Ozone stomatal flux-based critical levels translated into real-world forest impacts

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Introduction

Mediterranean forests are at the highest ozone (O_3) risk in Europe where ground-level O_3 is a pressing sanitary problem for ecosystem health. To protect vegetation, current European standards use the O_3 exposure index AOT40. O_3 effects on vegetation depend not only on the ambient air O_3 concentrations but results from the balance between internal O_3 dose, i.e. O_3 uptake through the stomata into leaves or needles, (stomatal flux) and detoxification and repair processes in the leaves.

A standard for forest protection is considered biologically relevant when it translates into real-world forest impacts. For this reason, detecting real plant damages in the field is necessary, to derive **new stomatal flux-based CLef** (stomatal flux-based critical level) for forest protection.

Three ozone indices, namely the accumulated exposure AOT40, and the accumulated stomatal flux with and without an hourly threshold of uptake (POD1 and POD0) were compared. Stomatal O_3 fluxes were modelled (DO3SE) and correlated (Spearman test) to forest-response indicators, i.e. crown defoliation, crown discoloration and visible foliar O_3 injury. **The aim of this study was** (1) to evaluate the robustness of O_3 risk indicators and (2) to suggest new O_3 critical levels for Mediterranean forest protection.

Methodology

Assessment of visible foliar ozone injury

Visible O_3 injury is usually the first unequivocal sign of the presence of phytotoxic levels of O_3. This innovative epidemiological assessment (ICP-Forest protocol) of forest responses to O_3 was carried out in South-eastern France (30 plots) and North-western Italy (24 plots) in 2012 and 2013. Each tree was assessed for leaf or needle injury to evaluate (1) the percentage of leaves/needles loss (**crown defoliation**), (2) the proportion of discolored leaves/needles (**crown discoloration**) and (3) the percentage of leaves/needles surface affected by O_3-induced symptoms (**visible foliar O_3 injury**).

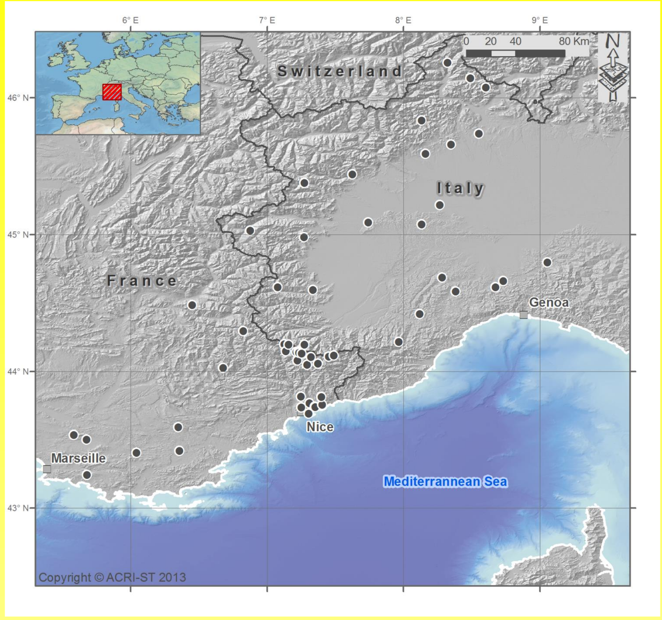


Figure 1: Location of experimental plots in South-eastern France and North-western Italy

AOT40 calculation

The O_3 exposure index AOT40 was calculated as sum of the exceedances above 40 ppb, for daylight hours (08:00 to 20:00 CET) during the assumed growing season: **AOT40 = Σ max ((C-40),0).dt** where C is hourly O_3 concentration (ppb) and *dt* is the time step (1 h).

Phytotoxic Ozone Dose calculation

$$\mathrm{POD}Y = \int_{i=1}^{n} \max\left((Fst - Y), 0\right) dt$$

PODY i.e. "**Phytotoxic O_3 Dose**" ($mmol.m^{-2}$) is the accumulated stomatal O_3 uptake above a detoxification threshold Y ($nmolO_3.m^{-2}$ $PLA.s^{-1}$) calculated from hourly values of F*st* (stomatal O_3 flux), *n* denotes the number of hours to be included in the calculation period and *dt* = 1h.

