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ORIGINAL PAPER

Epidemiological derivation of flux-based critical levels for visible ozone injury in European forests

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Abstract The European MOTTLES project set-up a new-generation network for ozone (O_3) monitoring in 17 plots in France, Italy and Romania. These monitoring stations allowed: (1) estimating the accumulated exposure AOT40 and stomatal O_3 fluxes (PODY) with an hourly threshold of uptake (Y) to represent the detoxification capacity of trees (POD1, with $Y = 1$ nmol O_3 m^{-2} s^{-1} per leaf area); and (2) collecting data of forest-response indicators, i.e. crown defoliation and visible foliar O_3-like injury over the time period 2017–2019. The soil water content was the most important parameter affecting crown defoliation and was a key factor affecting the severity of visible foliar O_3-like injury on the dominant tree species in a plot. The soil water content is thus an essential parameter in the PODY estimation, particularly for water-limited environments. An assessment based on stomatal flux-based standard and on real plant symptoms is more appropriated than the exposure-based method for protecting vegetation. From flux-effect relationships, we derived flux-based critical levels (CLef) for forest protection against visible foliar O_3-like injury. We recommend CLef of 5 and 12 mmol m^{-2} POD1 for broadleaved species and conifers, respectively. Before using PODY as legislative standard in Europe, we recommend using the CLec for $\geq 25\%$ of crown defoliation in a plot: 17,000 and 19,000 nmol mol^{-1} h AOT40 for conifers and broadleaved species, respectively.

Keywords POD · Critical levels · Ozone · Visible injury · Epidemiology

Introduction

Surface ozone (O_3) is a major air quality issue worldwide (Sicard et al. 2013, 2016a, 2017, 2020) with harmful effects on forest trees (Sicard et al. 2016b; Mills et al. 2018; Feng et al. 2019). The adverse effects can be a reduction of leaf chlorophyll content (Dalstein et al. 2005), sluggishness or impairment of leaf stomata (Hoshika et al. 2015), visible foliar O_3 injury (Calatayud and Cerveró 2007; Paoletti et al. 2009; Schaub et al. 2010; Sicard et al. 2016c; Moura et al. 2018), and a reduction of growth (Fares et al. 2013; Proietti et al. 2016; Braun et al. 2017; Cailleret et al. 2018). The O_3 exposure metric AOT40, i.e. the cumulated exposure to O_3 hourly concentrations exceeding 40 nmol mol^{-1} over the daylight hours during the growing season, is recommended for the protection of vegetation by the European Council Directive 2008/50/EC. For forest protection, an exposure-based critical level of 5000 nmol mol^{-1} h AOT40 is recommended by UNECE (2010).

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In Europe, the crown defoliation is recommended by the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP-Forests) as indicator of forest health in Europe (Fischer and Lorenz 2011). The visible foliar O_3 injury (e.g. stippling, necrosis) are the first indicator of phytotoxic O_3 levels (Grulke 2003), and can occur even for low AOT40 values e.g. in Lithuania (Araminienė et al. 2019). The O_3 effects on forest trees depend not only on the O_3 concentrations in ambient air, but also on O_3 uptake through stomata (Paoletti and Manning 2007). The Phytotoxic Ozone Dose, defined as the amount of O_3 absorbed into the leaves or needles through stomata over the growing season, and above a threshold Y of uptake (PODY), integrates the effects of climatic factors and vegetation characteristics on O_3 uptake (Emberson et al. 2000). Looking at the forest responses to O_3, the stomatal flux-based approach is more realistic compared to the exposure-based approach i.e. AOT40 (De Marco et al. 2015; Anav et al. 2016; Sicard et al. 2016c; Agathokleous et al. 2018; Paoletti et al. 2019). For these reasons, PODY is under discussion as new legislative standard in Europe (Lefohn et al. 2018; Paoletti et al. 2019). Following the revision of the National Emission Ceiling Directive in 2016, consistent flux-based critical levels for forest protection against visible O_3 damages are requested in a climate change context (De Marco et al. 2019). Epidemiological surveys of crown defoliation, visible foliar O_3-like injury and environmental variables, including O_3 metrics, can be used to derive robust stomatal flux-based critical levels (Mills et al. 2011; Braun et al. 2014; Sicard et al. 2016c; De Marco and Sicard 2019).

The European MOTTLES project (LIFE15 ENV/IT/000183), based on the O_3 risks on forests, set-up a new-generation network of 17 sites for O_3 monitoring in France, Italy and Romania, as described in Paoletti et al. (2019), allowing estimating stomatal O_3 fluxes (PODY) and collecting forest-response indicators under real conditions, i.e. crown defoliation and visible foliar O_3-like injury for the years 2017, 2018 and 2019 in order to derive proper critical levels for forest protection.

The aims of this study were (1) to determine the relative importance of different environmental variables in determining crown defoliation and visible O_3-like injury on dominant trees in a plot; and (2) to suggest proper epidemiologically-based O_3 critical levels for forest protection against O_3.

Materials and methods

Monitoring network

Seventeen forest sites were selected in France, Italy and Romania, representative of main biogeographical regions (Alpine, Atlantic, Continental and Mediterranean) along a gradient of O_3 pollution: 4 plots in France, 9 in Italy, and 4 in Romania (Table 1). Within the network, the dominant tree species in the plots are deciduous broadleaved species i.e. *Alnus glutinosa*, *Fagus sylvatica, Phillyrea latifolia, Quercus cerris*, *Q. ilex*, *Q. petraea* and *Q. robur* followed by conifer species i.e. *Larix decidua*, *Picea abies*, *Pinus pinea* and *P. sylvestris*. The meteorological and O_3 data are continuously measured in open areas, while soil moisture at 10 cm depth and forest-health indicators are measured into the nearby forest (Paoletti et al. 2019). Each integrated station is equipped with sensors for air temperature and relative humidity, rainfall, atmospheric pressure, solar radiation, wind speed and direction, soil moisture, while ground-level O_3 is recorded by an active monitor. The hourly averages are recorded by data loggers. A full description of the monitoring network set-up, with all information about equipment and sensors, is available in Paoletti et al. (2019).

