

Adapting Mediterranean forests to climate change and air pollution

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The ozone story: from simplicity to complexity to mystery



Epidemiological study may help to clarify the story

Epidemiology of ozone injury

- Epidemiology is the study of the patterns, causes, and effects of health and disease conditions in defined populations, to identify risk factors and targets for preventive healthcare. Epidemiology has helped developing methodology used in clinical research, public health studies and, to a lesser extent, basic research in biological sciences (see biomonitoring)
- Epidemiological investigations where large-scale biological responses are compared with ambient data in the field may provide useful information for establishing the best standards and thresholds for protecting plants from O₃
- Epidemiology of ozone injury may be very helpful in particular when forests are investigated, as large trees require expensive experimental facilities for realistic ozone simulation and a few individuals can be usually investigated
- The majority of previous epidemiological assessments used ambient O₃ exposure as a metric of injury (e.g. Arbaugh et al 1998, Karlsson et al 2006, Braun et al 2007, McLaughlin et al 2007, Bussotti & Ferretti 2009, Baumgarden et al 2009, Fishman et al 2010, Sun et al 2012, Kefauer et al 2013)

Epidemiology of exposure-based ozone injury



Ponderosa and Jeffrey pine

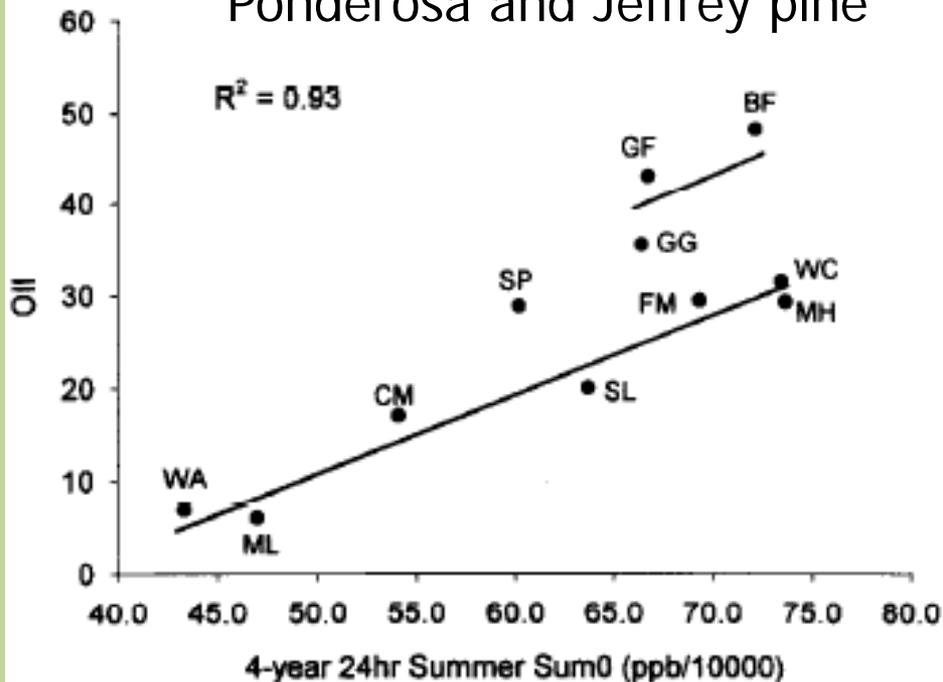


Fig. 5. Four-year cumulative SUM0 predictions of Ozone injury index (OII). Circles are observed values, and the lines represent predicted values. The upper line represents three sites that had >90% of trees injured, and the lower line sites with <90% of trees injured. See references.

