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ORIGINAL PAPER

Testing visible ozone injury within a Light Exposed Sampling Site as a proxy for ozone risk assessment for European forests

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Abstract Biologically meaningful and cost-effective indicators are needed for assessing and monitoring the impacts of tropospheric ozone (O_3) on vegetation and are required in Europe by the National Emission Ceilings Directive (2016). However, a clear understanding on the best suited indicators is missing. The MOTTLES (*MOnitoring ozone injury for seTTing new critical LEvelS*) project set up a new generation network for O_3 monitoring in forest plots in order to: 1) estimate the stomatal O_3 fluxes (Phytotoxic Ozone Dose above a threshold *Y* of uptake, PODY); and 2) collect visible foliar O_3 injury, both within the forest plot (ITP) and along the Light Exposed Sampling Site (LESS) along the forest edge. Nine forest sites at high O_3 risk were selected across Italy over 2017 – 2019 and significant correlations ($p < 0.05$) were found between the percentage of symptomatic plant species within the LESS, and POD1 (PODY, with $Y = 1$ nmol O_3 m^{-2} s^{-1}) calculated for mixed forest species ($r = 0.53$) and with the occurrence and severity of visible foliar O_3 injury on the dominant species in the plots ($r = 0.65$). A generic flux-based critical level for mixed forest species was derived within the LESS and it was recommended using 11 mmol m^{-2} POD1 as the critical level for forest protection against O_3 injury, similar to the critical level obtained in the ITP (12 mmol m^{-2} POD1). It was concluded that the frequency of symptomatic plant species within a LESS is a suitable and effective plant-response indicator of phytotoxic O_3 levels in forest monitoring. LESS is a non-destructive, less complex and less time-consuming approach compared to the ITP for monitoring foliar O_3 injury in the long term. Assessing visible foliar O_3 injury in the ITP might only underestimate the O_3 risk assessment at individual sites. These results are biologically meaningful and useful to monitoring experts and environmental policy makers.

Keywords Cost-effective indicator · Forest monitoring · Light-Exposed Sampling Site · Ozone · Phytotoxic ozone dose · Visible injury

Introduction

Tropospheric ozone (O_3) is a major air quality issue worldwide (Sicard et al. 2017, 2020a, 2021; Sicard 2021) with adverse effects on biodiversity (Agathokleous et al. 2020) and forest health (Paoletti 2007). Major damages can be the impairment of photosynthesis and stomatal functions (Hoshika et al. 2017), O_3 visible injury such as necrosis and stippling on leaves (Sicard et al. 2016a; Moura et al. 2018), and a reduction of growth (Proietti et al. 2016).

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For O_3 risk assessment to European forests, the Phytotoxic Ozone Dose (PODY), defined as the amount of O_3 absorbed into the leaves or needles through stomata over an accumulation time period and above a threshold Y of detoxification for trees ($Y = 1$ nmol O_3 m^{-2} s^{-1} per leaf area), is suggested as a new legislative standard in Europe (Lefohn et al. 2018; De Marco and Sicard 2019; Sicard et al. 2020b). As stated in Article 9 of the revised National Emission Ceilings Directive (NEC 2016), "*Member States shall ensure the monitoring of negative impacts of air pollution upon ecosystems through a cost-effective and risk-based approach, based on a network of monitoring sites…*" (De Marco et al. 2019). According to the Convention on Long-Range Transboundary Air Pollution (CLRTAP) and its International Cooperative Programs (ICP Forests, ICP Integrated Monitoring), the assessment, monitoring and analysis of the effects of air pollution on European forests includes the assessment of visible foliar O_3 injury and crown defoliation as forest-health indicators in forest monitoring (Schaub et al. 2016). Contrary to crown defoliation, visible foliar O_3 injury is an unequivocal sign of phytotoxic O_3 levels (Paoletti et al. 2019), is not caused by any other co-occurring factors (Sicard et al. 2016a) and can occur even at annual O_3 mean concentrations lower than 30 nmol mol^{-1}, e.g., in Baltic countries (Girgždienė et al. 2009; Araminienė et al. 2019).

For assessing the negative O_3 effects on vegetation, biologically meaningful and cost-effective indicators in line with the NEC Directive are needed. As requested by the ICP-Forests Manual on Assessment of Ozone Injury, at each ICP-Forests plot across Europe, visible foliar O_3 injury is assessed both 'In The Plot' (ITP) and along a Light Exposed Sampling Site (LESS) (Schaub et al. 2016). At each ITP, the percentage of foliar surface affected by O_3 injury is scored for 25 samples (five trees x five sunlight-exposed branches with at least 30 needles/leaves per branch or needle age class), while within the LESS, only the percentage of symptomatic species over the total number of species of the forest edge is reported (Schaub et al. 2016). According to ICP Integrated Monitoring, only the ITP assessment is required (www.syke.fi/nature/icpim).