Crown defoliation

In the 17 plots, 340 trees were investigated from the end of August to mid-September in 2017, 2018 and 2019. The assessment was based on the ICP-Forests Manual on Visual Assessment of Crown Condition (Eichhorn et al. 2016). Crown defoliation was assessed on 20 randomly selected trees of the dominant tree species per plot. Crown defoliation was assessed in 5% steps. A tree with a crown defoliation ≥ 25% is usually considered as damaged (Eichhorn et al. 2016). For each plot, a mean crown defoliation was calculated from the 20 scored trees.

Visible foliar ozone injury

The assessment was based on the ICP-Forests Guidelines (Schaub et al. 2016). Surveys were carried out by the same two trained observers in each country from the end of August to early-mid September. The observers were involved in validation activities, attended field courses and performed annual inter-comparison exercises, organized by ICP-Forests. Foliar injury was compared with the reference picture atlas provided by the validation center for Southern Europe (www.ozoneinjury.org) and for central Europe (www.wsl.ch). At each plot, 5 trees were randomly selected. For each tree, 5 light-exposed branches with ≥ 30 needles/leaves per branch or needle age class were removed from the upper crown. For each branch, the percentage of total leaf/needle surface affected by visible foliar O_3 injury was scored for current-year foliage (C), and one-year-old (C + 1) and two-year-old (C + 2) needles. If injury was due to another factor, different from O_3, the needle/leaf was excluded from scoring. For each plot, a mean percentage of needle/leaf surface affected by visible foliar O_3 injury was calculated.

Table 1 Annual average ± standard deviation (number of data n = 45) for air temperature (Temp, °C), relative humidity (RH, %), solar radiation (S rad, W m^{-2}), soil water content (SWC, %), rainfall (Rain, mm), 24-h ozone concentrations (Ozone, nmol mol^{-1}), AOT40 (nmol mol^{-1} h), POD1 (mmol m^{-2}), crown defoliation (mean percentage of missing tree crown per plot, %) and visible foliar ozone injury on the dominant tree species in a plot (VI_dom, mean percentage of injured light-exposed leaf surface, %) over the time period 2017–2019

Site code	Country	Latitude	Longitude	Elevation (m asl)	Dominant tree species	Temp	RH	S. rad	SWC	Rain	Ozone	AOT40	POD1	Crown defol.	VI_dom
ABR1	Italy	41.86°N	13.57°E	1500	*Fagus sylvatica*	7.2 ± 0.1	81.0 ± 2.3	155.3 ± 7.9	31.5 ± 1.0	1245 ± 158	55.7 ± 3.4	29,600 ± 9500	19.3 ± 7.9	24.4 ± 4.1	10.8 ± 5.5
CPZ1	Italy	41.70°N	12.36°E	0	*Quercus ilex*	16.2 ± 0.1	79.7 ± 0.8	175.1 ± 8.6	14.4 ± 0.6	817 ± 113	32.4 ± 2.4	20,500 ± 6900	21.6 ± 3.3	25.7 ± 0.8	0
CPZ2	Italy	41.70°N	12.36°E	0	*Phillyrea latifolia*	16.2 ± 0.1	79.7 ± 0.8	175.1 ± 8.6	14.4 ± 0.6	817 ± 113	32.4 ± 2.4	20,500 ± 6900	9.7 ± 1.6	35.0 ± 6.0	0
CPZ3	Italy	41.68°N	12.39°E	0	*Pinus pinea*	16.2 ± 0.1	79.7 ± 0.8	175.1 ± 8.6	19.9 ± 2.1	817 ± 113	32.4 ± 2.4	20,500 ± 6900	14.6 ± 3.0	33.6 ± 3.1	0.3 ± 0.2
EMI1	Italy	44.72°N	10.20°E	200	*Quercus petraea*	11.8 ± 1.7	70.4 ± 6.1	141.6 ± 19.9	15.6 ± 0.2	820 ± 296	40.6 ± 4.6	29,700 ± 5700	12.3 ± 4.2	29.5 ± 17.5	0
FAG	Romania	45.43°N	25.27°E	1300	*Fagus sylvatica*	7.1 ± 0.7	78.6 ± 3.8	161.7 ± 14.2	27.4 ± 6.5	876 ± 76	42.0 ± 0.9	10,600 ± 4000	19.5 ± 7.4	9.8 ± 0.6	0
GORUN	Romania	45.03°N	24.99°E	500	*Quercus petraea*	11.0 ± 1.0	78.8 ± 1.5	193.3 ± 71.3	19.2 ± 0.2	1258 ± 946	21.0 ± 1.8	3700 ± 2000	9.1 ± 3.2	13.2 ± 0.7	0
LAZ1	Italy	42.83°N	11.90°E	690	*Quercus cerris*	13.3 ± 0.1	76.5 ± 2.9	154.6 ± 5.3	18.6 ± 0.1	1981 ± 755	47.8 ± 2.1	21,600 ± 3600	16.2 ± 0.8	21.5 ± 0.4	0
LCAS	France	44.99°N	6.48°E	1755	*Larix decidua*	7.8 ± 1.4	63.4 ± 3.9	174.7 ± 15.6	20.8 ± 1.3	695 ± 205	48.1 ± 0.4	18,900 ± 4400	8.2 ± 6.6	14.1 ± 0.9	8.7 ± 0.7
MNTFR	France	45.80°N	2.06°E	810	*Pinus sylvestris*	10.5 ± 0.8	77.3 ± 1.8	130.3 ± 6.4	34.2 ± 2.2	1178 ± 332	38.4 ± 0.6	7900 ± 1400	12.6 ± 0.9	18.2 ± 0.6	21.0 ± 0.7
MOLID	Romania	45.51°N	25.59°E	1185	*Picea abies*	7.3 ± 0.5	80.5 ± 1.3	115.5 ± 5.6	23.3 ± 0.1	626 ± 12	27.4 ± 1.6	4200 ± 4600	12.0 ± 3.6	12.0 ± 1.0	0
MORV	France	47.27°N	4.10°E	620	*Alnus glutinosa*	9.6 ± 0.6	82.5 ± 3.8	141.7 ± 6.9	22.1 ± 2.8	295 ± 66	35.0 ± 1.9	12,100 ± 5100	12.4 ± 10.7	9.3 ± 2.8	4.6 ± 0.2
PIE1	Italy	45.68°N	8.07°E	1150	*Fagus sylvatica*	6.5 ± 0.1	73.4 ± 3.9	129.6 ± 4.0	29.5 ± 0.2	2639 ± 171	50.7 ± 0.1	25,400 ± 9500	19.5 ± 3.9	23.7 ± 0.1	1.9 ± 0.5
REV	France	49.91°N	4.63°E	390	*Picea abies*	11.0 ± 0.9	80.9 ± 2.1	123.3 ± 5.5	25.9 ± 0.8	995 ± 185	32.5 ± 1.7	10,100 ± 5300	6.2 ± 2.8	11.0 ± 1.5	8.4 ± 4.1
STEJAR	Romania	44.50°N	26.17°E	85	*Quercus robur*	13.4 ± 2.0	75.9 ± 1.3	192.3 ± 35.4	44.2 ± 1.5	968 ± 526	22.1 ± 2.2	4200 ± 1000	20.9 ± 2.2	17.9 ± 0.6	0
TRE1	Italy	46.36°N	11.49°E	1800	*Picea abies*	5.0	73.8	138.6	27.9	1062	52.2	39,800	24.2	14.4	2.9
VEN1	Italy	46.06°N	12.39°E	1100	*Fagus sylvatica*	7.6 ± 0.1	86.6 ± 0.8	134.5 ± 2.1	40.0 ± 0.8	2199 ± 403	36.0 ± 0.6	21,000 ± 1100	26.0 ± 3.6	24.3 ± 3.5	6.9 ± 0.7