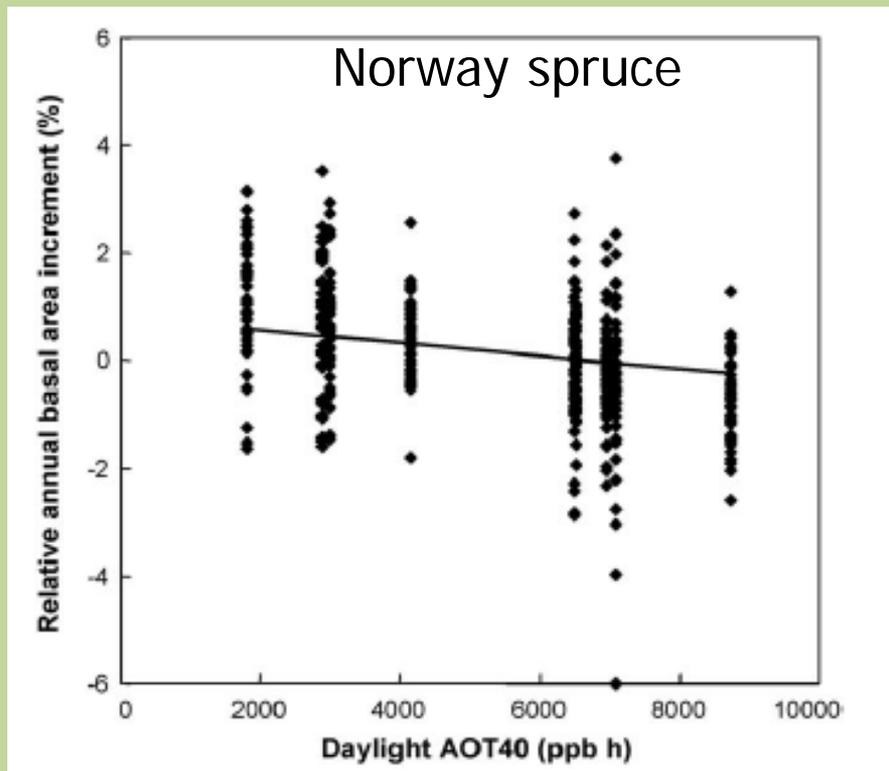


Fig. 3. A component-plus-residuals plot for the modelled relative annual basal area increment at the Asa Experimental Forest, Sweden in relation to the ozone exposure index AOT40. The plot shows the residuals around a line $\beta_r(x_{r,i} - x_{median,r,i})$, where β_r is the estimated value of its regression coefficient. It illustrates the relative magnitude of the residuals with respect to the explanatory power of the variable AOT40.

(Arbaugh et al. 1998)

(Karlsson et al. 2006)

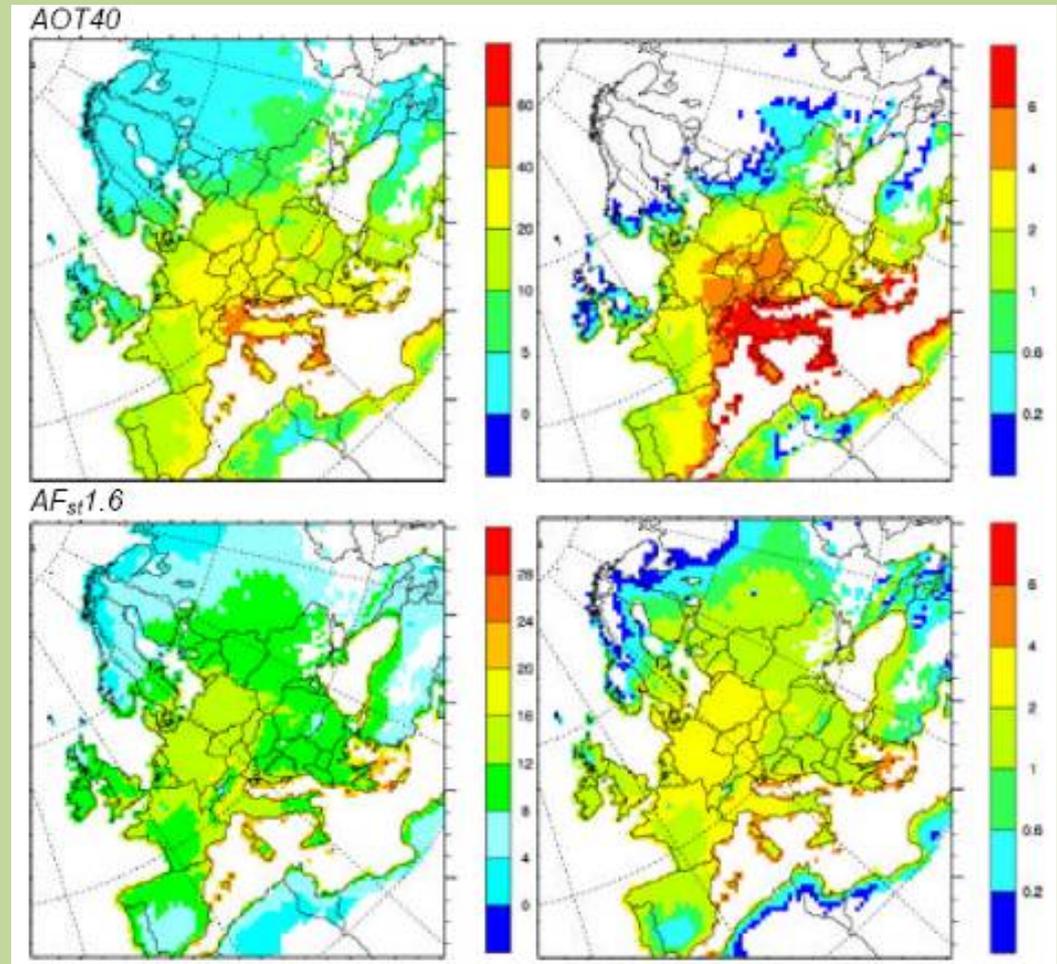
Stomatal ozone fluxes and epidemiology of forest injury

Phytotoxic Ozone Dose

$$POD_y = \sum [(gsto [O_3] CF)] - y$$

$$gsto = gmax * [\min (fphen, fO_3) * flight * \max [fmin (ftemp, fVPD, fSWC)]$$

DO₃SE model (Emberson et al 2000, Jarvis 1979)



Fagus sylvatica (Tuovinen et al. 2007)

