli et al., 2009; Paoletti et al., 2009; Penuelas et al., 1994; Ribas et al., 2005) or in the field (Calatayud et al., 2007; Cvitas et al., 2006; Vollenweider et al., 2003a; Waldner et al., 2007). Some forest trees and herbaceous species along the altitudinal gradient in the Pyrenees are also sensitive to O_3, e.g. *Fagus sylvatica*, *Pinus sylvestris*, and *Betula pendula* (Karlsson et al., 2003) and *Phleum alpinum*, *Leontodon hispidus*, *Valeriana officinalis*, *Silene acaulis*, and *Hieracium pilosella* (Hayes et al., 2007). O_3 detrimental effects on vegetation include physiological changes in leaves that eventually affect the amount of carbon available for growth and metabolic needs (Andersen, 2003). Since these effects differ among species in quality and magnitude, O_3 can alter plant interspecific competition giving place to shifts in community composition and losses of biodiversity (Wedlich et al., 2012).

The Mountain Pine (*Pinus uncinata* Ram.) is an autochthonous European species that dominate the subalpine forests in the central and eastern Pyrenees to 2400 m a.s.l. from 1600 to 1800 (depending on the area) (Burriel et al., 2004). Mountain pine forests play a key role in the central and eastern Pyrenees regarding timber production (between 190 000 and 215 000 m^3/year), the protective function against natural risks (floods, avalanches and erosion), biodiversity and landscape conservation, protection of threaten species (i.e. *Tetrao urogallus*), recreational uses and summer pastures (Coll et al., 2012). Nonetheless, the forest capacity to deliver these ecosystem services can be altered due to changes in their ecological function resulting from global change disturbances (Millar and Stephenson, 2015). The impacts of O_3 on these subalpine forests of *P. uncinata* in the Pyrenees and on the livelihoods of forest-dependent communities could have thus major ecological, economic, and social consequences.

The effects of O_3 on *P. uncinata* have been recently determined in several studies. Two-years old or older foliage of *P. uncinata* can develop diffuse light-green mottling characteristic of O_3 stress (Diaz-de-Quijano et al., 2011), similar to that reported in other pine species (Sanz and Calatayud, 2015). This diagnosis was confirmed experimentally (Diaz-de-Quijano et al., 2012b; Mortensen, 1994) and microscopically (Diaz-de-Quijano et al., 2011), with typical hypersensitive-like reactions underlying and causing the visible injury (Günthardt-Goerg and Vollenweider, 2007; Vollenweider et al., 2013). However, the extent of visible O_3 injuries to *P.*

uncinata stands in the Pyrenees and the general health of this tree species have not yet been determined.

The aims of this study were: 1) to evaluate the severity of visible O_3 injuries in two *P. uncinata* stands along an altitudinal gradient in the Pyrenees where O_3 concentrations have been monitored for several years, and 2) to assess crown defoliation and mortality as indicators of the health of the stands.

2. Materials and methods

2.1. Study area

The study area was in the county of La Cerdanya in the central Catalan Pyrenees (north-eastern Spain) (Fig. 1). This region is characterised by a mean annual temperature of 7.4 °C and a mean annual rainfall near 895 mm (climatic data for 1951-1999 from the Climatic Digital Atlas of Catalonia (CDAC)Ninyerola et al., 2000), corresponding to a Cfb climate of the Köppen-Geiger Classification System, defined as a temperate climate without a dry season (Agencia Estatal de Meteorología (España), 2011). Average monthly meteorological data for the study area for 1951-1999 are shown in Fig. 2.

We surveyed visible O_3-like injuries, crown defoliation, and tree mortality on altitudinal transects within two forest stands dominated by *P. uncinata* (Diaz-de-Quijano et al., 2009). One transect (Guils transect) had a north-eastern aspect and ranged from 1500 to 2200 m a.s.l., and the other transect (Meranges transect) had a southern aspect and ranged from 1700 to 2300 m a.s.l. Nine sites were distributed along both transects and were surveyed for visible O_3 injury (Fig. 1). Crown defoliation was surveyed in eighteen plots and tree mortality in sixty plots (the same eighteen plots as before plus forty-two new plots) distributed in six sites at altitudes ranging from 1500 to 2200 m (Fig 1). Each plot was 20×20 m and was separated by at least 30 m within homogenous, similarly oriented, and sloping parts of the stands and showed no signs of recent disturbance or sylvicultural treatment. In most cases, plots for crown defoliation and mortality sampling could not be located in the sites where visible foliar injury was assessed because O_3 passive sampling with their coresponding visible foliar injury sites did not always show the set of required characteristics just mentioned above.