AOT40 calculation

The O_3 exposure index AOT40 (here in nmol/mol hour, abbreviated to nmol mol^{-1} h) was calculated as sum of the hourly exceedances above 40 nmol mol^{-1} for daylight hours when global radiation is higher than 50 W m^{-2} (CLRTAP 2017) during the actual growing season of the dominant tree species at each site (Paoletti et al. 2019). In our study, the accumulation period started from the actual start date of the growing season (aSGS, as defined in Paoletti et al. 2019) until the day when the survey of forest-health responses was carried out at a site.

$$\mathrm{AOT40} = \int_{t=aSGS}^{surveydate} \max\left(\left(\left[O_3\right] - 40\right), 0\right).dt \tag{1}$$

where $[O_3]$ is hourly O_3 concentration (nmol mol^{-1}) and dt is time step (1−h). The function "maximum" ensures that only values exceeding 40 nmol mol^{-1} are taken into account.

Phytotoxic ozone dose calculation

The actual stomatal conductance (g_{sto}) was calculated as a species-specific function where the maximum value of stomatal conductance (g_{max}) is reduced by limiting functions, scaled from 0 to 1 as described in Eq. 2.

$$g_{sto} = g_{max} \times f_{phen} \times f_{light} \times max\left\{f_{min}, \left(f_{temp} \times f_{VPD} \times f_{SWC}\right)\right\} \tag{2}$$

where g_{max} is the maximum stomatal conductance to O_3 expressed on a total leaf surface area (mmol O_3 m^{-2} s^{-1}). The maximum stomatal conductance (g_{max}) is based on the average above the 90th or 98th percentile of g_{sto} measurements under optimum environmental conditions for stomatal opening (CLRTAP 2017).

The functions f_{phen}, f_{light}, f_{temp}, f_{VPD} and f_{SWC}, expressed in relative terms (i.e. values between 0 and 1), are the variation in g_{max} with leaf age, irradiance (photosynthetically flux density at the leaf surface, µmol photons m^{-2} s^{-1}), air temperature (T, °C), vapor pressure deficit estimated through the relative air humidity (VPD, kPa), and volumetric soil water content (SWC, m^3 m^{-3}), respectively. The function f_{min} is the minimum stomatal conductance, expressed as a fraction of g_{max}. The following formulas were applied:

$$f_{light} = 1 - exp^{-light_a \times PPFD} \tag{3}$$

$$f_{temp} = \left(\frac{T - T_{min}}{T_{opt} - T_{min}}\right)\left\{\left(\frac{T_{max} - T}{T_{max} - T_{opt}}\right)^{\left(\frac{T_{max}-T}{T_{opt}-T_{min}}\right)}\right\} \tag{4}$$

$$f_{VDP} = \min\left[1, \max\left\{f_{min}, \left(\frac{\left(1 - f_{min}\right)\left(VPD_{min} - VPD\right)}{VPD_{min} - VPD_{max}}\right) + f_{min}\right\}\right] \tag{5}$$

$$f_{SWC} = \min\left[1, \left(f_{min}, \left(\left(1 - f_{min}\right)\left(\frac{SWC - WP}{FC - WP}\right) + f_{min}\right)\right)\right] \tag{6}$$

where $light_a$ is an a-dimensional constant; PPFD is hourly photosynthetic photon flux density estimated through the solar radiation; T_{opt}, T_{min}, and T_{max}, represent the optimum, minimum, and maximum temperature for stomatal conductance, respectively; VPD_{min} and VPD_{max} are minimum and maximum vapor pressure deficit for stomatal conductance, respectively; WP is SWC at wilting point and FC is SWC at field capacity.