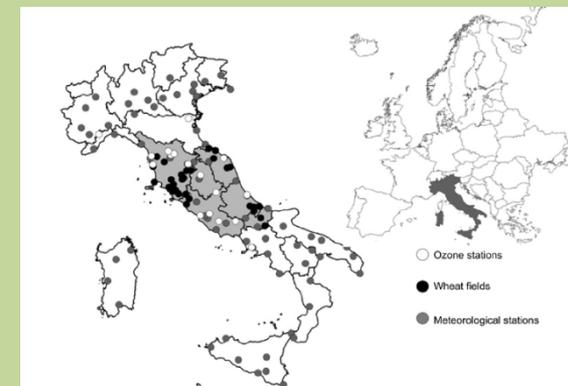
Epidemiology of yield reduction in durum wheat

Percent of wheat annual yield variability that was explained by environmental variables in a stepwise multiple linear regression over the three months of durum wheat growing season in central Italy, April–June, 2000–2004. The model was run for each of the six ozone standards for wheat protection that were selected in this study, i.e. AOT40EC; AOT40UNECE; accumulated stomatal O₃ flux; NAAQSO₃; W126M-S; W126A-J; and environmental parameters (period 2000–2004). See Table 1 for explanation of metrics. Only variables that correlated with yield ($p < 0.1$, Table 2) were included in the model.

Variables	Ozone standard in the regression model					
	AOT40EC	AOT40UNECE	Stomatal O ₃ flux	NAAQSO ₃	W126M-S	W126A-J
Total precipitation	21.97***	21.97***	21.97***	21.97***	21.97***	21.97***
AOT40EC	0.54 ns					
AOT40UNECE		rm				
Stomatal O ₃ flux			0.84 ns			
NAAQSO ₃				0.12 ns		
W126M-S					0.08 ns	
W126A-J						rm
Daily solar radiation average	rm	rm	0.08 ns	rm	rm	rm
Daily soil water content average	0.40 ns	0.40 ns	0.08 ns	0.40 ns	0.40 ns	0.40 ns
Diurnal air temp. average	0.16 ns	0.15 ns	0.22 ns	0.15 ns	0.15 ns	0.15 ns
Diurnal air RH average	rm	rm	0.06 ns	rm	rm	rm
Multiple R ²	0.23	0.23	0.23	0.23	0.23	0.23
F	14.61	19.28	11.52	14.26	14.09	18.99
N	200	203	235	200	198	200
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Level of significance at each model step: ***, $p < 0.0001$; ns, $p > 0.1$; rm, removed from the model because of F to enter < 0.1 ; empty spaces show the variables that were not included in a model.

Precipitation explains most of the regression variance, as wheat in central Italy is not irrigated. Several ozone indices were tested. The best one was stomatal flux. Overall the ozone indexes explained ab. 5% of total variance.



De Marco A., Screpanti A., Paoletti E.: 2010, **Geostatistics as a validation tool of ozone standards for durum wheat protection.**