2.2. Characterisation of site conditions

Topographic wetness indices were used to characterise spatial soil-moisture conditions at a catchment scale (Beven and Kirkby, 1979; O'Loughlin, 1986). These indices assume that topography plays a key role in controlling and modifying the hydrology at a hillslope scale (Grayson et al., 1999). We thus obtained a GIS-derived topographic index (topographic wetness index, TWI) that accounts for the contributing area of the catchment that drains into a given point and for the slope of the terrain, following the method by Galiano et al.(2010). The water availability during summer was estimated by the ratio of summer (July to September) total precipitation to average potential evapotranspiration (P/PET) for 1951-1999.

Soil depth was estimated by forcing a 130 cm steel rod into the soil to the bedrock and averaging readings from five locations. Estimates were obtained at the nine sites where visible O_3 injuries were assessed and at the plots used for the assessments of crown defoliation and tree mortality. Maximum water-holding capacity (MWHC) of the soil was estimated by dividing the mass of water retained after 24 h in a soil core to a depth of 20 cm by the dry mass of the soil.

We used passively sampled O_3 concentrations monitored at nine sites (Fig. 1) between 2004 and 2008 to calculate the derived O_3 variables. Five sites were located in the Guils transect (north-eastern aspect from 1500 to 2200 m a.s.l.) and four sites, in the

Meranges transect (southern aspect from 1700 to 2300 m a.s.l.). The sampling sites were located every 200 m in altitude in a forested area dominated by *Pinus uncinata* and in relatively accessible sites to facilitate fortnightly sampling(for further details about the sampling locations and procedure see Diaz-de-Quijano et al., 2009). Radiello radial symmetry passive samplers (Cocheo et al., 1996) were used to analyse O_3 at all sampling sites. Frequency of sampling was two weeks during the warm period (April to September) and once a month in the cold period (October to March). The derived O_3 variables comprised the average of the mean annual concentrations for 2005-2007, the average of the mean summer (April to September) concentrations for 2004-2008, and the accumulated sum of the mean fortnightly concentrations for 2004-2008. The average mean annual O_3 concentrations for 2005-2007 were selected for comparison with the O_3-induced injuries on the basis of previously identified correlations between these two estimates (Kefauver et al., 2014). We used the sum of mean fortnightly O_3 concentrations for 2004-2008 for comparison with estimates of crown defoliation and tree mortality. The mean O_3 concentrations from April to September for 2004-2008 were calculated in order to better characterize each site .

2.3. Assessment of the severity of visible O_3 injury

A total of 27 *P. uncinata* trees were examined for visible O_3 injury in May 2007. Three trees 1) close to the measuring station, 2) at least 2 m high, 3) with a diameter at breast height (DBH) >10 cm, and 4) with accessible and unshaded branches were selected at each of the nine sites equipped with O_3 passive samplers (Fig. 1; Díaz-de-Quijano et al., 2009). Outer and non-terminal branches with a minimum of five needle generations were sampled from the northern and southern sides of the trees at mid-canopy height and from the tree tops using a tree-pruning pole. The severity of visible O_3 injury (VI-sev) is one of the two scaled scorings of visual chlorotic mottling (VI), which is part of the Ozone Injury Index (OII) (Arbaugh et al., 1998; Duriscoe et al., 1996 and see http://www.fs.fed.us/psw/publications/documents/gtr-155/ for further details). VI-sev is calculated by estimating the average percentage of chlorotic mottling for all symptomatic needles and converting the estimates into a semi-quantitative variable with five grades of intensity (1:1-6, 2:7-25, 3:26-50, 4:51-75, and 5:>75%). A computer-generated chart with different percentage covers of chlorotic mottling was used to assess the VI-sev in order to reduce the source of personal error.

2.4. Assessment of crown defoliation and tree mortality

Crown defoliation and tree mortality were assessed in July 2008. Crown defoliation was estimated by a method slightly modified from that described in the UNECE/CLRTAP manual (UNECE/CLRTAP, 2006). We chose four subplots oriented along the main compass directions 8 m from the centre of each plot. The six trees nearest to the subplot centre were selected as sample trees, for a total of 24 sample trees per plot. Defoliation was estimated in 5% classes relative to a reference tree as suggested in the manual (UNECE/CLRTAP, 2006). The reference tree was a healthy tree with no defoliation, located in the lowest altitude site of the Meranges transect. It was representative of approximately 75% of the trees in this site. Ratings were averaged at the plot level. Tree mortality was assessed by counting the total numbers of live and dead trees and measuring the DBH of each tree. The tree density, percentage of dead trees, and total basal area (BA) were then calculated and averaged at the plot level.

