An epidemiological assessment of stomatal ozone flux-based critical levels for visible ozone injury in Southern European forests

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Background & Context

Surface ozone (O_3) is the **most worrying** air pollutant, with harmful effects on human health, crops & forests in Europe (e.g. Paoletti, 2006; Mills et al., 2011, WHO, 2013) and may become worse in the future.

In southern Europe, surface O_3 levels are high enough to induce adverse effects in the field (Sicard et al., 2013).

The calculation of vegetation-relevant metrics, over a region, estimates the relative severity of O_3 exposures.

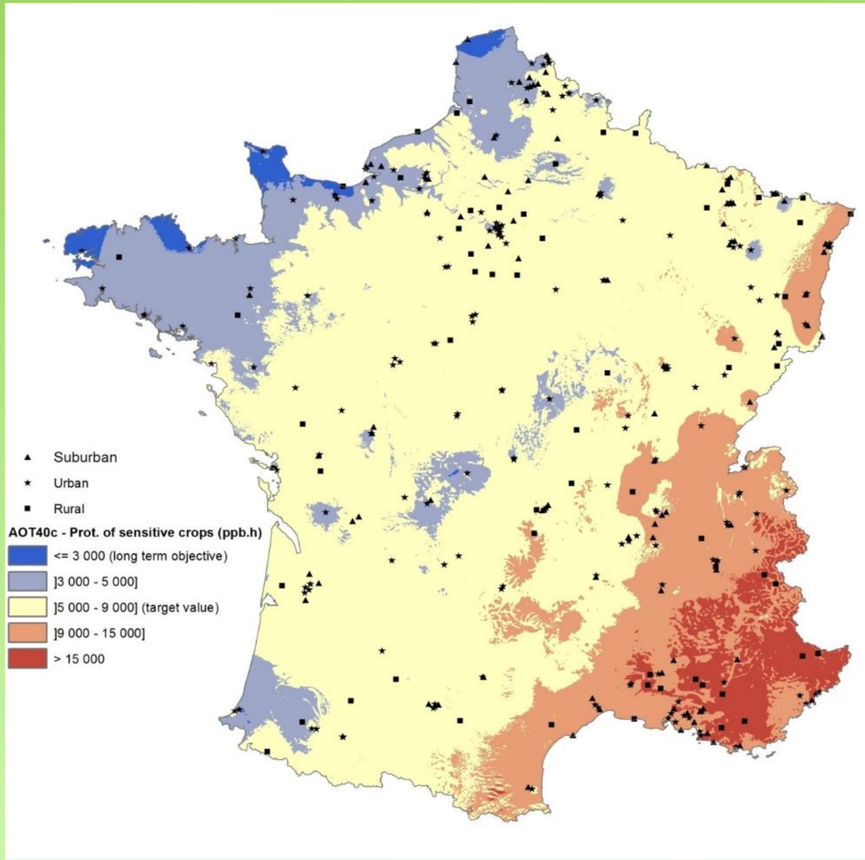
However, the current exposure-based standards for protecting vegetation are **not representative** of actual field conditions. A biologically-sound stomatal flux-based standard was proposed, although critical levels (CL) for protection still need to be validated.