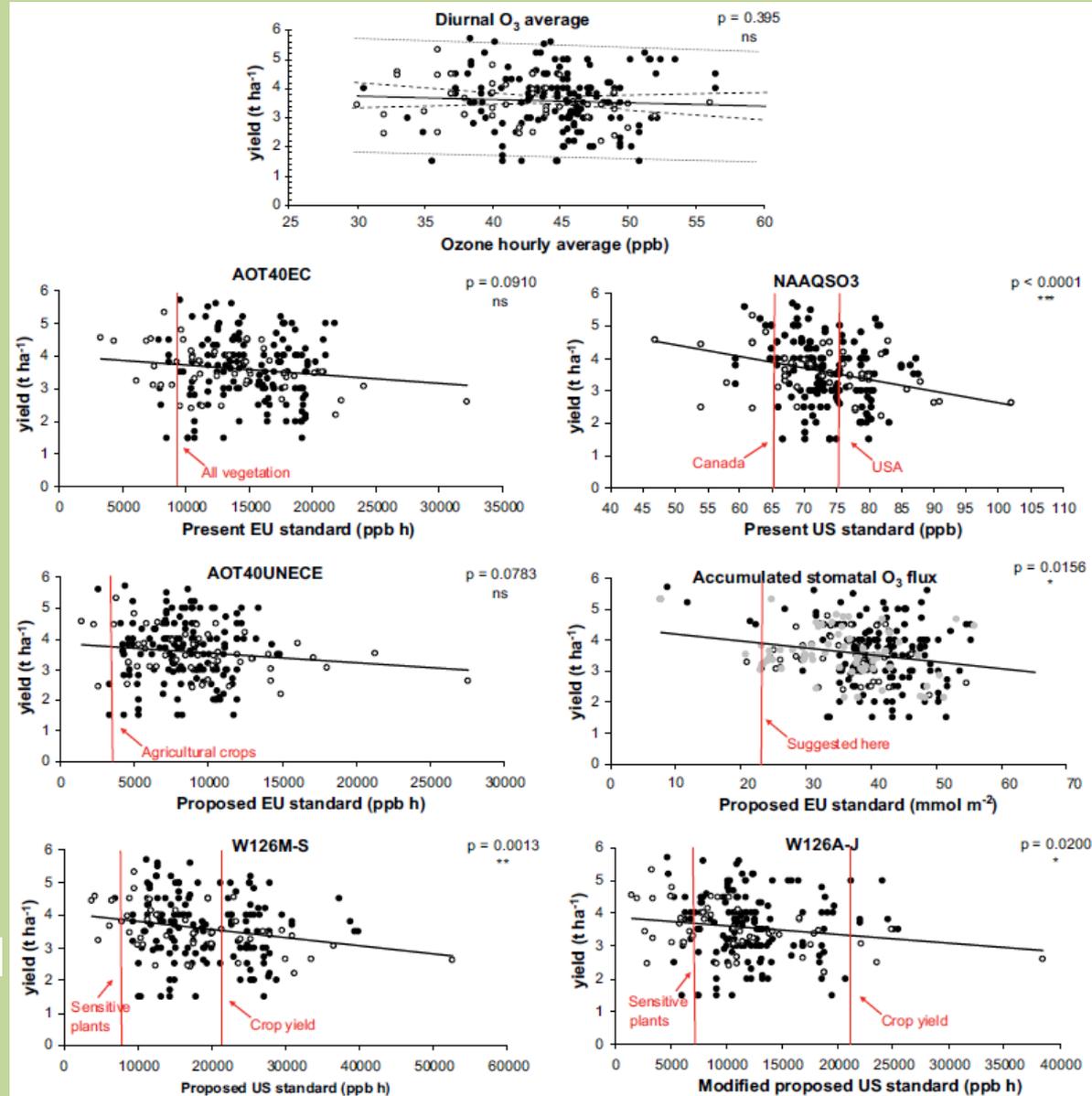
Environmental Pollution 158: 536–542

Selection of the best standards

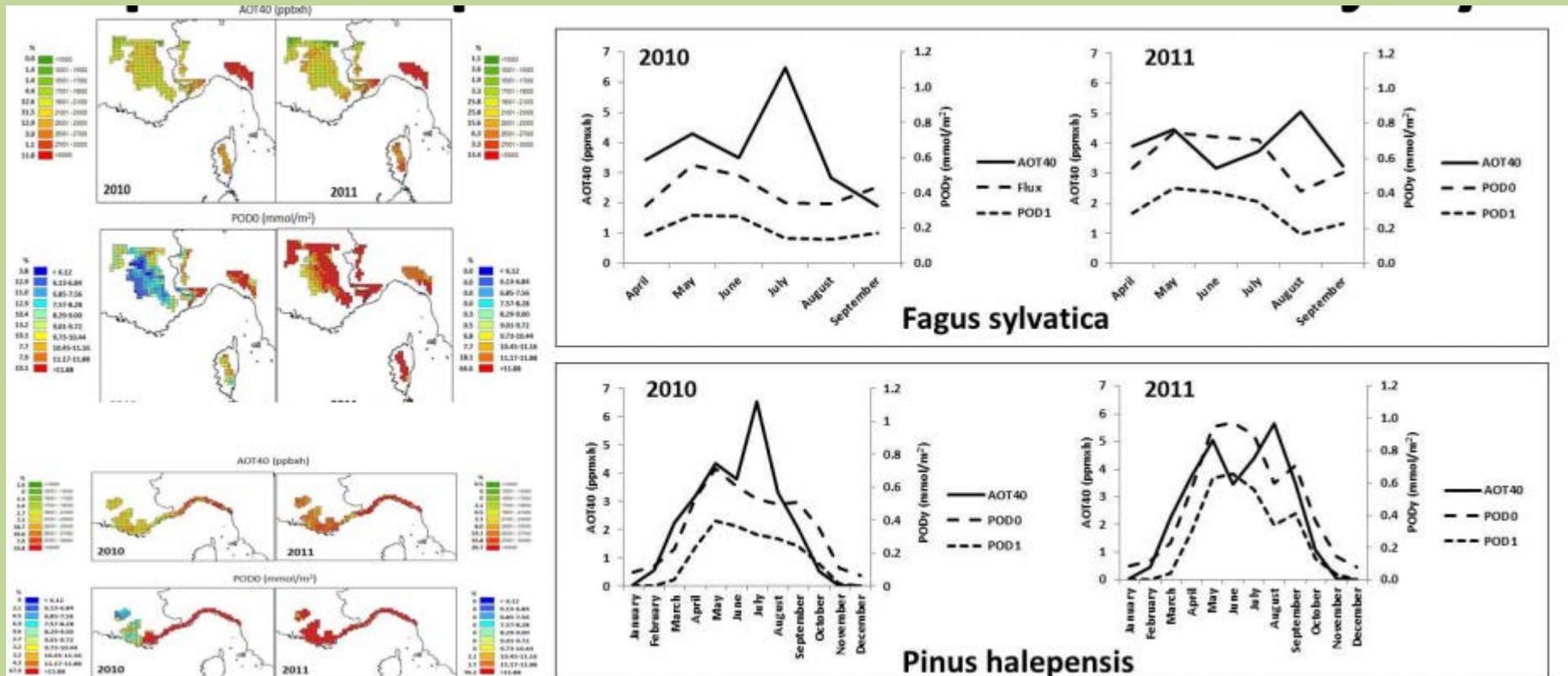
Triticum durum Creso cv: 45 ppb (ambient) decreased yield by 10.1% relative to the pre-industrial concentration (10 ppb) [-18% in a meta-analysis on *Triticum aestivum*, Feng et al. 2008]

USA standard explained yield decline better than EU standards, although the legal threshold for protections in the USA (75 ppb) protected only 39% of sites. AOT40-based EU standards protected >90% of sites. Canadian standard (65 ppb) protected 91%. A flux-based critical level of 22 mmol m⁻² would protect 97% of sites.