The objective of this study was to determine if epidemiological surveys of visible foliar O_3 injury within a LESS can be used as suitable indicators of O_3 risk assessment to forests in order to derive POD-based critical levels for forest protection. Here, the critical level is defined as the "cumulative stomatal O_3 flux above which visible foliar O_3 injury may occur on sensitive tree species" (Sicard et al. 2016a). For this study, we used the data collected within the European LIFE MOTTLES (*MOnitoring ozone injury for seTTing new critical LEvelS*) project (Paoletti et al. 2019) that set up a network of forest sites for O_3 monitoring in France, Italy and Romania to estimate POD1 (PODY, with $Y = 1$ nmol O_3 m^{-2} s^{-1}) and collect forest-response indicators within ITP and LESS over the period 2017 – 2019.

Materials and methods

Monitoring network and data collection

Within the MOTTLES network, we selected forest sites at higher O_3 risk, i.e., sites where O_3 levels were high enough to negatively affect trees by inducing typical visible foliar O_3 injury in the ITP and/or in the LESS, and with at least 75% of validated hourly O_3 and meteorological data per year over the period 2017 – 2019 (Table 1). The nine selected Italian sites represent a complex patchwork of climate and vegetation between Africa and European mid-latitudes (Paoletti 2006). Following recommendations of the ICP Forests monitoring manual (Ferretti et al. 2017), meteorological and O_3 values are recorded in open areas nearby ITP and LESS, while soil moisture is recorded in the ITP. A full description of the monitoring stations is available in Paoletti et al. (2019). Each station is equipped with sensors for air temperature, relative humidity, rainfall, solar radiation, wind speed and direction, soil moisture at a 10-cm depth, and surface O_3 concentrations. All data are continuously measured and are available as hourly values.

Visible foliar ozone injury

The assessment of visible foliar O_3 injury was carried out annually at each site, both in the plot (ITP) and along the Light Exposed Sampling Site (LESS).

Within the ITP, the scoring of visible foliar O_3 injury was performed each year on the same five trees, randomly selected, of the dominant species at that site (Table 1). On each tree, five branches of the sun-exposed upper part of the crown were removed and observed by two trained surveyors. For deciduous species, current year leaves/needles were assessed. For evergreen species, current, one-year-old and two-year-old leaves/needles were assessed and scored, separately. For each leaf/needle and age class, the percentage of area affected by O_3 injury was scored and averaged for the five branches, resulting in one mean value per tree. A mean percentage of needle/leaf surface affected by O_3 injury was calculated per plot (Schaub et al. 2016). If injury was unclear or doubtful, the sample was excluded.

As defined by Schaub et al. (2016), the LESS is a light-exposed forest edge (maximum radius of 500 m). Length and width of the LESS were 30 m and 1 m, respectively (Fig. 1). A total of 15×2 m^2 non-overlapping quadrates was defined and two randomly excluded. In each quadrate, the plant species were listed and the presence or absence of visible O_3 injury was recorded on the same day that the ITP survey

Table 1 Annual average ± standard deviation for air temperature (Temp, °C), relative humidity (RH, %), solar radiation (S. rad, W m^{-2}), soil water content (SWC, %), rainfall (Rainfall, mm), 24-h ozone concentrations (Ozone, nmol mol^{-1}), POD1 (mmol m^{-2}) in the plot (POD1_ITP) and within the Light Exposed Sampling Site (POD1_LESS), and visible foliar ozone injury on the dominant tree species in the plot (VI_ITP, mean percentage of injured light-exposed leaf surface, %) and the percentage of symptomatic plant species within the Light Exposed Sampling Site (VI_LESS) over the time period 2017 – 2019 across Italy