2.5. Statistical analyses

General linear models were used to study the relationships between site characteristics and severity of visible injury, defoliation, and mortality. Parameters of the fitted models (β) were estimated using maximum likelihood. The selection of the model was based on a stepwise procedure using the Akaike information criterion (AIC). Data were transformed when needed to satisfy the assumption of normality (log(VI-sev), log(defoliation), log(mortality+1)). All analyses were performed with R, version 2.12.2 (2011, The R Foundation for Statistical Computing).

3. Results

3.1. Assessment of severity of visible O_3 injury

VI-sev ranged between 1 and 2 on the Guils transect and 1.4 and 3.2 on the Meranges transect (Table 1). VI-sev was higher in sites at higher altitude (Table 1) on both transects, but the average VI-sev was lower on the Guils (mean±SE of 1.26±0.2) than the Meranges (mean±SE of 2.08±0.4) transect. The final model for VI-sev fitted using stepwise model selection is shown in Table 2. The interactions between the explanatory variables in the model were significant. A higher VI-sev was thus associated with higher [O_3] only when summer P/PET was >0.96. Individual relationships of VI-sev with summer P/PET and mean annual [O_3] for 2005-2007 are shown in Fig. 3.

3.2. Defoliation and tree mortality

A summary of the defoliation and tree mortality grouped by altitude are shown in Table 3. Defoliation ranged between 20 and 66% and was generally higher on the Guils than the Meranges transect. Defoliation and tree mortality also clearly tended to increase with altitude on the Guils transect (Table 3). This pattern was not as clear on the Meranges transect, where the mid-altitude site had the highest defoliation. Tree mortality increased with altitude on both transects but was higher on the Guils transect, ranging between 1-30 and 1-7.5% on the Guils and Meranges transects, respectively. The Guils transect generally had clearer increasing trends with altitude and higher defoliation and mortality than the Meranges transect (Table 3). The Guils transect also had wetter conditions than Meranges, as indicated by the generally higher values for the variables associated with site water availability (e.g. topographic wetness index, soil depth, MWHC) (Table 3).

Both defoliation and mortality were mostly affected by the sum of the mean fortnightly [O_3] for 2004-2008 but were also associated with the explanatory variables defining site water availability and stand characteristics (Table 2). Increases in defoliation and mortality were associated with higher accumulated exposures to O_3 and with higher water availability, which was represented by MWHC for defoliation and by the topographic wetness index and summer P/PET for mortality (Figs. 4 and 5). Both defoliation and mortality showed the highest values above a threshold of sum of fortnightly [O_3] of 2900 ppb. Defoliation increased abruptly above an MWHC threshold of 0.58 g $H_2O\cdot g$ soil^{-1}, and mortality increased above a threshold of 12.5 of the topographic wetness index. Stand basal area was negatively correlated with defoliation in the defoliation model, although only marginally, whereas mean DBH was positively correlated with mortality.

4. Discussion

4.1. Dependence of VI-sev on summer P/PET and mean annual [O_3] for 2005-2007

The effects of O_3 on vegetation depend on the amount of O_3 entering the leaves and the plant’s sensitivity to O_3 (Matyssek et al., 2008). O_3 uptake is highly influenced by the availability of soil moisture, because it directly affects stomatal conductance (Nunn et al., 2005; Patterson et al., 2000; Schaub et al., 2007, 2003). Soil-water availability may also be one of the most important site factors influencing the response of trees to O_3 stress (Lefohn et al., 1997; Ollinger et al., 1997; Vollenweider et al., 2003a, 2003b). This influence is in agreement with our results showing that the severity of visible O_3 injury increased with increasing [O_3] under situations of relatively high summer P/PET (>0.96). Stomatal conductance, and the consequent O_3 uptake, were likely high under high summer P/PET. The lower VI-sev with increasing [O_3] under conditions of low summer P/PET could similarly be due to lower stomatal conductances under a certain level of water availability. Under a situation of low water availability, O_3 uptake will remain low and cause fewer injuries even if atmospheric [O_3] is high.