A. De Marco et al. / Environmental Pollution 158 (2010) 536–542

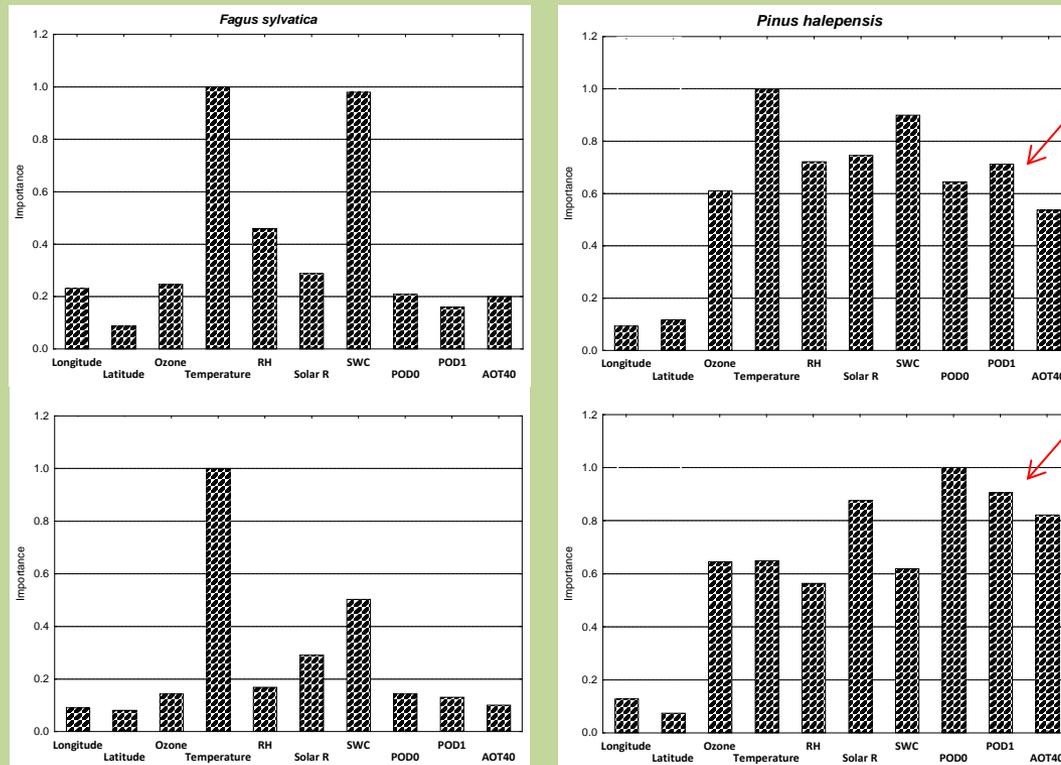


Testing Canopy Moisture Content as a plant response indicator of O₃ injury



- ✓ Both AOT40 and POD1 exceeded the critical levels in the entire domain
- ✓ AOT40 overestimated O₃ risk as compared to PODY
- ✓ The use of AOT40 significantly changed ozone risk assessment for vegetation relative to PODY, while no spatial and temporal differences occurred when using POD1 rather than POD0

Testing Canopy Moisture Content as a plant response indicator of O₃ injury



- CMC response to ozone was species-specific, being negligible in *F. sylvatica* and significant in *P. halepensis*, and was affected by complex linear and non-linear interactions with other ecological drivers. AOT40 has an importance slightly lower than POD

Visible injury vs ozone metrics

In **FO₃REST**, stomatal O₃ fluxes were modelled & correlated to real-world forest impacts in terms of visible injury to define more realistic thresholds for vegetation protection.