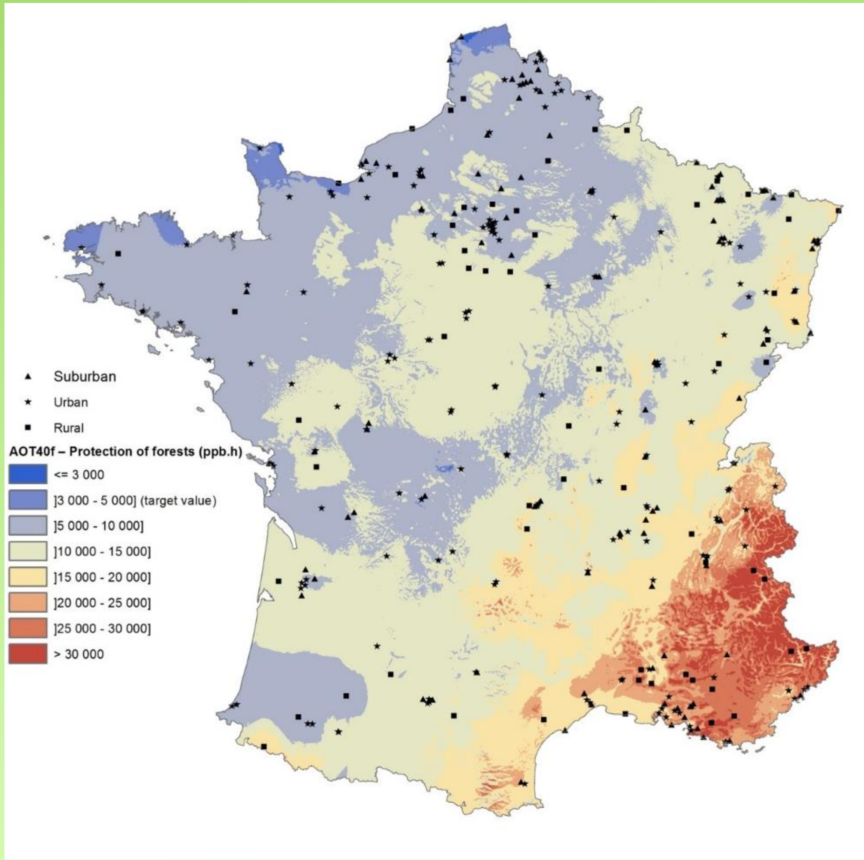
By a **large-scale field investigation** in Southern Europe, **the main objectives are** :

- To evaluate the performance of O_3 risk metrics, i.e. POD0, POD1 and AOT40.
- To define which threshold Y is the most biologically-based.
- To define the best time-window of PODY accumulation.
- To suggest new epidemiologically-based O_3 CLs for forest protection against visible O_3 injury.

Background & Context

Surface O_3 levels in the **South-eastern France are higher** than in the rest of Europe and represent a **potential threat** to vegetation (*see Poster session*).





Figures 1: **Spatial distribution** (**by local interpolation)** of surface ozone metrics in France based on 332 stations over the time period 1999-2012: AOT40 for agricultural crops (left) and forests (right).

Description of the works

A standard for forest protection is biologically relevant when it translates into real-world forest impacts.

To derive new stomatal flux-based critical levels (CLef), stomatal O_3 fluxes were modelled & correlated to real plant damages (visible injury).

Main steps:

(1) In field campaigns - 2 years : **visible injury** (visible foliar O_3 injury, crown defoliation & discoloration) were evaluated. In agreement with the European protocol defined by the ICP-Forests & carried out by 2 trained **experts**.

(2) Meteorological data, soil data and O_3 concentrations were obtained from the coupled **WRF-CHIMERE** modelling system & Stomatal O_3 fluxes were modelled (**DO3SE**).

(3) Three O_3 indices, i.e. the accumulated exposure AOT40 & the accumulated stomatal flux with & without an hourly threshold of uptake (POD1 & POD0) were correlated to measured forest-response indicators.