Visible O_3 injury could thus be much better predicted using a stomatal flux-based model that includes the factors influencing stomatal conductance and the specific hourly [O_3] at each site. More effort should thus focus on characterising the hourly [O_3] at each site and the micro-environmental conditions that affect stomatal conductance, which are usually influenced by local topography and stand structure. This would certainly permit to better analyse the relationship between visible O_3 injury and the specific environmental conditions at each site. The mean percentage of the area of all symptomatic needles with chlorotic mottling at each site was ≤30% (VI-sev score of 3.22), but visible injury could have appeared much later than below-ground responses to O_3, and negative effects on a cellular and histological level may have already begun (Andersen, 2003; Laurence and Andersen, 2003).

4.2. Higher crown defoliation and tree mortality associated with higher accumulated O_3 exposure and water availability

The mean values of crown defoliation between 20 and 66% at our study sites were not surprising, because the defoliation of *P. uncinata* crowns increased in the Iberian Peninsula from 15 to 25% between 1996 and 2006 (Carnicer et al., 2011). The rate of mortality followed the same pattern as defoliation, being higher at those sites with higher defoliation. The average mortality rate for all sites was 9.19%, which is similar to the 6% for 1997-2007 for the same species throughout the Iberian Peninsula (Carnicer et al., 2011). In fact, several studies have reported significant correlations between deteriorating crown conditions and tree mortality (Dobbertin and Brang, 2001; Drobyshev et al., 2007; Eckmullner and Sterba, 2000). The high crown defoliation and tree mortality, with defoliation >25% considered to be indicative of poor tree health (Innes, 1998), show that the stands of *P. uncinata* in our study generally had poor vitality.

Crown defoliation and tree mortality were correlated most with the accumulated O_3 exposure during the last five years and with variables characterising soil-water availability. Plant responses to O_3 depend on the amount of O_3 entering the leaves and the plant’s sensitivity to O_3 (Matyssek et al., 2008). The amount of O_3 entering the leaves is mainly affected by the atmospheric O_3 concentration and by the stomatal conductance (Ro-Poulsen et al., 1998), which is controlled by a range of environmental variables such as light intensity, temperature, vapour-pressure deficit, and soil-water availability (Zierl, 2002). Soil-water availability subsequently affects O_3 uptake by plants (Nunn et al., 2005; Panek and Goldstein, 2001; Patterson et al., 2000; Schaub et al., 2007, 2003). The higher defoliation and mortality at our sites with higher soil-water availabilities and accumulated O_3 exposures could thus be due to higher uptakes of O_3. In effect, the Guils transect, which was significantly wetter than the Meranges transect, had the most crown defoliation and tree mortality.

We could not, however, identify O_3 exposure as the main causing factor of crown defoliation and subsequent tree mortality. Crown assessment based on crown defoliation is one of the best indicators of tree vitality (Dobbertin, 2005), but tree vitality is influenced by a multitude of stress factors (meteorological (e.g. air temperature and frost), hydrological (e.g. droughts and floods), biological (e.g. fungal disease and insects), chemical (e.g. air or soil pollution and soil nutrients), and physical (e.g. wind)) (Aamlid et al., 2000; De Vries et al., 2000; Landmann and Bonneau, 1995; Wellbum, 1994; Zierl, 2002). Hence, O_3 exposure cannot be established as the main cause of crown defoliation and tree mortality in our study: a multitude of other environmental or anthropogenic stresses difficult to detect and quantify could also be contributing to the poor tree vitality. Further research should be thus conducted in order to determine the contribution of other stress factors as well as to diminish the sources of uncertainty. Hourly measurements of $[O_3]$ at each site would supply more precise data on O_3 exposure than sum of mean fortnightly $[O_3]$. The use of this kind of data would diminish the uncertainties entailed by the use of mean fortnightly $[O_3]$ measured by passive sampling and it could probably help to better disentangle the relationship between tree vitality and O_3 exposure.

5. Conclusions

This study on the severity of visible O_3 injury, crown defoliation, and tree mortality along two altitudinal and O_3 gradients in stands of *P. uncinata* in the Catalan Pyrenees indicates that O_3 contributes in part to the reduced tree vitality in this region. The severity of visible O_3 injuries increased with mean annual $[O_3]$ when summer P/PET was above a threshold of 0.96, whereas higher $[O_3]$ in drier conditions did not cause more visible O_3 injury. Crown defoliation and tree mortality were positively correlated with the accumulated O_3 exposure during the last five years and with variables associated with soil-water availability, which suggests a likely higher uptake of O_3, because soil-water availability highly influences stomatal conductance. The effect of O_3 could not, however, be established conclusively and definitively as the main cause of the crown defoliation and tree mortality in our study, because a multitude of other stress factors could also be contributing to the poor tree vitality. We can nonetheless conclude that O_3 is probably one of the factors involved in the crown defoliation and tree mortality in this area, although further research is clearly warranted to determine the contributions of the various other stress factors.