Activities

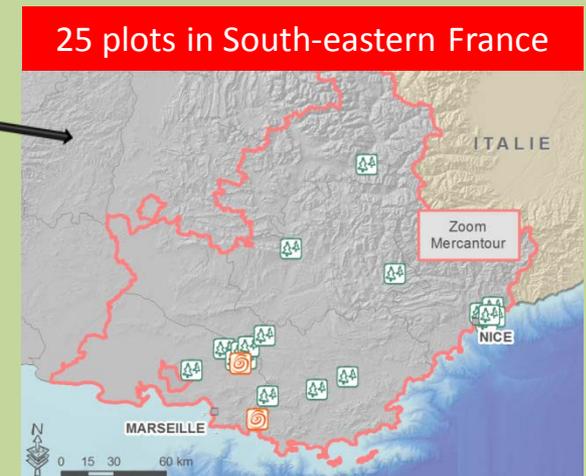
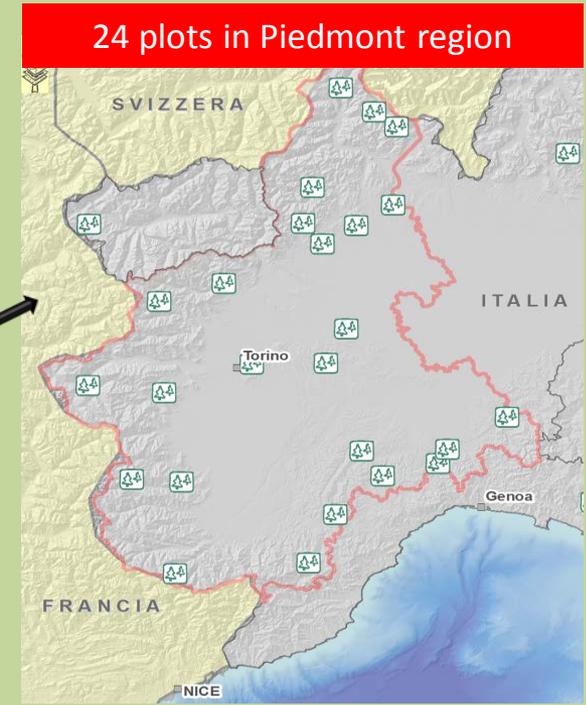
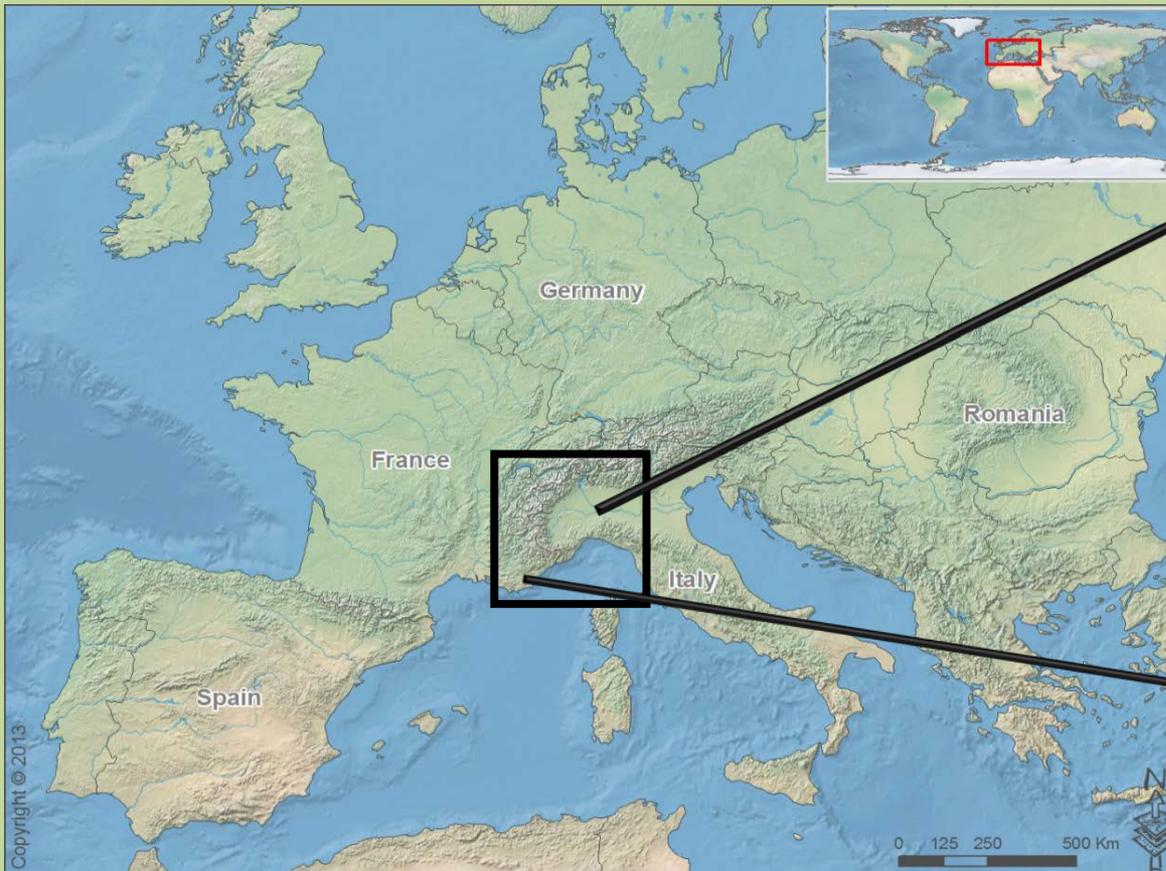
In field campaign (2012 and 2013) for **O₃ visible injury** (stippling/mottling, crown discoloration & leaf loss) evaluation in agreement with ICP Forest methodology

France: 14 plots with *Pinus cembra* & 11 with *Pinus halepensis*.

Italy: 19 Deciduous & 5 Conifers.

Meteorological data, soil data and O₃ concentrations were obtained from the coupled **WRF-CHIMERE** modelling system.

Location of experimental plots



Distributed at different altitudes & main ecological zones to consider climate impact on the symptoms occurrence.