Site code	Latitude	Longitude	Dominant tree species	Elevation (m asl)	Temp	RH	S. rad	SWC	Rainfall	Ozone	POD1_ITP	VI_ITP	POD1_LESS	VI_LESS
ABR1	41.86°N	13.57°E	*Fagus sylvatica*	1500	7.2 ± 0.1	81.0 ± 2.3	155.3 ± 7.9	31.5 ± 1.0	1245 ± 158	55.7 ± 3.4	15.8 ± 7.5	10.8 ± 5.5	22.7 ± 9.6	25.0 ± 11.7
CPZ1	41.70°N	12.36°E	*Quercus ilex*	0	16.2 ± 0.1	79.7 ± 0.8	175.1 ± 8.6	14.4 ± 0.6	817 ± 113	32.4 ± 2.4	10.7 ± 10.8	0	9.7 ± 7.9	0
CPZ2	41.70°N	12.36°E	*Phillyrea latifolia*	0	16.2 ± 0.1	79.7 ± 0.8	175.1 ± 8.6	14.4 ± 0.6	817 ± 113	32.4 ± 2.4	5.3 ± 6.4	0	9.8 ± 7.9	0
CPZ3	41.68°N	12.39°E	*Pinus pinea*	0	16.2 ± 0.1	79.7 ± 0.8	175.1 ± 8.6	19.9 ± 2.1	817 ± 113	32.4 ± 2.4	4.2 ± 1.7	0.3 ± 0.2	10.1 ± 4.1	0
EMI1	44.72°N	10.20°E	*Quercus petraea*	200	11.8 ± 1.7	70.4 ± 6.1	141.6 ± 19.9	15.6 ± 0.2	820 ± 296	40.6 ± 4.6	14.0 ± 4.9	0	7.8 ± 3.5	11.4 ± 12.6
LAZ1	42.83°N	11.90°E	*Quercus cerris*	690	13.3 ± 0.1	76.5 ± 2.9	154.6 ± 5.3	18.6 ± 0.1	1981 ± 755	47.8 ± 2.1	16.0 ± 0.7	0	9.4 ± 0.7	9.7 ± 2.0
PIE1	45.68°N	8.07°E	*Fagus sylvatica*	1150	6.5 ± 0.1	73.4 ± 3.9	129.6 ± 4.0	29.5 ± 0.2	2639 ± 171	50.7 ± 0.1	16.5 ± 0.6	1.9 ± 0.5	23.3 ± 1.0	27.7 ± 7.8
TRE1	46.36°N	11.49°E	*Picea abies*	1800	5.0 ± 0.1	73.8 ± 3.1	138.6 ± 6.1	27.9 ± 0.1	1062 ± 194	52.2 ± 0.2	23.4 ± 0.5	2.9 ± 0.2	28.8 ± 2.1	20.0
VEN1	46.06°N	12.39°E	*Fagus sylvatica*	1100	7.6 ± 0.1	86.6 ± 0.8	134.5 ± 2.1	40.0 ± 0.8	2199 ± 403	36.0 ± 0.6	27.6 ± 3.2	6.1 ± 1.4	37.1 ± 3.9	25.0

Fig. 1 Forest edge with Light Exposed Sampling Site (LESS) in yellow; length is 30 m and width 1 m; The number of possible 2-m non-overlapping quadrates is 13 (15 in total, 2 randomly excluded - marked with red cross - to adjust sample size)

was carried out. Finally, the frequency of symptomatic species i.e., percentage of symptomatic species over the total number of species on the forest edge was reported (Table 2).

Phytotoxic ozone dose calculation

A full description of PODY calculation, including parameterization for dominant tree species in the ITP, was published by Sicard et al. (2020b). PODY (mmol m^{-2}) was accumulated from the start date of the growing season (SGS) until the time of the visible O_3 injury survey (S) using hourly data:

$$PODY = \int_{t=SGS}^{S} \max[((g_{sto} \times [O_3]) - Y), 0] \cdot dt \qquad (1)$$

where, PODY is the accumulated stomatal O_3 flux above a detoxification threshold Y (nmol O_3 m^{-2} s^{-1}) over the accumulation period in hours with at least 50 W m^{-2} solar radiation, g_{sto} represents hourly values of stomatal conductance (mmol m^{-2} s^{-1}), [O_3] is hourly O_3 concentrations (nmol mol^{-1}) and dt is the time step (1-h). Stomatal conductance (g_{sto}) was calculated by the Jarvis (1976) multiplicative model, depending on functions related to phenology, irradiance, air temperature, vapor pressure deficit, and volumetric soil water content (Sicard et al. 2020b). A latitude model for phenology was used according to CLRTAP (2017). As recommended by CLRTAP (2017), we calculated PODY with $Y = 1$ nmol O_3 m^{-2} s^{-1} per leaf area, assuming that any O_3 molecule below this threshold will be detoxified by the plant. To calculate POD1, species-specific parameterizations available in the literature were used (Table S1) for each dominant tree species in the ITP (POD1_ITP). Based on plant species

Table 2 Symptomatic and asymptomatic species occurring along the Light-Exposed Sampling Sites (LESS) over the period 2017 – 2019