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Table 1. Description of sites assessed for severity of visible ozone injury along the Guils and Meranges transects. Numbers in parentheses are standard errors of the means.

Sites	Latitude	Longitude	Altitude (m a.s.l)	Aspect	Slope (º)	Topographic Wetness Index	Summer P/PET	Soil depth (cm)	MWHC (g H_2O·g soil^{-1})	Mean annual [O_3] 2005-2007 (ppb)	Severity of visible injury
Guils								*n=5*	*n=3*		*n=3*
G1	42.458532	1.877621	1500	NE	25	12.74	0.69	50.6(6.9)	0.312(0.04)	46.1	1(0.0)
G2	42.460940	1.864956	1700	NE	15	11.58	0.76	36.4(8.8)	0.416(0.01)	47.2	1(0.2)
G3	42.458108	1.856287	1800	NE	15	11.37	0.85	65.8(5.6)	0.436(0.08)	53.7	1.1(0.3)
G5	42.458333	1.842645	2000	NE	5	13.37	0.92	68.4(3.9)	0.635(0.12)	53.9	1.2(0.2)
G6	42.462582	1.808833	2200	NE	2	11.44	1.12	56(10.0)	0.662(0.02)	50.9	2(0.5)
Meranges											
M1	42.452438	1.792290	1700	SW	42	8.42	0.91	42.6(4.5)	0.286(0.04)	46.9	1.4(0.3)
M2	42.456236	1.789355	1900	SE	30	9.68	1.02	21.0(0.5)	0.467(0.05)	50.8	1.5(0.5)
M4	42.464095	1.785139	2100	S	5	9.55	1.09	30.4(3.4)	0.644(0.07)	54.7	2.1(0.4)
M6	42.465586	1.778331	2300	SE	35	9.71	1.18	42.0(5.2)	0.687(0.03)	62.1	3.2 (0.4)

Table 2. General linear models for severity of visible injury, defoliation, and mortality. The data for the dependent variables were normalised by log transformation.

Model term	β	SE	*p*
Severity of visible injury (VI-sev) model			
Intercept	19.427	5.136	<0.05
Mean annual $[O_3]$ 2005-2007	-0.418	0.105	<0.05
Summer P/PET	-18.769	4.619	<0.01
Mean annual $[O_3]$ 2005-2007*Summer P/PET	0.436	0.093	<0.01
Defoliation model			
Intercept	-0.486	0.772	0.538
Sum of mean fortnightly $[O_3]$ 2004-2008	$6.93\cdot10^{-4}$	$3.06\cdot10^{-4}$	<0.05
Basal area	$-4.2\cdot10^{-3}$	$1.98\cdot10^{-3}$	<0.1
Maximum water-holding capacity	0.537	0.265	<0.1
Mortality model			
Intercept	-2.110	2.317	<0.001
Sum of mean fortnightly $[O_3]$ 2004-2008	$5.21\cdot10^{-3}$	$9.05\cdot10^{-4}$	<0.001
Topographic wetness index	0.379	0.114	<0.01
Summer P/PET	1.871	0.751	<0.05
Mean DBH	0.095	0.047	<0.1

A stepwise model selection was used starting from the set of variables in Table 1 (for the VI-sev model) and Table 3 (for the defoliation and mortality models). Only the final models are shown. AICvi-sev=-16.65, AICdefoliation=-68.95, AICmortality=-137.66.