In-field ozone-induced visible injury assessment



Estimation of PODY

Estimation of PODY: DO3SE model

PODY (nmolO₃.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 hourly thresholds:

- 1 nmolO₃.m⁻².PLA.s⁻¹ as recommended by UNECE (2010), *but now changing to 2 nmolO₃.m⁻².PLA.s⁻¹*
- 0 nmolO₃.m⁻².PLA.s⁻¹ - Any O₃ molecule entering into leaves may induce a metabolic response (Musselmann *et al.*, 2006)

DO3SE model was applied for:

- Whole day (when solar radiation >0)
- Day hours from 08:00-20:00 (CET)
- Day hours with a global radiation > 50 W.m⁻²

Estimation of PODy

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):

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

Species-specific parameterization

Parameter	<i>Mediterranean Europe</i>			<i>Continental Central Europe</i>	
	<i>F. sylvatica</i>	<i>P. halepensis</i>	<i>Holm oak</i>	<i>Conifers</i>	<i>Deciduous</i>
g_{max}	145	215	180	200	200
$light_a$	0.006	0.013	0.012	0.01	0.006
T_{opt}	21	27	23	14	16
T_{min}	4	10	1	0	5
T_{max}	37	38	39	35	33
VPD_{min}	4.0	3.2	2.2	3.0	3.1
VPD_{max}	1.0	1.0	4.0	0.5	1.0
f_{min}	0.02	0.15	0.02	0.16	0.13
Period	April-September	All the year	All the year	April-September	April-September

g_{max} : maximum stomatal conductance

f_{min} : minimum stomatal conductance

f_{phen} , f_{light} , f_{temp} , f_{VPD} , f_{SWC} are the variation in g_{max} with leaf age, irradiance, temperature, water vapour pressure deficit and soil water content.

Results of visible injury 2012-2013

Number	Main tree specie	Survey 2012				Number	Main tree specie	Survey 2013			
		Leaf Loss (%)	Discoloration scoring	O ₃ induced damages				Leaf Loss (%)	Discoloration scoring	O ₃ induced damages	
				C+1 (%)	C+2 (%)					C+1 (%)	C+2 (%)
1	<i>Robinia pseudoacacia</i>	7	0.2	0		1	<i>Robinia pseudoacacia</i>	9.8	0.35	1.60	
2	<i>Fraxinus excelsior</i>	2	0	1.84		2	<i>Fraxinus excelsior</i>	4.5	0.05	10.80	
3	<i>Robinia pseudoacacia</i>	12	1	1.52		3	<i>Robinia pseudoacacia</i>	6.0	0.05	0.0	
4	<i>Fraxinus excelsior</i>	2	0.4	18.8		4	<i>Fraxinus excelsior</i>	11.8	0.20	38.50	
5	<i>Quercus cerris</i>	3	0	0		5	<i>Quercus cerris</i>	17.8	0.30	4.30	
6	<i>Fagus sylvatica</i>	9	1.4	1.8		6	<i>Fagus sylvatica</i>	7.5	0.40	21.40	
7	<i>Fraxinus ornus</i>	10	0.2	0.3		7	<i>Fraxinus ornus</i>	10.5	0.15	17.10	
8	<i>Robinia pseudoacacia</i>	10	0.2	0.3		8	<i>Robinia pseudoacacia</i>	10.5	0.15	31.40	
9	<i>Pinus cembra</i>	10	0.2	0.3		9	<i>Pinus cembra</i>	10.5	0.15	0.00	2.92
10	<i>Fraxinus excelsior</i>	10	0.2	0.3		10	<i>Fraxinus excelsior</i>	10.5	0.15	7.60	
11	<i>Pinus sylvestris</i>	10	0.2	0.3		11	<i>Pinus sylvestris</i>	10.5	0.15	0.24	7.08
12	<i>Pinus sylvestris</i>	14	0	0	0	12	<i>Pinus sylvestris</i>	12.2	0.05	1.56	4.76
13	<i>Fagus sylvatica</i>	21	1.4	6.32		13	<i>Fagus sylvatica</i>	26.8	0.45	6.32	
14	<i>Fagus sylvatica</i>	3	0.2	0		14	<i>Fagus sylvatica</i>	13.3	0.40	2.08	
15	<i>Robinia pseudoacacia</i>	18	0	0		15	<i>Robinia pseudoacacia</i>	13.8	0.35	0.00	
16	<i>Fraxinus ornus</i>	2	0.2	5.8		16	<i>Fraxinus ornus</i>	10.8	0.30	14.80	
17	<i>Quercus petraea</i>	14	0.8	0		17	<i>Quercus petraea</i>	9.3	0.60	0.60	
18	<i>Fagus sylvatica</i>	5	1	0		18	<i>Fagus sylvatica</i>	25.5	0.80	0.00	
19	<i>Robinia pseudoacacia</i>	15	0.2	0		19	<i>Robinia pseudoacacia</i>	7.8	0.00	0.00	
20	<i>Fraxinus excelsior</i>	19	0.2	0		20	<i>Fraxinus excelsior</i>	10.5	0.25	0.00	
21	<i>Picea excelsa</i>	10	0	0	0	21	<i>Picea excelsa</i>	18.2	0.20	0.00	0.00
22	<i>Fraxinus excelsior</i>	15	0.4	16.6		22	<i>Fraxinus excelsior</i>	15.8	0.30	24.0	
23	<i>Fraxinus excelsior</i>	20	0.2	2.8		23	<i>Fraxinus excelsior</i>	46.5	2.00	1.20	
24	<i>Abies alba</i>	4	0	0	0	24	<i>Abies alba</i>	1.75	0.15	0.00	0.00