Site code	Symptomatic plant species	Asymptomatic plant species
ABR1	*Cornus sanguinea* *Fagus sylvatica* *Sorbus aucuparia*	*Prunus* spp.; *Rosa canina; Rubus hirtus; Rubus ideaus; Sorbus aria;Taxus baccata*
CPZ1 CPZ2 CPZ3		*Asparagus acutifolius; Phillyrea latifolia; Pinus pinea; Pistacia lentiscus* *Quercus ilex; Rosmarino officina; Smilax aspera; Viburnum* spp.
EMI1	*Carpinus betulus* *Rubus ulmifolius*	*Cornus sanguinea* *Crataegus* spp.; *Fraxinus excelsior;Fraxinus ornus; Juglans regia;Prunus spinosa; Quercus ilex; Quercus petraea; Robinia pseudoacacia; Rosa canina*
LAZ1	*Clematis vitalba* *Prunus spinosa* *Rubus ulmifolius*	*Cornus* sp.; *Crataegus monogyna; Euonymus* sp.; *Juniperus communis; Ligustrum* sp.; *Quercus cerris; Quercus pubescens; Rosa canina; Vitis vinifera*
PIE1	*Corylus avellana* *Fagus sylvatica*	*Betula pendula; Cytisus scoparius; Rubus hirtus; Rhododendron ferrugineum; Sorbus aucuparia; Vaccinium myrtillus*
TRE1	*Vaccinium myrtillus*	*Buxus* sp.; *Calluna vulgaris; Erica* sp.; *Juniperus communis; Picea abies; Pinus cembra*
VEN1	*Fagus sylvatica*	*Acer pseudoplatanus; Cornus sanguinea; Picea abies; Rubus ideaus; Sambucus nigra*

occurring within the LESS (Table 2), an averaged value for each parameter for mixed species (POD1_LESS) was calculated using species-specific parameterizations available in the literature (Table S1).

Statistical analysis

The data coverage in ABR1, PIE1 and LAZ1 was lower than 75% in 2017, and were excluded from the statistical analysis (n = 240). A multivariate statistical technique, the principal component analysis (PCA), was used to analyze the dependence among variables (air temperature, relative humidity, solar radiation, soil water content, elevation, rainfall, 24-h O_3 concentrations, POD1_LESS, VI_ITP, VI_LESS) within different sampling times (three years) and nine experimental sites. The PCA was used to visualize the dependence between variables affecting the occurrence and severity of visible O_3 injury within the LESS and ITP. The selection of the principal factors was based on those with eigenvalues greater than 1. The non-parametric Spearman rank correlation test was applied to this dataset to measure statistical dependence among variables. Following the methodology established by Sicard et al. (2016a, 2020b), we correlated POD1_ITP to visible foliar O_3 injury in the ITP and POD1_LESS with the percentage of symptomatic plant species within the LESS, by joining data from all sites and years to derive POD1-based critical levels (CLef). These values were calculated from significant flux-effect functions ($p < 0.05$) for 0% of visible foliar O_3 injury. *Statgraphics Centurion* was used for statistical analyses.

Results and discussion

Description of visible foliar ozone injury

Injuries includes stippling, chlorosis and necrosis (Sicard et al. 2010), and can be visually differentiated from other biotic and abiotic stressors, e.g., from road salt, drought, desiccation, fungi and insects, winter flecks and lightning, by in-hand observation of the symptoms: color, shape, pattern of development on the foliage, and occurrence in the crown (Schaub et al. 2010; Vollenweider et al. 2013). In broad-leaved species, O_3 injury is limited to the upper leaf surface, categorized as stippling, chlorosis, and fleck (Fig. 2). Stippling is characterized by interveinal, dot-like areas of tan, red, brown, purple or black pigmentation on the upper surface of the leaf. Chlorosis is a loss of chlorophyll (non-green pigmentation) and appears in relatively discrete patches known as mottles. Fleck is characterized by small, discrete areas of dead tissue in the palisade mesophyll. Ozone injury on conifer needles appears as tipburn (acute exposure) or chlorotic mottling (chronic exposure). Chlorotic mottling (discrete patches, yellow or light green) is the most common visible injury for conifers (Miller et al. 1996; Wieser et al. 2006). In the framework of the MOTTLES project, an atlas of visible O_3 injury has been elaborated and is available on https://mottles-project.wixsite.com/life/atlas-ozone-injury. Over the past decades a number of reviews have been published, ranking plant species according to their sensitivity to O_3, for instance based on the amount of ambient O_3 required to induce visible foliar injury (e.g., VanderHeyden et al. 2001; Bussotti and Gerosa 2002; Bussotti et al. 2003; Gerosa et al. 2003).