We assumed that f_{phen} was 1 throughout the growing season, i.e. from the start date of the growing season (aSGS) until the time of the visible foliar O_3 injury survey. Phenology for the survey accumulation periods was directly assessed at the Romanian sites. At the French and Italian sites, when no direct observation was carried out, we used a latitude model according to CLRTAP (2017).

At each site, hourly air temperature, relative air humidity, solar radiation, wind speed and direction, SWC, and ground-level O_3 concentrations are measured. Furthermore, for each dominant tree species, we used the fixed species-specific flux parameterization (Table 2) available in literature (CLRTAP 2017; Hoshika et al. 2018).

Once the stomatal conductance was computed, similarly to AOT40, the stomatal O_3 flux PODY was accumulated from the actual start date of the growing season (aSGS) until the time of the visible foliar O_3 injury survey. PODY (mmol m^{-2}) was calculated from hourly data as:

$$PODY = \int_{t=aSGS}^{surveydate} \left[\left(\left(g_{sto} \times \left[O_3\right] - Y\right), 0\right].dt\right) \tag{7}$$

where PODY is the accumulated stomatal O_3 flux above a detoxification threshold Y per leaf area (nmol O_3 m^{-2} s^{-1}) over the accumulation period for hours with 50 W m^{-2} solar radiation, g_{sto} represents hourly values of stomatal conductance, $[O_3]$ is hourly O_3 concentrations (ppb) and dt is the time step (1−h). We are able to calculate PODY with any Y uptake threshold, however we calculated PODY with Y = 1 nmol O_3 m^{-2} s^{-1} per leaf area, below which it is assumed that any O_3 molecule absorbed by the plant will be detoxified according to present knowledge, as recommended by CLRTAP (2017).

AOT40 and PODY were estimated by extrapolating the values measured at 2 m a.g.l. up to the top of the canopy, by making use of neutral stability profiles, from wind speed and O_3 concentration at a height z, and aerodynamic resistance (CLRTAP 2017).

Table 2 Summary of parameterizations for the dominant species at each MOTTES site

Site code	Dominant tree species	g_{max} (mmol O_3 m^{-2} s^{-1})	f_{min} (fraction)	f_{light_a} (dl)	T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)	VPD_{max} (kPa)	VPD_{min} (kPa)	FC (m^3 m^{-3})	WP (m^3 m^{-3})
ABR1	*Fagus sylvatica*	145	0.02	0.0060	4	21	37	1.0	4.0	0.439	0.066
CPZ1	*Quercus ilex*	195	0.02	0.0120	1	23	39	2.2	4.0	0.439	0.066
CPZ2	*Phillyrea latifolia*	150	0.01	0.0016	0	23	40	1.2	5.3	0.404	0.067
CPZ3	*Pinus pinea*	130	0.03	0.0032	6	20	39	0.6	4.2	0.439	0.066
EMI1	*Quercus petraea*	265	0.13	0.0060	0	22	35	1.1	3.1	0.434	0.047
FAG	*Fagus sylvatica*	145	0.02	0.0060	4	21	37	1.0	4.0	0.434	0.047
GORUN	*Quercus petraea*	265	0.13	0.0060	0	22	35	1.1	3.1	0.434	0.047
LAZ1	*Quercus cerris*	265	0.13	0.0060	0	22	35	1.1	3.1	0.465	0.103
LCAS	*Larix decidua*	140	0.10	0.0050	0	22	35	0.8	3.5	0.465	0.103
MNTFR	*Pinus sylvestris*	180	0.10	0.0060	0	20	36	0.6	2.8	0.439	0.066
MOLID	*Picea abies*	130	0.16	0.0100	0	14	35	0.5	3.0	0.439	0.066
MORV	*Alnus glutinosa*	300	0.13	0.0024	5	29	40	1.8	5.7	0.439	0.066
PIE1	*Fagus sylvatica*	145	0.02	0.0060	4	21	37	1.0	4.0	0.439	0.066
REV	*Picea abies*	130	0.16	0.0100	0	14	35	0.5	3.0	0.465	0.103
STEJAR	*Quercus robur*	200	0.03	0.0035	0	22	50	0.8	7.0	0.434	0.047
TRE1	*Picea abies*	130	0.16	0.0100	0	14	35	0.5	3.0	0.434	0.047
VEN1	*Fagus sylvatica*	145	0.02	0.0060	4	21	37	1.0	4.0	0.465	0.103

The g_{max}, maximum stomatal conductance; f_{min} minimum stomatal conductance; f_{light_a} parameter determining the shape of the hyperbolic relationship of stomatal response to light (dl = dimensionless); T_{max}, T_{opt} and T_{min} are maximum, optimal and minimum temperature; VPD_{min} and VPD_{max} are the vapor pressure deficit for attaining minimum and full stomatal aperture; FC and WP are the soil field capacity and wilting point and depend on the soil type

Statistical analysis

The sites with at least 75% of validated hourly O_3 and meteorological data per year were selected. The non-parametric Spearman rank correlation test was applied to measure statistical dependence between pairs of variables. Random Forests Analysis (RFA) is a non-parametric tree-based ensemble learning method for classification (Breiman 2001) and can be used to rank the importance of variables in a regression or classification (Vitale et al. 2014; Sicard and Dalstein-Richier 2015). RFA was performed to determine the importance of environmental variable averaged over the year-round and over the actual growing season (mean O_3 concentrations, air temperature, relative humidity, solar radiation, rainfall, soil water content) in determining the severity of visible foliar O_3 injury (i.e. surface affected by visible injury) and crown defoliation. The highest predictor importance is assigned a value of 1, and the importance of all other predictors is expressed relative to the most important predictor (Breiman 2001). The non-parametric Spearman rank correlation test can be applied to a small dataset (here, n = 45) to assess the relationships between AOT40, POD1 and the crown defoliation and visible O_3 injury. We used *Statgraphics Centurion* for statistics analyses, and *ArcGIS* (Environmental Systems Research Institute) for PODY mapping.