The leaf-level stomatal conductance for water vapor (g_{sw}) was estimated from the **Jarvis' multiplicative model** where g_{sw} is estimated from functions describing the response of stomata to key species-specific and environmental variables: $g_{sw} = g_{max} * [\min(f_{phen}, f_{O3})] * f_{light} * \max\{f_{min}, (f_{temp} * f_{VPD} * f_{SWP})\}$.

PODY was estimated for two thresholds Y = 1 (UNECE, 2010) **and 0** (any O_3 molecule entering into the leaf induces a metabolic response, Musselmann et al., 2006). PODY was calculated for **daylight hours** (8am-8pm, for daylight hours with a **global radiation exceeding 50 Wm^{-2}** (UNECE, 2010) or **exceeding 0 Wm^{-2}** over the 24-h exposure period of time.

Table 1: Parameters used in the stomatal flux-based model, according to UNECE (2010)

Parameter	*Mediterranean Europe*	*Continental Central Europe*	
	Pinus halepensis	*Conifers*	*Deciduous*
g_{max} [mmol m^{-2} s^{-1}]	215	200	200
$light_a$ [dl]	0.013	0.010	0.006
T_{opt} [°C]	27	14	16
T_{min} [°C]	10	0	5
T_{max} [°C]	38	35	33
VPD_{min} [kPa]	3.2	3.0	3.1
VPD_{max} [kPa]	1.0	0.5	1.0
f_{min} [mmol m^{-2} s^{-1}]	0.15	0.16	0.13
SGS	1st January	1st April	1st April
EGS	Time of the survey	Time of the survey	Time of the survey

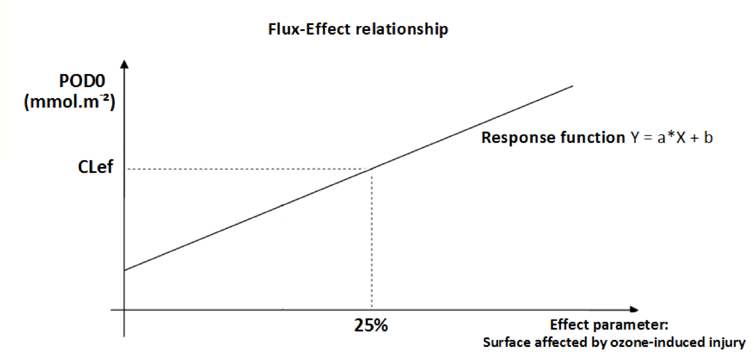
Statistical analysis

The Spearman's rank correlation test is a non-parametric statistical test, based on ranked variables, to measure the statistical dependence between two variables. The test is robust and suitable for non-normally distributed data with missing and extreme values and can be applied to a small number of observations.

Derivation of critical levels

From the **Flux-Effect function**, statistically significant at $p < 0.05$, we derived stomatal flux-based critical levels (CLef). The CL was rounded up or down to the nearest whole number. For the visible O_3 injury, damage to the tree is considered to occur when the surface of needles/leaves affected exceeds 25%.

Meteorological data, soil data and O_3 concentrations were obtained from **the WRF-CHIMERE modelling system** (1-h temporal resolution and a spatial resolution of 6x6km).



In Piedmont region, we used the available parameterization for continental deciduous broadleaf forests at 19 plots (*Fagus sylvatica, Quercus cerris, Q. petraea, Fraxinus excelsior, Fraxinus ornus, Robinia pseudoacacia*) and for continental conifers at 5 plots (*P. cembra, P. sylvestris, Abies alba and Picea abies*).

In South-eastern France, we used parameterization for continental conifers in 18 high-lying sites (*P. cembra, P. sylvestris and Abies alba*) and specific parameterization for *P. halepensis* at 12 sites in Mediterranean area.

Results and discussion

Comparison of the exposure and flux-based approaches

PODY was better correlated with the severity of O_3-induced symptoms whereas AOT40 was better correlated with discoloration and defoliation, i.e. typical aspecific indicators caused by multiple factors (tree species, forest management, meteorology, soil characteristics, water limitation…). The responses of vegetation to O_3 depend not only on atmospheric O_3 concentrations but also on the absorbed O_3 uptake through stomata, detoxification and repair processes into the leaves (Paoletti et al., 2008).