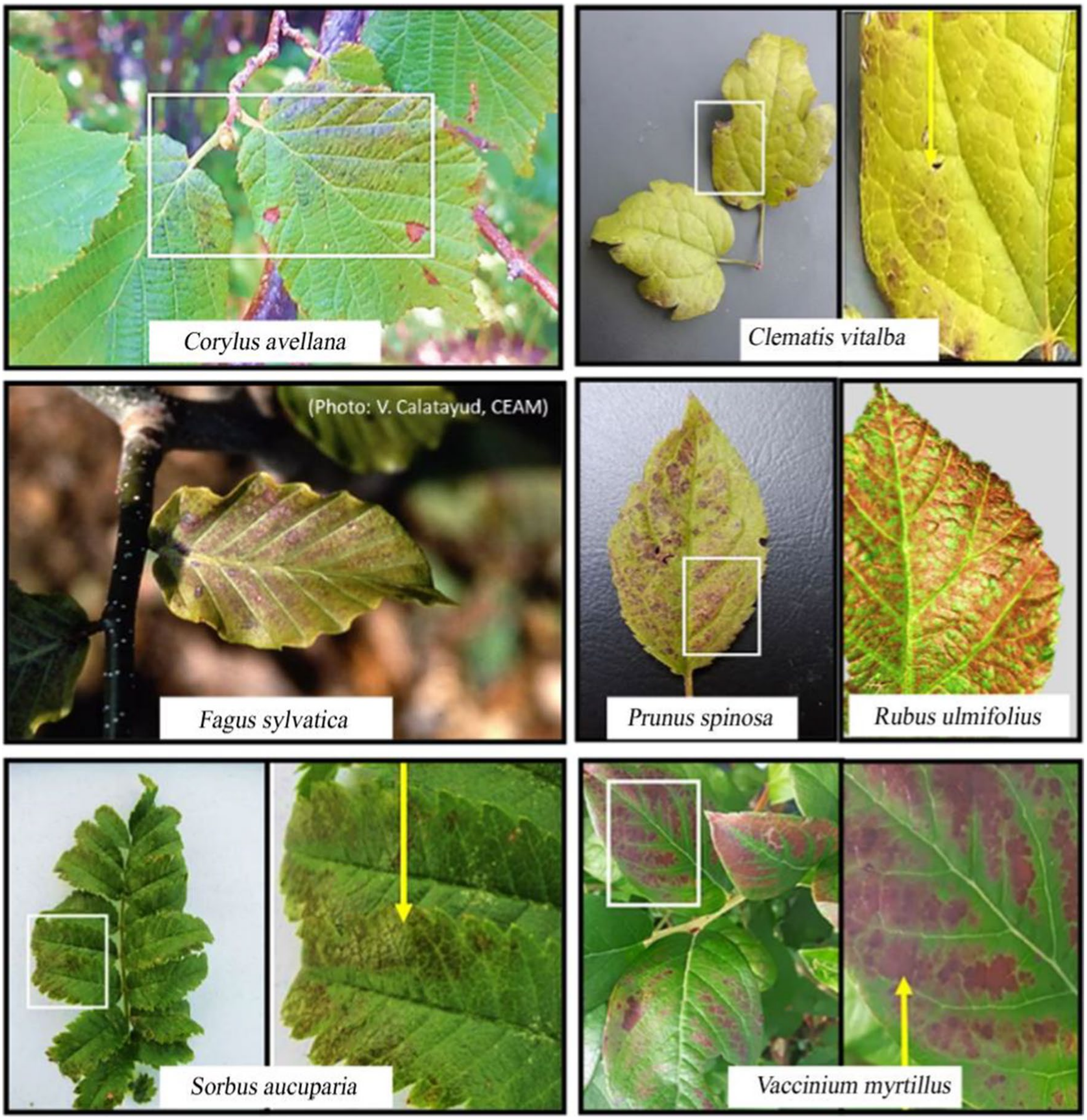

Fig. 2 Examples of visible foliar injury on species within the Light Exposed Sampling Sites in Italy (Pictures by E. Carrari-CNR, Y. Hoshika-CNR). Interveinal dark/brown/purple stippling on the upper leaf surface (e.g., *Fagus sylvatica, Corylus avellana*, *Prunus spinosa*) or interveinal reddening on the upper leaf surface (*Vaccinium myrtillus*). © 2019 by LIFE15 ENV/IT/000,183 MOTTLES