Derivation of critical levels

Following the methodology established by Sicard et al. (2016c), we correlated AOT40 and POD1 to forest-response parameters (crown defoliation and visible foliar O_3-like injury) by joining data from all sites and years to derive exposure-based (CLec) and flux-based (CLef) critical levels. As a tree with defoliation above 25% is usually rated as damaged (Eichhorn et al. 2016), CLec was calculated based on a threshold of 25% crown defoliation. The CLef values were calculated from flux-effect functions for 0% and 15% of visible foliar O_3-like injury (Sicard et al. 2016c).

Results

Ozone metrics and forest-response indicators

The highest O_3 mean concentrations (55.7 nmol mol^{-1}) were measured in central Italy (ABR1) while the lowest concentrations (21.0 nmol mol^{-1}) were observed in Romania (GORUN) over the time period 2017–2019

(Table 1). The highest AOT40 value was observed in Italy (39,800 nmol mol^{-1} hat TRE1) and the lowest value was in Romania (3700 nmol mol^{-1} h at GORUN). The highest POD1 mean value (26.0 mmol m^{-2} POD1) was found in Italy (VEN1) while the lowest POD1 value (6.2 mmol m^{-2} POD1) was measured in Northern France (REV). The mean crown defoliation ranged from 9.3% (*Alnus glutinosa* in MORV) to 35.0% (*Phillyrea latifolia* in CPZ2) while the highest percentages of visible foliar O_3 injury in the dominant species (21.0%) were observed in a highly O_3-sensitive tree species, i.e. *Pinus sylvestris*, in central France. As the data of 2017 were published in Paoletti et al. (2019), we present here the AOT40 and POD1 values for 2018 and 2019 (Fig. 1). Results indicate a remarkable spatial inconsistency between both metrics, except for STEJAR in Romania and PIE1 in Italy.

Analysis of the effect of environmental parameters on effect parameters

The defoliation severity was mainly influenced by the annual SWC, followed by the annual mean air temperature and the annual total amount of rainfall (Fig. 2). The RFA highlighted that the mean O_3 concentrations and variables averaged over the growing season did not influence so much the defoliation severity. The most important factors determining the severity of visible O_3-like injury were the annual SWC and global radiation over the entire year followed by the rainfall during the growing season, air temperature (annual and growing season) and the mean annual O_3 concentrations, while the O_3 concentrations during the growing season was less influential. The severity of visible O_3-like injury on leaves/needles was influenced by a combination of multiple climatic factors compared to crown defoliation (Fig. 2). The SWC was by far the most influential environmental parameter affecting the severity of visible O_3-like injury and crown defoliation on dominant tree species.

Epidemiologically-based critical levels for forest protection

AOT40 was better correlated with crown defoliation ($r = 0.58$ for conifers and broadleaved species; $p < 0.05$) than visible injury (non-significant; $p > 0.1$). POD1 was better correlated with visible O_3-like injury ($r = 0.61$ for conifers, $r = 0.41$ for broadleaved species; $p < 0.05$) than crown defoliation (non-significant, $p > 0.1$). We thus selected crown defoliation as the effect parameter for defining CLec (Table 3) and visible