Location of experimental plots

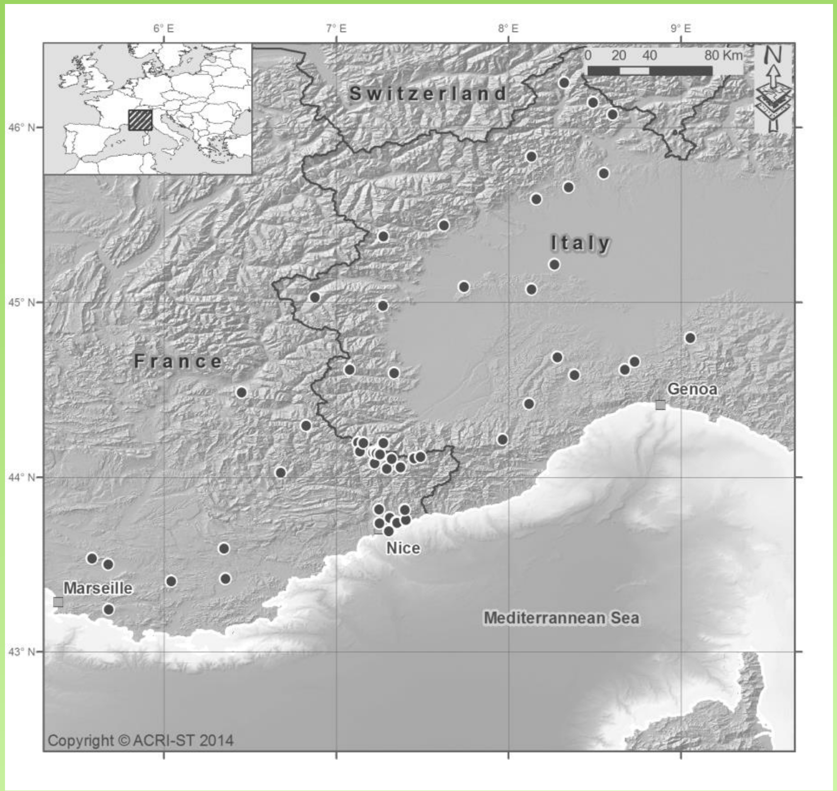


Figure 2: Location of experimental plots in South-eastern France and North-western Italy

<u>North-western Italy</u> (24 plots)

Broadleaved trees: *Fraxinus excelsior*, *Robinia pseudoacacia*, *Fagus sylvatica*, *Quercus petraea*, *Q. cerris)* + Conifer species: *P. sylvestris, Picea abies, Abies alba* and *P. cembra*.

<u>South-eastern France</u> (30 plots)

Focused on 2 O_3-sensitive conifer tree species: *Pinus halepensis* and *Pinus cembra*.

O_3 produces specific symptoms on needles of *P. cembra & P. halepensis:* **valuable bioindicator species** for O_3 stress.