Within the ITP, the mean percentage of leaf surface of *Fagus sylvatica* L. affected by O_3 injury ranged from 1.9% (PIE1) to 10.8% (ABR1). *Picea abies* (L.) H. Karst. (2.9%) and *Pinus pinea* L. (0.3%) were less affected by O_3 injury, and sites with *Quercus* species (*Q. ilex*, *Q. cerris* and *Q. petraea*) and *Phillyrea latifolia* L. did not show any foliar injury in the ITP. These observations concur with the classification of *Quercus* species (i.e., *Q. ilex*, *Q. cerris* and *Q. petraea*) and *Picea abies* as O_3-tolerant and *F. sylvatica* as O_3-sensitive (VanderHeyden et al. 2001; Bussotti et al. 2003; Calatayud et al. 2011) reported in a previous epidemiological study carried out in 54 plots in south-eastern France and north-western Italy in 2012 and 2013 (Sicard et al. 2016a). The evergreen broadleaved species (e.g., *Quercus ilex*) are more O_3 tolerant than mesophilic broadleaf trees (e.g., *F. sylvatica*) in Italy (Paoletti 2006).

Within the LESS, the highest frequency of symptomatic plant species was observed in Piedmont (PIE1, 27.7%), Abruzzo (ABR1, 25.0%) and Veneto (VEN1, 25.0%) regions, while three sites close to Rome (CPZ1, CPZ2 and CPZ3) did not show foliar injury on any species. Visible foliar O_3 injuries were mainly on *F. sylvatica* and *Rubus ulmifolius* Schott (Table 2). In many LESS areas, injured individuals were also observed on species known to be sensitive to O_3 such as *Corylus avellana* L. and *Carpinus betulus L.* (VanderHeyden et al. 2001; Bussotti et al. 2003). In addition, a few shrubs were O_3-injured such as European blueberry (*Vaccinium myrtillus* L.) and sorb (*Sorbus aucuparia* L.), and the vine (*Clematis vitalba* L.). Among the symptomatic plant species observed in the MOTTLES network, *F. sylvatica* is considered as more O_3-sensitive relative to *C. betulus* and *C. avellana* (VanderHeyden et al. 2001; Bussotti et al. 2003). *S. aucuparia*, and *Vaccinium myrtillus* have often shown visible O_3 injury in the field (Bussotti et al. 2003) and the latter is more sensitive to O_3 than *S. aucuparia* (Hoshika et al. 2020a). In other LESS areas of the network in France and Romania, foliar O_3 injuries were also identified on species such as *Alnus glutinosa* (L.) Gaertn., *Fraxinus excelsior* L., *P. abies*, and *Sorbus aria* (L.) Crantz (Paoletti et al. 2019).

Within the LESS, the frequency of symptomatic plant species depends on the occurrence of O_3-sensitive ones relative to the total number of species at these sites. For the same species, the occurrence and severity of O_3 injury depend on various parameters and interactions, site and environmental conditions. For instance, *Cornus sanguinea* L. was found symptomatic in ABR1 and asymptomatic in EM1, LAZ1, and VEN1. By comparing ABR1 and VEN1 (mountainous stations), higher mean O_3 concentrations were recorded in ABR1 (56 nmol mol^{-1}) than in VEN1 (36 nmol mol^{-1}) where *C. sanguinea* was found as symptomatic. The species must be (1) genetically predisposed to be O_3-sensitive, (2) under optimal environmental conditions for O_3 uptake; and (3) exposed to ambient O_3 levels exceeding the threshold required for injury occurrence (VanderHeyden et al. 2001). Responses to O_3 vary by species, genotype, phenology, leaf age, position in the canopy, and nutrient availability (Tjoelker and Luxmoore 1991; Karnosky et al. 1996; Wieser et al. 2002; Percy et al. 2003; Schaub et al. 2005; Zak et al. 2011; Yuan et al. 2016).

Monitoring visible ozone injury within the LESS for ozone risk assessment for forests

The highest O_3 mean concentrations (55.7 nmol mol^{-1}) were measured in a high-altitude remote area of central Italy (ABR1), while the lowest (32.4 nmol mol^{-1}) were observed close to Rome (CPZ) over the period 2017–2019 (Table 1). The highest average concentrations are recorded in remote areas, in particular at high elevation stations (above 1200 m a.s.l.) with concentrations exceeding 40 nmol mol^{-1}, and lower levels are found in suburban areas (Sicard et al. 2016b). Our results are in agreement with previous studies performed in Italy (Sicard et al. 2020a). For instance, annual O_3 mean concentrations recorded were 33.4 nmol mol^{-1} and 24.9 nmol mol^{-1} in rural and suburban stations, respectively, over the period 2005–2014. Higher biogenic volatile emissions, lower O_3 titration by nitrogen monoxide (NO), and O_3 and/or precursors transported from urban areas are main factors to explain higher O_3 levels at remote sites compared to urban and suburban areas. Altitude reduces the O_3 destruction by deposition and NO and at high-elevation sites, the stratospheric O_3 inputs within troposphere and the solar radiation efficiency are more important (Sicard et al. 2016b).