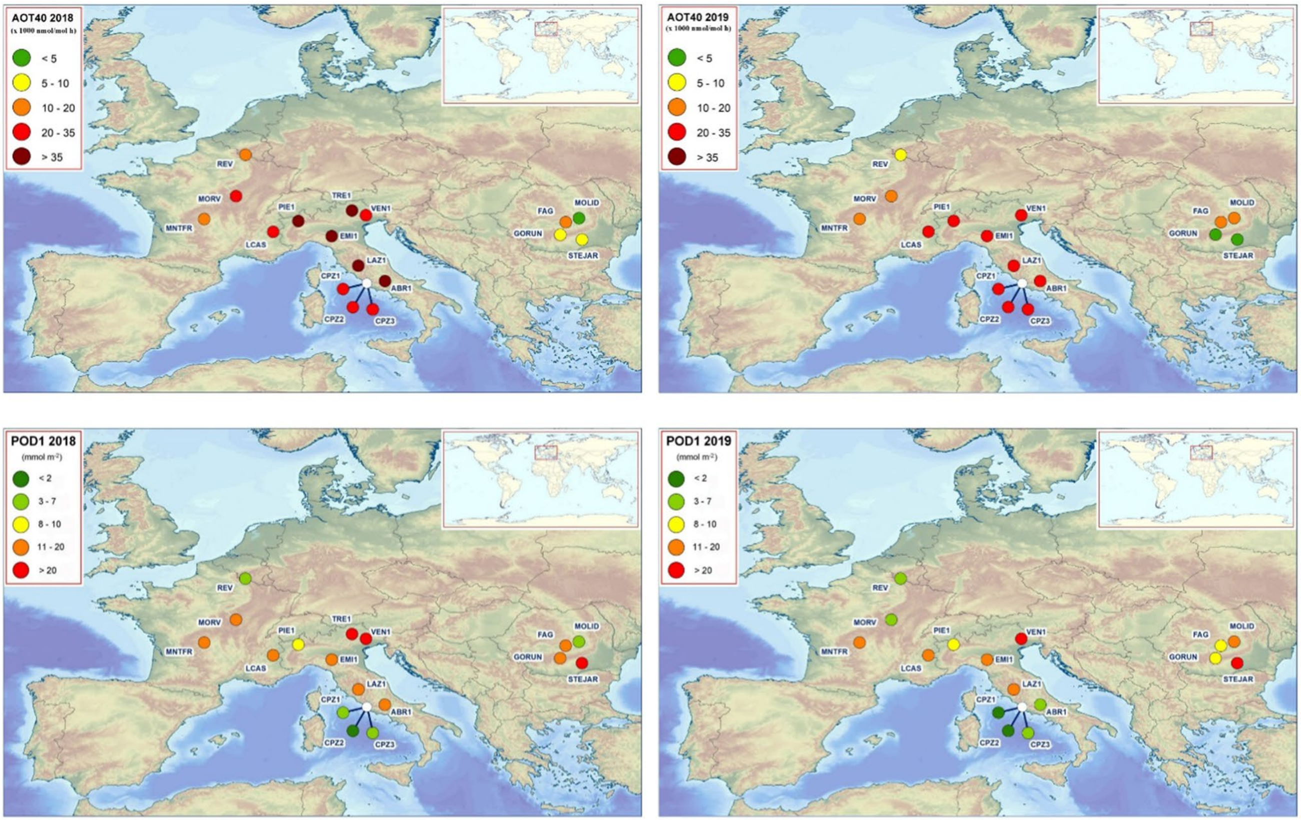

Fig. 1 Annual AOT40 (nmol mol^{-1} h) and POD1 (mmol O_3 m^{-2}), i.e. the accumulated stomatal O_3 flux above a threshold $Y = 1$ nmol O_3 m^{-2} s^{-1} (per leaf area) calculated over the actual growing season of the dominant tree species at each site for the years 2018 and 2019

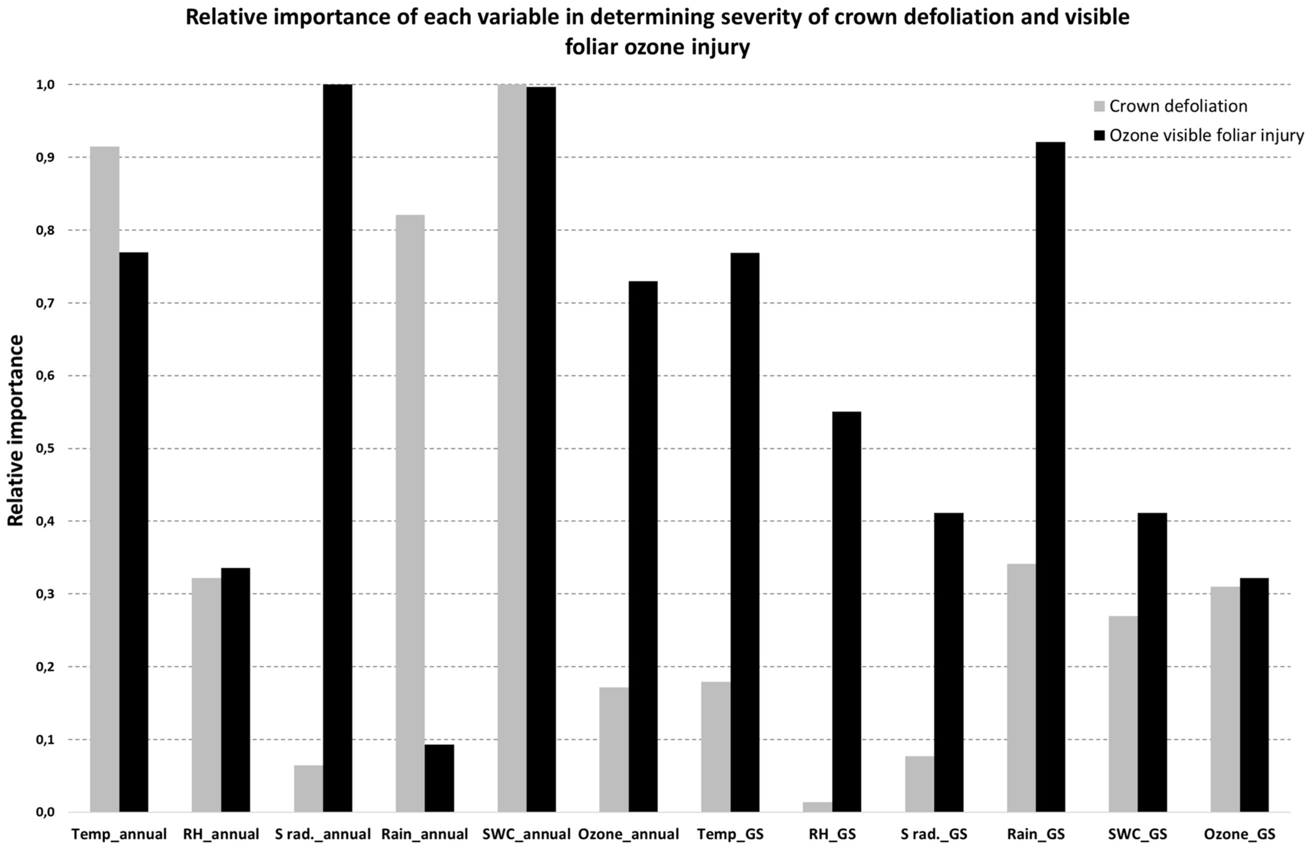

Fig. 2 Random Forest Analysis - Relative importance of each environmental variable, averaged year-round (annual) and over the growing season (GS), in determining severity of crown defoliation (mean percentage of missing tree crown per plot) and visible foliar O_3 injury on the dominant tree species in a plot (mean percentage of injured light-exposed leaf surface) over the time period 2017–2019. The environmental variables are air temperature (Temp), relative humidity (RH), solar radiation (S rad.), rainfall (Rain), soil water content (SWC), 24-h ozone concentrations (Ozone)

Table 3 Recommended exposure-based critical levels (CLec) for effects on forest tree species, calculated by joining all stations and years (*n* = number of data)

Tree species	CLec (nmol mol^{-1} h AOT40)	Response function	*r*	*p* value
Conifers (*n* = 15)	16,800	$Y = 316.3X + 8909$	0.58	0.032
Broadleaves (*n* = 30)	19,000	$Y = 486.2X + 6842$	0.58	0.002

The response functions were calculated between AOT40 (variable *Y*) and the annual averages of crown defoliation for broadleaved species and conifers in a plot (variable *X*) over the time period 2017–2019. The CLec was established for ≥ 25% of crown defoliation in a plot. Spearman coefficients (*r*) and level of significance (*p*) for the exposure–response relationship