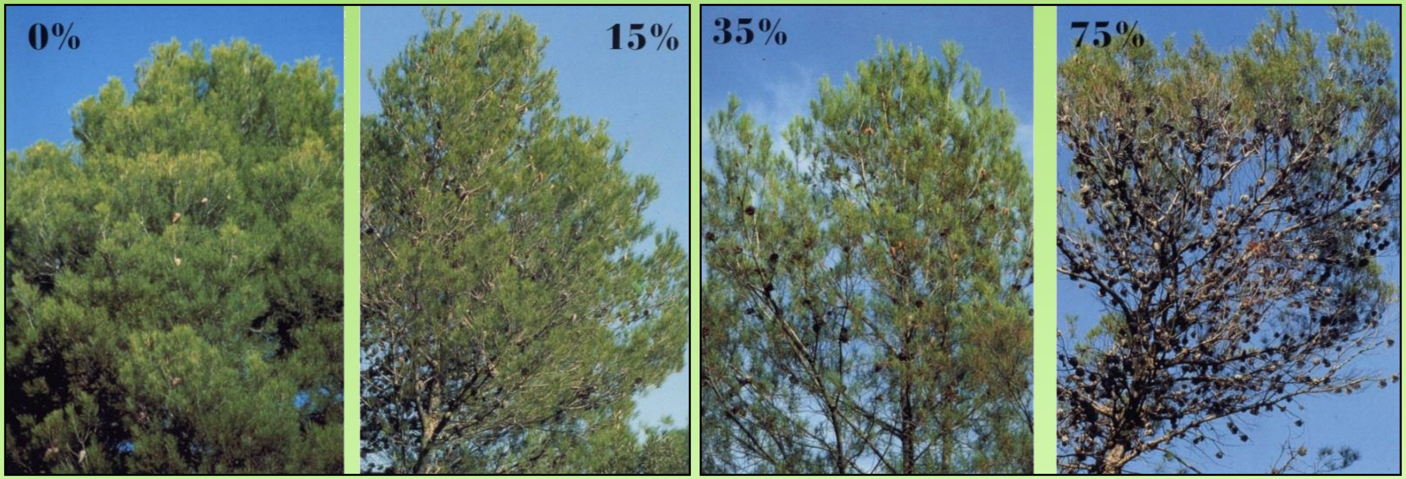
In-field campaigns: visible injury

The survey was based on **1080 trees** over 54 plots (20 trees per site).

Discoloration: to estimate the global color with 5 scorings: 0 (green) to 4 (yellow tree).

Crown defoliation: Assessed in 5% steps (0-10%: healthy, 15-25%: warning phase, 30-60%: alert phase).

Pinus halepensis



In Europe, forest monitoring has concentrated on crown defoliation & discoloration as indicators of forest **health and vitality**, while visible foliar O_3 injury is usually the **first unequivocal marker** of O_3 phytotoxic levels (Grulke, 2003; Matoušková et al., 2010).

Campaigns for O_3-induced injury

For broadleaf plants

Symptoms observed and scored on 5 trees & 5 branches (30 leaves):

- Red or brown spots - Interveinal **stipple**
- **Chlorosis**: loss of chlorophyll
- **Flecking**: brown or black areas on the upper surface (death of cells)

Fagus sylvatica

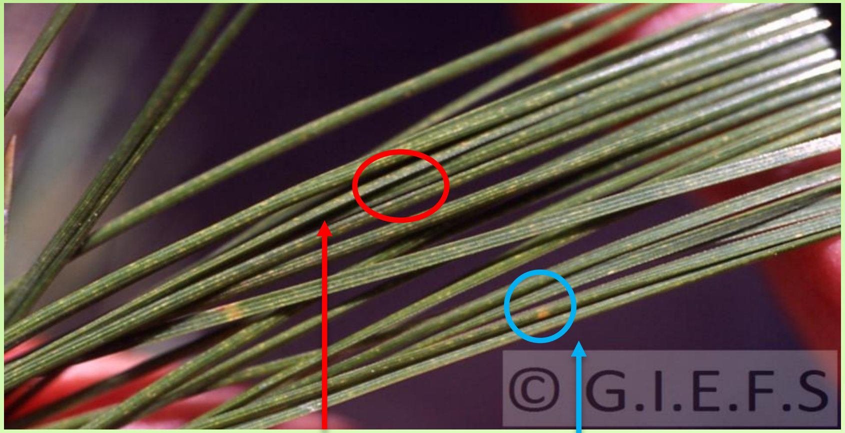
Bronzing + stippling

For conifers

5 trees & 5 branches (30 needles) are removed from the upper third of the crown layer.

- **Tipburn** : death of cells (red or brown)
- **Chlorotic mottle**: discrete patches of yellow tissue on needles