The highest POD1 mean values in the plot (27.6 mmol m^{-2} POD1), and within the LESS (37.1 mmol m^{-2} POD1), were found in the Veneto region (VEN1), while the lowest POD1 values were measured in CPZ3 (4.2 mmol m^{-2} POD1_ITP) and EMI1 (7.8 mmol m^{-2} POD1_LESS). Even if lower O_3 mean concentrations were recorded, the highest POD1 values were measured in northeastern Italy (TRE1, VEN1), mostly due to the Alpine climate not limiting stomatal uptake as strongly as in the Mediterranean climate (e.g., CPZ1-3). In the Piedmont region, the modelled POD1 mean values in the plot with *Fagus sylvatica* (9.6 mmol m^{-2} POD1) in 2012–2013 (Sicard et al. 2016a) were lower than in PIE1 in 2018–2019 (16.5 mmol m^{-2} POD1). This is similar to the POD1 values for *F. sylvatica* (15 to 20 mmol m^{-2} POD1) at a humid site in Germany (Vollenweider et al. 2019). The large difference of POD1 is mainly due to the parameterization of the soil water content function. Soil water deficit may cause stomatal closure, thus limiting O_3 uptake (Hoshika et al. 2020b). A high POD1 difference (about 100%) was previously recorded

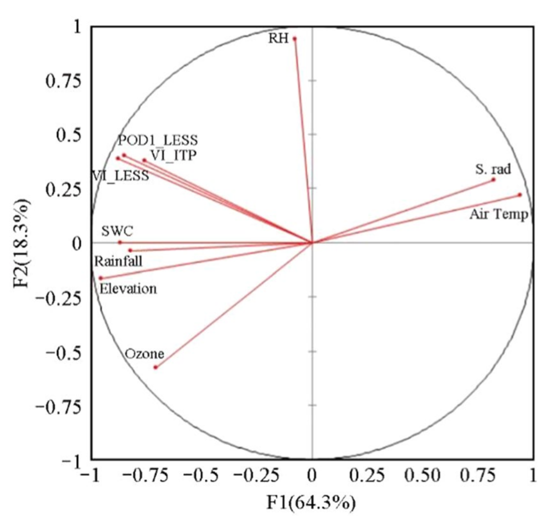

Fig. 3 Principal Component Analysis—Air temperature (Air temp), relative humidity (RH), solar radiation (S. rad), soil water content (SWC), site elevation, rainfall, 24-h ozone concentrations (Ozone), POD1 within the Light Exposed Sampling Site (POD1_LESS), and severity of visible foliar ozone injury on the dominant tree species in the plot (VI_ITP) and the percentage of symptomatic plant species within the Light Exposed Sampling Site (VI_LESS) over the period 2017–2019

Table 3 Flux-based critical levels (CLef) established by joining all Italian stations and years (n = 24); response functions were calculated in the plot (ITP) and within the Light Exposed Sampling Site (LESS) between POD1 and the mean percentage of visible ozone on the dominant tree species in a plot (VI_ITP), and the percentage of symptomatic plant species within the LESS (VI_LESS) over the period 2017 – 2019

Mixed species	Clef (mmol m^{-2} POD1)	Response function	*r*	*p* value	Standard error
ITP	11.8	POD1_ITP = 11.81 + 0.97 * VI_ITP	0.58	0.005	0.204
LESS	11.0	POD1_LESS = 11.06 + 0.51 * VI_LESS	0.53	0.007	0.170

Standard error, Spearman coefficients (r) and level of significance (p) for the flux-response relationships

for temperate *F. sylvatica* in northern Italy (De Marco et al. 2016).