Table 2: Spearman coefficients and *p*-value for correlations between crown discoloration, crown defoliation, visible foliar ozone injury for conifers (one year old needles C+1; two year old needles C+2) and AOT40, POD0 and POD1, calculated over different time windows: for hours with a global radiation exceeding 0 $W.m^{-2}$ (A), 8-20h (B) and for hours with a GR exceeding 50 $W.m^{-2}$ (C). $p < 0.01$***; $0.01 < p < 0.05$**; $0.05 < p < 0.1$*; $p > 0.1$: non-significant (ns)

	AOT40	A_POD0	A_POD1	B_POD0	B_POD1	C_POD0	C_POD1
Conifers							
Discoloration	0.6463 ***	0.4149 ***	0.3021 **	0.4083 ***	0.3195 ***	0.3794 ***	0.2278 *
Defoliation	0.7170 ***	ns	ns	ns	ns	ns	ns
O_3 visible injury C+1	ns	0.4412 ***	0.3784 ***	0.3686 ***	0.3360 ***	0.3641 ***	0.3824 ***
O_3 visible injury C+2	ns	0.4646 ***	0.4076 ***	0.3748 ***	0.3561 ***	0.3672 ***	0.4035 ***
Pinus cembra							
Discoloration	ns	0.4532 **	ns	0.3110 *	0.3866 **	0.3903 **	ns
Defoliation	0.4945 ***	ns	ns	ns	ns	ns	ns
O_3 visible injury C+1	ns	0.5912 ***	0.3143 *	0.3808 **	0.3531 *	0.4663 **	0.3255 *
O_3 visible injury C+2	ns	0.5652 ***	ns	0.3457 *	0.3349 *	0.4447 **	0.3061 *
Pinus halepensis							
Discoloration	0.3075 *	ns	0.3853 *	ns	ns	ns	ns
Defoliation	0.3389 **	ns	ns	ns	ns	ns	ns
O_3 visible injury C+1	ns	0.4120 **	0.3900 *	ns	ns	ns	0.3831*
O_3 visible injury C+2	ns	0.6067 **	0.6207 ***	0.5426 **	0.5771 **	0.5751 **	0.5859 **
Pinus sylvestris							
Discoloration	0.7427 **	ns	ns	ns	ns	ns	ns
Defoliation	0.3120 *	ns	ns	ns	ns	ns	ns
O_3 visible injury C+1	ns	0.3833 *	0.3667 *	ns	ns	ns	ns
O_3 visible injury C+2	ns	0.4500 *	ns	ns	ns	ns	ns

Analysis of the best threshold

The flux-response relationships were strongest when there was no threshold (POD0) as compared to POD1. The sensitivity of PODY to the inputs typically increases with the value of the threshold (Tuovinen et al., 2007). As a consequence, it is desirable to keep thresholds as low as possible from a robustness point of view. The results suggest that any O_3 molecule entering into the leaf induces a metabolic response and confirm the work carried out by Musselmann et al. (2006), using no detoxification threshold.

Analysis of the best time window

The robustness of the time window, used in the dose-response approaches, was evaluated. The flux-response relationships were strongest when POD0 was calculated for hours with a global radiation exceeding 0 Wm^{-2} during the growing season. Since ground-level O_3 can remain high at night and stomata are partially open at night, a 24-h O_3 exposure period of time should be used for flux-based O_3 approaches. The two other approaches underestimated the actual ozone dose.

Derivation of CLef

For O_3 sensitive conifers, a CLef of 22 $mmol.m^{-2}$ PLA for *P. cembra* (high O_3-sensitivity) and 28 $mmol.m^{-2}$ PLA for *P. halepensis* (moderate O_3-sensitivity) were calculated. Regarding broadleaved species, we obtained a CLef of 24 $mmol.m^{-2}$ PLA for *Fagus sylvatica* (moderate O_3-sensitivity) and 19 $mmol.m^{-2}$ PLA for *Fraxinus excelsior* (high O_3-sensitivity).

This **innovative study** provided useful information for establishing the best standards and thresholds for forest protection to O_3. Derivation of the new flux-based critical levels, for Mediterranean tree species, represents **considerable progress** in the development of methods for quantifying effects of O_3 on vegetation at the regional scale.

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