Pinus halepensis

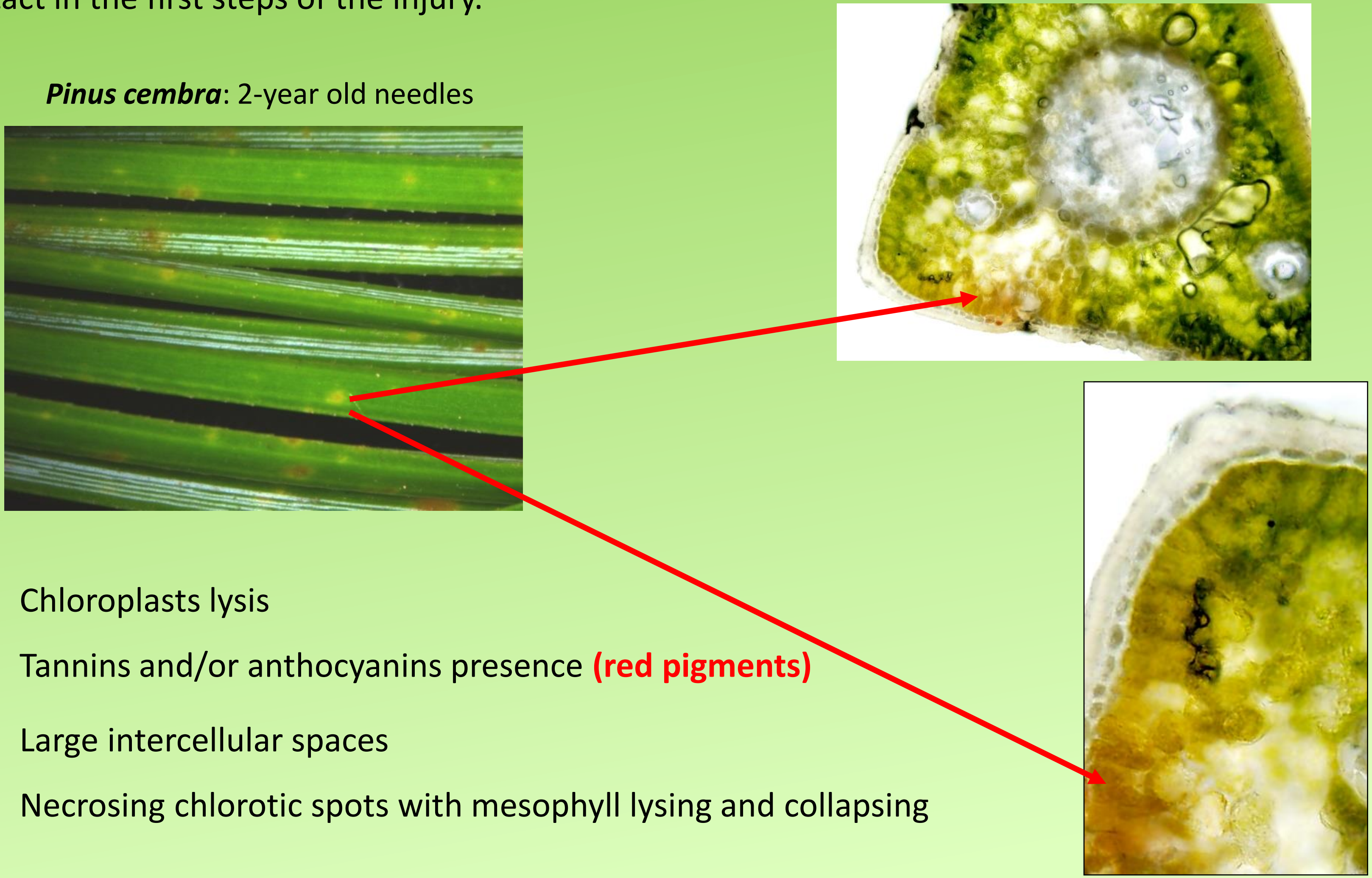


Mottling (diffuse) + insect

Microscopical analysis

First visible effects of O_3 appear on the palisade mesophyll chloroplasts. Epidermis and spongy mesophyll remain intact in the first steps of the injury.

Pinus cembra: 2-year old needles



- ✓ Chloroplasts lysis
- ✓ Tannins and/or anthocyanins presence **(red pigments)**
- ✓ Large intercellular spaces
- ✓ Necrosing chlorotic spots with mesophyll lysing and collapsing

Symptomatology

Our results allowed ranking the tree species on the basis of their **sensitivity to ambient O_3**.