Increase of defoliation, discoloration and the surface affected by ozone-induced symptoms between 2012 and 2013

Results: Spearman's coefficients

	AOT40	A_POD0	A_POD1	B_POD0	B_POD1	C_POD0	C_POD1
<i>Pinus cembra</i>							
Discoloration	ns	0,4532	ns	0,3110	0,3866	0,3903	ns
Needle loss	0,4945	ns	ns	ns	ns	ns	ns
O ₃ symptoms C+1	ns	0,5912	0,3143	0,3808	0,3531	0,4663	0,3255
O ₃ symptoms C+2	ns	0,5652	ns	0,3457	0,3349	0,4447	0,3061
<i>Pinus halepensis</i>							
Discoloration	0,3075	ns	0,3853	ns	ns	ns	ns
Needle loss	0,3389	ns	ns	ns	ns	ns	ns
O ₃ symptoms C+1	ns	0,4120	0,3900	ns	ns	ns	0,3831
O ₃ symptoms C+2	ns	0,6067	0,6207	0,5426	0,5771	0,5751	0,5859

A - Whole day; B - 08:00-20:00; C - hours with a GR > 50 W.m⁻²

Analysis of exposure and flux-based ozone approaches

PODY is correlated with the occurrence and the severity of O₃-induced symptoms.

AOT40 is stronger correlated with discoloration and defoliation, i.e. typical aspecific indicators.

Analysis of the best threshold

For all tree species, POD0 is better correlated with the occurrence and the severity of visible symptoms.

Analysis of the best time window

For all species, O₃-symptoms are stronger correlated with PODY calculated for the whole day - Night-time

O₃ uptake may be physiologically relevant.

Results: Spearman's coefficients

Analysis of the meteorological parameters

	G. Radiation	Rainfall	RH	SWC	Temperature
<i>Conifers</i>					
Discoloration	0,5299	ns	ns	ns	0,4172
Needle loss	0,4603	ns	- 0,3057	ns	0,5022
O ₃ symptoms C+1	ns	0,3424	0,3504	0,4906	- 0,2562
O ₃ symptoms C+2	ns	0,3383	0,3402	0,4403	- 0,2912
<i>Pinus cembra</i>					
Discoloration	ns	ns	ns	ns	0,3552
Needle loss	0,3621	- 0,3160	ns	ns	0,4011
O ₃ symptoms C+1	ns	0,2101	ns	0,3876	ns
O ₃ symptoms C+2	ns	ns	ns	0,2969	ns
<i>Pinus halepensis</i>					
Discoloration	ns	ns	0,2105	0,3170	0,4145
Needle loss	ns	ns	ns	ns	ns
O ₃ symptoms C+1	ns	ns	ns	ns	0,3488
O ₃ symptoms C+2	ns	- 0,2249	ns	0,3152	ns

The most important factor affecting the occurrence and the severity of ozone-induced symptoms, in all tree species, was the **soil water content**.

The **function SWC** must be included in the DO3SE model because it is critical for Mediterranean environments, characterized by summer water stress.

Derivation of Critical limits from specific symptoms

Tree species	CLef (POD0, mmol.m ⁻²)	Effect parameter	% of surface affected for CLef
All tree species	22	Severity of ozone- induced injury	25% (annual)
Conifers	23		
C+1 needles	25		
C+2 needles	22		
Pinus cembra	22		
C+1 needles	26		
C+2 needles	21		
Pinus halepensis	28		
C+1 needles	35		
C+2 needles	26		
Deciduous	21		
Fagus sylvatica	24		
Fraxinus excelsior	19		
F. excelsior & ornus	20		

A POD0 limit value of **23 mmol.m⁻² PLA** has been identified for conifers and of **21 mmol.m⁻²** for deciduous.

22 mmol.m⁻² PLA for *P. cembra* (high O₃-sensitive) and of **28 mmol.m⁻²** for *P. halepensis* (moderate O₃-sensitive).

24 mmol.m⁻² for *Fagus sylvatica* (moderate O₃-sensitive) and of **19 mmol.m⁻²** for *Fraxinus excelsior* (high O₃-sensitive).

Conclusions & discussions

- most experiments to establish biologically relevant plant responses have been performed under controlled conditions, **not representative** of field conditions
- the results may not provide the development of appropriated standards for vegetation protection “in field”
- AOT40 inconsistent with forest condition: it does not account for the different kinds of tree species, genotypes, forest types and site conditions
- **stomatal flux-based approach** would be a useful tool for O₃ risk assessment.

Conclusions & discussions

- PODy is well correlated with O₃-induced symptoms whereas AOT40 is stronger correlated with discoloration and defoliation (a-specific indicators).
- POD1 is not working better than POD0 in protecting vegetation against ozone induced damage, thus use of the threshold is not recommended.
- POD0 cumulated all day long perform (solar radiation >0) well than POD0 cumulated in the time range 8-20.
- In Mediterranean environment SWC must be included to obtain a good estimation of POD.
- Further field-based validation of O₃ flux-effect relationships is required via epidemiological studies.



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