Sites	Number of plots	Altitude (m a.s.l.)	Topographic wetness index	Summer P/PET	Soil depth (cm)	MWHC (g H_2O·g soil^{-1})	Individuals· ha^{-1}	DBH	Basal area	Mean [O_3] April-Setember 2004-2008 (ppb)	Sum of fortnightly [O_3] 2004-2008 (ppb)	Defoliation (%)
Guils												
G6	3	2211(1.45)	13.09(0.39)	1.12(0.00)	41.2(14.4)	0.618(0.03)	850(281)	19.4(3.7)	32.2(11.8)	50.9	2953	66.4(15.8)
G4	3	1867(15.0)	11.56(1.50)	0.90(0.02)	63.8(6.5)	0.381(0.08)	2083(563)	18.8(4.5)	66.6(21.1)	49.8	2919	32.4(3.7)
G1	3	1535(7.3)	10.64(0.34)	0.72(0.00)	53.7(5.1)	0.312(0.06)	1416(448)	15.4(1.8)	32.9(4.9)	48.7	2886	36.8(3.4)
Meranges												
M5	3	2231(12.2)	11.29(0.73)	1.13(0.00)	54.6(22.1)	0.481(0.15)	2191(700)	18.9(1.3)	67.4(12.6)	56.5	2926	29.8(8.1)
M3	3	1998(1.45)	10.30(0.72)	0.99(0.01)	45.4(17.2)	0.308(0.00)	2225(651)	14.8(1.5)	44.7(8.0)	51.6	2759	35.2(11.0)
M1	3	1797(3.52)	10.93(0.78)	0.86(1.49)	39.4(3.6)	0.286(0.07)	1133(14)	18.8(1.2)	37.6(5.4)	47.3	2615	20.4(5.4)
												Mortality (%)
Guils												
G6	10	2213(3.88)	12.92(0.29)	1.12(0.00)	41.2(8.2)	0.618(0.03)	887(453)	19.1(3.0)	33.0(15.3)	50.9	2953	29.6(15.1)
G4	10	1869(30.69)	11.92(1.05)	0.90(0.02)	63.8(4.6)	0.381(0.08)	1997(599)	17.9(2.9)	59.5(15.6)	49.8	2919	15.2(8.6)
G1	10	1536(8.49)	10.82(0.25)	0.72(0.00)	53.7(2.9)	0.312(0.06)	1502(346)	15.8(2.1)	36.5(6.1)	48.7	2886	1.48(2.5)
Meranges												
M5	10	2228(26.88)	11.17(0.60)	1.13(0.00)	54.6(12.7)	0.481(0.15)	2350(585)	18.5(1.2)	71.1(13.6)	56.5	2926	7.5(6.0)
M3	10	2009(20.83)	10.37(0;60)	0.99(0.01)	45.4(9.9)	0.308(0.00)	2110(364)	15.9(1.2)	50.5(10.1)	51.6	2759	0.8(1.2)
M1	10	1793(19.92)	11.16(0.71)	0.86(0.00)	39.4(2.0)	0.286(0.07)	1557(887)	17.5(1.6)	42.3(17.4)	47.3	2615	0.6(1.9)

Table 3. Mean (standard deviation) values of the variables defining plot conditions distributed along six sites.

Figure captions

Fig. 1. Location of the two transects at La Cerdanya in the Central Catalan Pyrenees of Spain. The sites of assessment of visible ozone injury (VI), crown defoliation (tree icon), tree mortality (tree icon), and O_3 concentrations (O_3)(Diaz-de-Quijano et al., 2009) are indicated. Distribution of the eighteen plots of crown defoliation (three plots per site) and the sixty plots of tree mortality (ten plots per site) are not visible in the figure.

Fig. 2. Averaged accumulated rainfall (bars) and mean temperatures (lines) from January to December for 1951-1999 (data from the Climatic Digital Atlas of Catalonia (CDAC)(Ninyerola et al., 2000).

Fig. 3. Correlation between the severity of visible injury (VI-sev) and summer P/PET (log VI-sev = 0.9*P/PET-0.7; $p<0.001$; $R^2=0.88$) and mean annual [O_3] for 2005-2007 (log VI-sev = 0.03*(mean annual [O_3] 2005-2007)-1.5; $p<0.05$; $R^2=0.59$). Datapoints represent observations at plots from both the Guils and Meranges transects ($n=9$).

Fig. 4. Correlation between defoliation and MWHC ($\text{Defoliation}=169.3*\text{MWHC}^2-71.7*\text{MWHC}+35.4$; $p<0.01$; $R^2=0.46$) and the sum of mean fortnightly [O_3] for 2004-2008 ($\text{Defoliation}=0.079e^{0.0021\text{SumOzone}}$; $p<0.05$; $R^2=0.41$). Datapoints represent observations at plots from both the Guils and Meranges transects ($n=18$).

Fig. 5. Correlation between mortality and the topographic wetness index ($\text{Mortality}=5.5*\text{TWI}^2-118.2*\text{TWI}+640.8$; $p<0.001$; $R^2=0.66$) and the sum of mean fortnightly [O_3] for 2004-2008 ($\text{Mortality}=5\cdot10^{-4}*\text{Sum0408}^2-2.8*\text{Sum0408}+3804.8$; $p<0.001$; $R^2=0.46$). Datapoints represent observations at plots from both the Guils and Meranges transects ($n=60$).