For conifers species:

P. cembra can be considered as highly O_3-sensitive species
P. halepensis = moderate O_3-sensitivity species
P. sylvestris = low O_3-sensitivity species
Abies alba & *P. abies* were not impacted and may be classified as O_3-tolerant species

For broadleaved species:

F. excelsior = highly O_3-sensitive species
F. sylvatica & *R. pseudoacacia* = moderate O_3-sensitivity species
Quercus species (i.e. *Q. cerris* & Q. petraea) = O_3-tolerant species

F. sylvatica is sometimes considered as a highly sensitive species (e.g. Nunn et al., 2002; Deckmyn et al., 2007).

Estimation of AOT40

Estimation of AOT40

Sum of the exceedances above 40 ppb over the daylight hours during the growing season (UNECE, 2010):

$$AOT40 = \int \max\left((C - 40), 0\right) dt$$

where C is hourly O_3 concentration (ppb) and *dt* is the time step (1h).

Accumulating evidence suggests that the responses of vegetation to O_3 is more related to the **absorbed dose**, through stomata, than exposure.

Estimation of PODY – DO3SE model

Estimation of PODY: DO3SE model

PODY ($nmolO_3.m^{-2}.s^{-1}$): accumulated stomatal ozone uptake above a species-specific threshold Y:

$$PODY = \int (POD - Y) \cdot dt$$

DO3SE model was applied with 2 thresholds ($nmolO_3.m^{-2}.PLA.s^{-1}$):

- 1 as recommended by UNECE (2010)
- 0 - Any O_3 molecule entering into leaves may induce a metabolic response (Musselmann *et al.*, 2006)

DO3SE model was applied for 3 time windows :

- Hours from **08:00 to 20:00 CET**, a practical definition adopted by the Directive 2008/50/CE
- Hours with a **global radiation > 50 $W.m^{-2}$**, as recommended by UNECE (2010)
- Hours with a **global radiation > 0 $W.m^{-2}$**, assuming that stomata open even with < 50 $W.m^{-2}$ light (Launiainen et al., 2013; Elhaddad et al., 2014)

Estimation of PODY – DO3SE model

Leaf-level stomatal conductance to water vapour (g_{sw}) was estimated using the multiplicative model (Emberson *et al.*, 2000) and the parameters suggested in UNECE (2010):

$$g_{sw} = g_{max} \cdot f_{phen} \cdot f_{light} \cdot \max\{f_{min}, (f_{temp} \cdot f_{VPD} \cdot f_{SWC})\}$$

Soil water content = a key variable affecting the severity of visible foliar O_3 injury was included in DO3SE.

Species-specific parameterization

Table 2: 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

Spearman test: coefficients

AOT40 significantly correlated with aspecific symptoms, i.e. crown discoloration and crown defoliation.

AOT40 not correlated with visible foliar O_3 injury.

Specific O_3-induced symptoms are **only** correlated with PODY.

POD0 better correlated with visible O_3 injury than POD1 and AOT40.

Stronger coefficients obtained with POD0 calculated for hours with a global radiation **exceeding 0 W.m^{-2}**.

POD0 and POD1, calculated over different time windows: **8am-8pm (A)**and for hours with a global radiation exceeding **50 W.m^{-2} (B)** and exceeding **0 W.m^{-2} (C)** over the 24h exposure period of time.