Table 4 Recommended flux-based critical levels (CLef) established with two different thresholds of visible injury in a plot (0% and 15%) by joining all stations and years (*n* = number of data). The response functions were calculated between POD1 (variable *Y*) and the mean percentage of visible ozone injury on the dominant broadleaved species and conifers in a plot (variable *X*) over the time period 2017–2019. For conifers, the percentage of total needle surface affected by visible foliar O_3 injury was scored in current-year (C), one-year-old (C + 1) and two-year-old needles (C + 2). Spearman coefficients (r) and level of significance (p) for the flux-response relationship

Tree species	CLef (mmol m^{-2} POD1)		Response function	*r*	*p* value
	0%	15%			
Conifers (*n* = 15)	4.8	9.0	$Y = 0.28{*}X + 4.8$	0.61	0.041
Broadleaves (*n* = 30)	11.7	18.6	$Y = 0.46{*}X + 11.7$	0.48	0.050

foliar O_3-like injury as the effect parameter for deriving CLef values (Table 4). The average CLec, established for ≥ 25% of crown defoliation in a plot, was higher for deciduous broadleaves (19,000 nmol mol^{-1} h AOT40) than for conifers (16,800 nmol mol^{-1} h AOT40). The average CLef was 11.7–18.6 mmol m^{-2} POD1 and 4.8–9.0 mmol m^{-2} POD1 for deciduous broadleaves and conifers, respectively, with

0–15% thresholds of visible injury. By calculating the CLef exceedance, we obtained a higher correlation between the amount of visible O_3 injury and the exceedance of the CLef established with 0% ($r = 0.31$; $p < 0.1$) than 15% ($r = 0.11$; $p > 0.1$) as threshold of visible injury. By using these CLef, POD1 values have largely exceeded the CLef in 2018 at MNTFR, TRE1, VEN1 and STEJAR, while no exceedance was observed at REV, LCAS, CPZ1, CPZ2, CPZ3, LAZ1, FAG and GORUN (Fig. 3). The spatial distribution of CLef exceedances in 2019 was similar to 2018, except at MOLID and ABR1 (Fig. 3). The highest percentages of visible foliar O_3 injury on the dominant species (e.g. MNTFR) was associated to high CLef exceedance (Table 1).

Discussion and conclusions

The crown defoliation is a response to different biotic and abiotic factors, including climatic conditions (e.g. drought, frozen), pests and diseases, deposition of air pollutants. The crown defoliation is thus an aspecific indicator of O_3 (Schaub et al. 2010). In broadleaved species, specific visible foliar injury caused by O_3 is generally categorized as stipple, necrosis, chlorosis and bronzing. Specific O_3 injury on conifer needles generally appears as tipburn or chlorotic mottling (Günthardt-Goerg and Vollenweider 2007; Schaub et al. 2010). The RFA allowed discerning the main variables influencing the severity of crown defoliation and severity of visible foliar O_3-like injury, i.e. the surface affected by visible injury, under actual field conditions (Vitale et al. 2014). In this study, the most important variables determining the defoliation severity were SWC and surface air temperature, while the severity of visible foliar O_3 injury on trees were influenced by a combination of multiple co-factors (e.g. SWC, air temperature, solar radiation, O_3 concentration) through the entire year. The severity of visible O_3-like injury depends on the O_3 uptake through stomata, thus to O_3 levels but also multiple climatic factors and environmental parameters, vegetation characteristics and soil conditions (Emberson et al. 2000; Matyssek et al. 2007; Hoshika et al. 2017), detoxification and repair processes (Musselman et al. 2006; Paoletti and Manning 2007). The RFA outputs highlighted the critical role of the soil water availability in determining the stomatal O_3 uptake, and thus the SWC function is essential in the PODY estimation (De Marco et al. 2016; Anav et al. 2018), in particular for water-limited environments