Based on the PCA (Fig. 3) and Spearman correlations (Table 3), the frequency of symptomatic species within the LESS shows significant correlation with POD1 values calculated for mixed species within the LESS ($r = 0.53$; $p < 0.05$). As previously reported by Sicard et al. (2020b), visible foliar O_3 injury on the dominant tree species in the plot (VI_ITP) was correlated to POD1_ITP ($r = 0.58$; $p < 0.05$). The frequency of symptomatic plant species within the LESS was significantly correlated to the occurrence and severity of visible O_3 injury on the dominant tree species in the plot ($r = 0.65$; $p < 0.05$), even if a difference of relative severity can be noted between LESS and ITP (Table 1). The difference of severity within the LESS and ITP may be explained by: (1) a high number of different plant species within the LESS increasing the probability of finding O_3-sensitive species (Paoletti et al. 2019); (2) young trees, frequently within a LESS, are more sensitive to O_3 compared to mature trees (Nunn et al. 2005); (3) removing five branches of mature ITP trees every year is destructive sampling that may be damaging for the plant and not representative of the conditions of large crowns; and, iv) more visible O_3 injury is found on light-exposed leaves (Yuan et al. 2016). The assessment of visible O_3 injury on plants only in the ITP might underestimate the risk of O_3 impacts on forest trees.

Bussotti and Ferretti (2009) reported that the previous exposure-based index (i.e., AOT40) did not significantly correlate with the frequency of symptomatic species within the LESS in Italian forest sites. However, the good performance of PODY in explaining O_3 damages on forest trees has been recently recognized according to field monitoring data across Europe (Sicard et al. 2016a; Araminiene et al. 2019; Paoletti et al. 2019). As POD1_ITP and POD1_LESS were well- correlated to visible foliar O_3 injury on the dominant tree species in the ITP and the percentage of symptomatic plant species within the LESS, respectively, flux-response relationships were established to derive POD1-based critical levels in both areas for mixed species (Table 3, Fig. 4). We obtained a POD1-based critical level of 12 mmol m^{-2} POD1 in the ITP, mainly represented by broadleaved species, and 11 mmol m^{-2} POD1 within the LESS, also represented only by broadleaved species. For forest protection against O_3 injury in Europe, Sicard et al. (2020b) recommended a critical level (Clef) of 12 mmol m^{-2} POD1 for broadleaved species in the ITP. In addition, at the local scale, Hoshika et al. (2020c) recommended a CLef of 11 mmol m^{-2} POD1 for the LESS in the Piedmont region in north-western Italy. Previously, a CLef of 13.7 mmol m^{-2} POD1 was reported for deciduous oaks in the Mediterranean region related to a 4% reduction in annual tree growth (CLRTAP 2017).

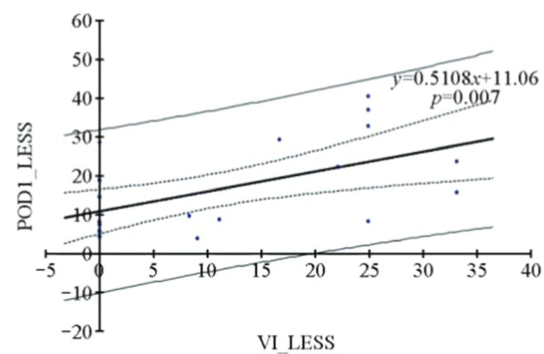

Fig. 4 Linear flux-response relationship (Spearman correlation) between POD1 within the Light Exposed Sampling Site (POD1_LESS) and the percentage of symptomatic plant species within the LESS (VI_LESS) over the period 2017–2019 (n = 24), with 95% confidence interval of observed (gray line) and predicted (dot-dashed gray line) values

Conclusions

As stated in Article 9 under the revised NEC Directive (2016), a cost-effective and risk-based approach is needed for assessing and monitoring harmful O_3 damage to vegetation. Many plants species respond to ground-level O_3 pollution with specific visible foliar injury, easily diagnosed in the field by trained surveyors. The ITP assessment of O_3 injury can lead to an underestimate of the O_3 risk to forest trees. The frequency of injured species at the forest edge, i.e., within the LESS, may be considered as an unequivocal plant-response indicator of phytotoxic O_3 levels in forest monitoring. Assessing visible foliar O_3 injury within the

LESS is less time-consuming (30 min) compared to the ITP assessment, from 30 min (no injury) to 60 min when the target species show O_3 injury. In most forest types (i.e., beech, spruce or fir forests), light exposed branches in the ITP are above 20 m. In these cases, samples cannot be taken with a pruner and more complex methods are required, such as tree climbers or leaf shooting. Furthermore, based on visual observations, the LESS assessment is not destructive and can be repeated over the long- term without affecting tree health. In addition, POD1-based critical levels for forest protection against visible O_3 injury are similar in the plot and in the LESS. These results are biologically meaningful and useful to monitoring experts and environmental policy-makers.

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