	AOT40	A_POD0	A_POD1	B_POD0	B_POD1	C_POD0	C_POD1
All species							
Discoloration	0,3921 ***	0.3339 **	0.2494 **	0.3827 **	0.2488 **	0.3562 **	0.2427 **
Defoliation	0.4391 ***	0.3493 **	0.2444 **	0.3695 **	0.2383 **	0.3390 **	0.2245 **
O_3 visible injury	ns	0.4236 ***	0.3142 ***	0.3596 ***	0.3432 ***	0.4958 ***	0.3590 ***
Conifers							
Discoloration	0.6463 ***	0.3783 ***	0.3195 ***	0.3794 ***	0.2278 *	0.4149 ***	0.3021 **
Defoliation	0.7170 ***	ns	ns	ns	ns	ns	ns
O_3 visible injury C+1	ns	0.3786 ***	0.3360 ***	0.3641 ***	0.3824 ***	0.4412 ***	0.3784 ***
O_3 visible injury C+2	ns	0.4048 ***	0.3561 ***	0.3672 ***	0.4035 ***	0.4646 ***	0.4076 ***
Pinus cembra							
Discoloration	ns	0.3110 *	0.3866 **	0.3903 **	ns	0.4532 **	ns
Defoliation	0.4945 ***	ns	ns	ns	ns	ns	ns
O_3 visible injury C+1	ns	0.3408 **	0.3331 *	0.4663 **	0.3255 *	0.5912 ***	0.3143 *
O_3 visible injury C+2	ns	0.3857 *	0.3549 *	0.4447 **	0.3061 *	0.5652 ***	ns
Pinus halepensis							
Discoloration	0.3075 *	ns	ns	ns	ns	ns	0.3853 *
Defoliation	0.3389 **	ns	ns	ns	ns	ns	ns
O_3 visible injury C+1	ns	ns	ns	ns	0.3831*	0.4120 **	0.3900 *
O_3 visible injury C+2	ns	0.5426 **	0.5771 **	0.5751 **	0.5859 **	0.6067 **	0.6207 ***
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	ns	ns	ns	ns	0.3833 *	0.3667 *
O_3 visible injury C+2	ns	0.3950 *	ns	ns	ns	0.4500 *	ns
Broadleaves							
Discoloration	ns	ns	ns	ns	ns	ns	ns
Defoliation	0.3587 **	ns	ns	0.2793 *	0.2874 *	0.2862*	ns
O_3 visible injury	ns	0.3233 *	ns	ns	ns	0.3600 **	ns

$p < 0.01$***; $0.01 < p < 0.05$**; $0.05 < p < 0.1$*; $p > 0.1$: non-significant (ns)

Derivation of Critical Levels

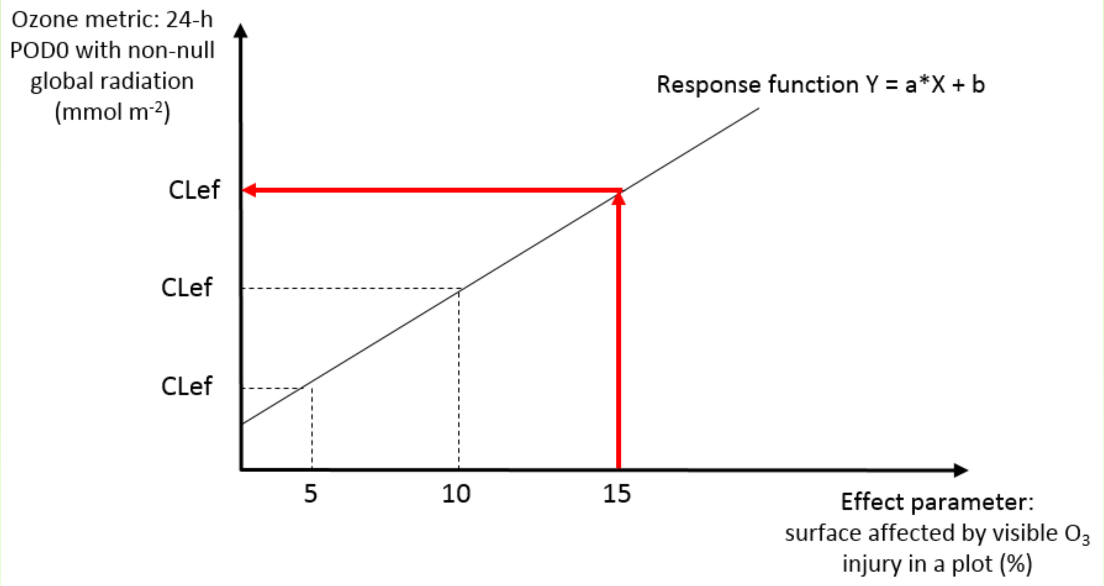
On the basis of data from controlled-condition experiments, the UNECE calculated AOT40- and PODY-based critical levels for growth and yield reductions of several crop and forest species. Similarly, **we correlated PODY & AOT40 to real-world forest impacts** in terms of different effect parameters.