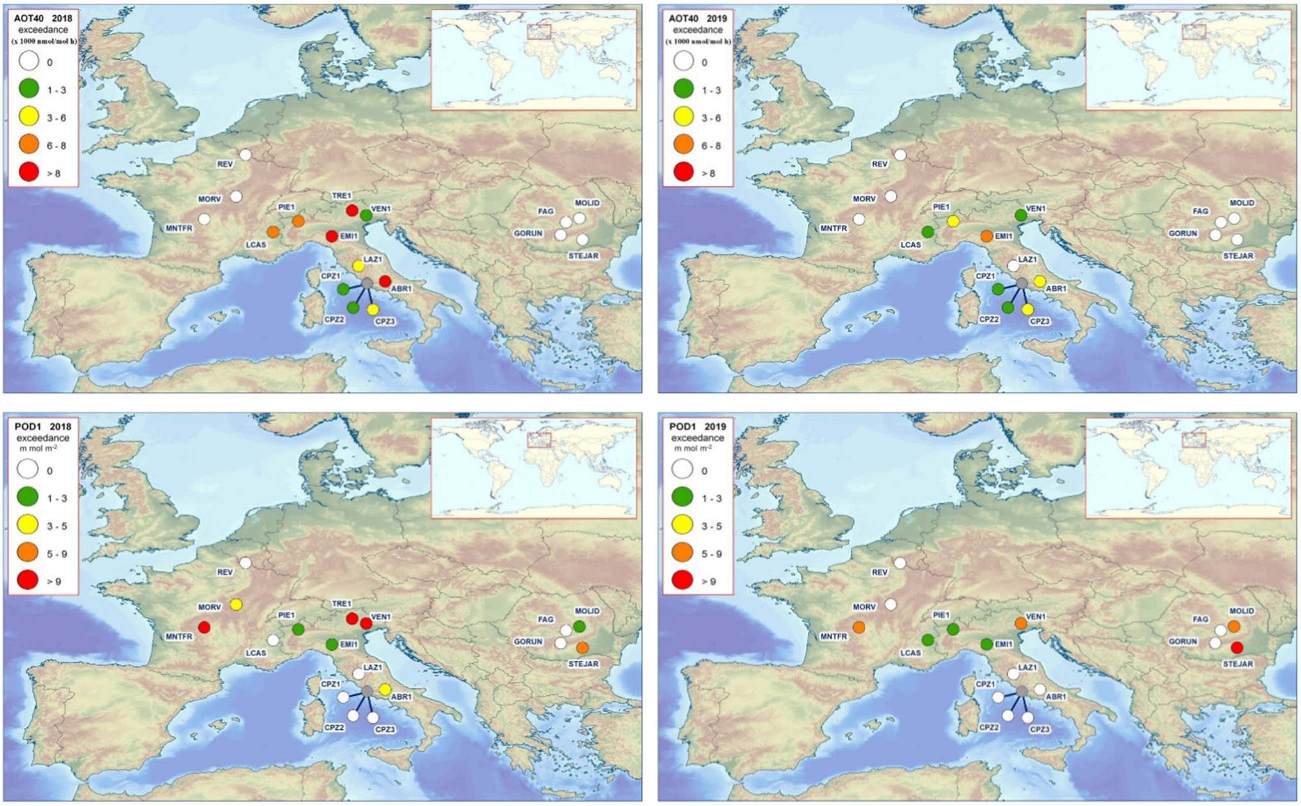

Fig. 3 Exceedance of the suggested AOT40-based critical levels (CLec: 17,000 and 19,000 nmol mol^{-1} h for conifers and broadleaved species) and flux-based critical levels (CLef: 5 and 12 mmol m^{-2} for conifers and broadleaved species) for dominant tree species at each site for the years 2018 and 2019

such as the Mediterranean region (González-Fernández et al. 2013; Ochoa-Hueso et al. 2017).

Between 2000 and 2014, Anav et al. (2019) found a decline of AOT40 (−22%) and O_3 concentrations (−1.6%) and an increase of POD1 (+7.3%) in Europe, mainly due to climate change (Fu et al. 2017; Anav et al. 2019). In addition to a longer growing season (Anav et al. 2019), higher air temperature and global radiation increase the stomatal conductance (Hoshika et al. 2017). The O_3 precursors control strategies could be offset by climate change, leading to higher O_3 risk to European forests (Proietti et al. 2016; Anav et al. 2019). To consistently protect forests against surface O_3 pollution, proper standards (PODY) and realistic critical levels (CLef), representative of real-world conditions, are urgently needed (De Marco and Sicard 2019). Thanks to the new-generation network MOTTLES set-up at 17 plots in France, Italy and Romania, we (1) estimated AOT40 and PODY as descriptors of O_3 risk for vegetation and (2) derived CLec and CLef from forest-health responses (crown defoliation and visible foliar O_3-like injury). In previous studies, critical levels were derived under controlled conditions, that may be not representative of actual field conditions, and from biomass loss (Karlsson et al. 2006; Calatayud et al. 2011; Büker et al. 2015) as aspecific indicator of O_3 i.e. coupled with co-factors (e.g. nutrients and water availability). To overcome these issues, epidemiologically-based flux-response functions were established between O_3 metrics and real-world plant symptoms. As PODY is better than AOT40 as metric for O_3 risk assessment to European forests (De Marco et al. 2015; Sicard et al. 2016c; Paoletti et al. 2019), and due to the biological support for a Y = 1 to represent the detoxification capacity of trees (Karlsson et al. 2007; CLRTAP 2017), we derived CLef from the flux-effect function between POD1 and visible foliar O_3-like injury as specific indicator of phytotoxic O_3 levels (Günthardt-Goerg and Vollenweider 2007; Schaub et al. 2010; Sicard et al. 2016c).

For forest protection against visible O_3 injury in Europe, we recommend CLef of 5 and 12 mmol m^{-2} POD1 for broadleaved species and conifers, respectively. At 54 plots in Southeastern France and Northwestern Italy in 2012–2013, Sicard et al. (2016c) found CLef of 7 and 9 mmol m^{-2} POD1 for broadleaved tree species and conifers, respectively. Previously, critical levels were derived for the cumulative O_3 flux responsible for a reduction of 2% (Norway spruce) or 4% (beech and birch) in annual growth of young trees under experimental conditions: POD1 = 5.2 mmol m^{-2} for beech and birch, and 9.2 mmol m^{-2} for Norway spruce in continental and Atlantic areas; 13.7 mmol m^{-2} for deciduous oaks in Mediterranean area (CLRTAP 2017). Braun et al. (2014) performed an epidemiological analysis of stem increment data for adult trees in Switzerland over the time period 1991–2011. They estimated 4.4% growth reduction for *Fagus sylvatica* at POD1 = 4.0 mmol m^{-2} and 1.9% of growth reduction for *Picea abies* at POD1 = 8.0 mmol m^{-2}. To date, policymakers continue to use the AOT40 index in Europe, which is more practical in use, thus we recommend using generic CLec of 17,000 and 19,000 nmol mol^{-1} h AOT40 for conifers and broadleaved species, respectively. Sicard et al. (2016c) suggested CLec of 15,000 and 24,000 nmol mol^{-1} h AOT40 for coniferous and broadleaved tree species, respectively. They proposed CLec of 12,000 nmol mol^{-1} h for high O_3 sensitivity coniferous (*Pinus cembra*); 24,000 nmol mol^{-1} h for moderate O_3 sensitivity coniferous (*Pinus halepensis*); 21,000 nmol mol^{-1} h for high O_3 sensitivity broadleaved species (*Fraxinus excelsior*); and 23,000 nmol mol^{-1} h for moderate O_3 sensitivity broadleaved species (*Fagus sylvatica*). A monitoring network, like MOTTLES, but at larger scale and additional epidemiological studies are needed to refine the CLef by expanding the range of vegetation, climatic and soil characteristics, and O_3 data.

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