Based on previous results, we showed that POD0 (for hours with a non-null solar radiation) was strongly correlated with visible O_3 injury → **Flux-effect functions**.

New **species-specific CLef** were derived from flux-effect functions, statistically significant (p < 0.05) by joining data from all plots and years.

Hoshika et al. (2012) showed that gs decreased sharply above **5% injury** and did not change any more above 15% injury.

CLef was derived from flux-effect functions for **5, 10 and 15%** of visible foliar O_3 injury.



Recommended flux-based critical levels (CLef) for forest protection against visible O_3 injury

Recommended CLef, established with different thresholds of visible injury, and percentages of plots that can be protected using the CLef values.

Tree species	CLef (mmol m^{-2} B_POD0)			Response function	r	*p* value	Percentage of protected plots		
	5%	10%	15%				5%	10%	15%
All species	**20.0**	**21.0**	**22.0**	Y = 0.19*X + 19.1	0.51	< 0.01	45	53	74
Conifers	**20.8**	**22.4**	**23.9**	Y = 0.31*X + 19.3	0.44	< 0.01	48	58	80
Pinus cembra	**15.9**	**17.3**	**18.7**	Y = 0.28*X + 14.5	0.60	< 0.01	25	45	62
Pinus halepensis	**26.5**	**29.2**	**31.9**	Y = 0.54*X + 23.8	0.59	< 0.05	70	81	92
Broadleaves	**18.4**	**19.1**	**19.9**	Y = 0.15*X + 17.6	0.38	< 0.05	71	740	90
Fagus sylvatica	**21.6**	**23.4**	**25.2**	Y = 0.36*X + 19.8	0.56	< 0.05	50	66	79
Fraxinus excelsior	**16.9**	**17.7**	**18.6**	Y = 0.16*X + 16.2	0.61	< 0.05	52	60	76

To **maximize** the percentage of protected sites, CLef is derived from flux-effect functions with 15% as a threshold of visible foliar O_3 injury per plot.

As an example, we proposed:

CLef of **32 mmol.m^{-2} PLA** for moderate O_3-sensitive species (e.g. *P. halepensis*)
CLef of **19 mmol.m^{-2} PLA** for high O_3-sensitive species (e.g. *P. cembra*)

CLef of **25 mmol.m^{-2} PLA** for moderate O_3-sensitive species (e.g. *Fagus sylvatica*)
CLef of **19 mmol.m^{-2} PLA** for high O_3-sensitive species (e.g. *Fraxinus excelsior*)

Conclusions & Perspectives

We showed that the performance of POD0, as a descriptor of O_3 risk to forests, is better than POD1 and AOT40 when visible foliar O_3 injury is considered.

For **forest protection against visible O_3 injury**, we recommend the **use of POD0** calculated for hours with a non-null solar radiation over 24-h. **Nocturnal O_3 levels** will be higher in the future (*see Poster session*).

In the short-term, AOT40 should be replaced with the flux concept & POD0 has both **biological significance** and **practicality** in usage.

As a **main conclusion**, we propose to use generic epidemiologically-based CLef, i.e. **20 & 24 mmol.m^{-2}** for broadleaved species & conifers, respectively. Eventually, a species-specific CLef based on the O_3-sensitivity.

To date, CL were derived from controlled-condition experiments and from **biomass loss** as plant parameter (e.g. UNECE, 2010) while visible foliar O_3 injury & crown symptoms, under field conditions, were used here.

The presence of foliar injury does not always coincide with measurable biomass losses due to O_3 (Chappelka and Samuelson, 1998). Therefore, we recommend that a **large-scale epidemiological investigation**, similar to our approach, is also applied to biomass losses as measured in real-world forests.

Спаси́бо за приём

Thanks