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

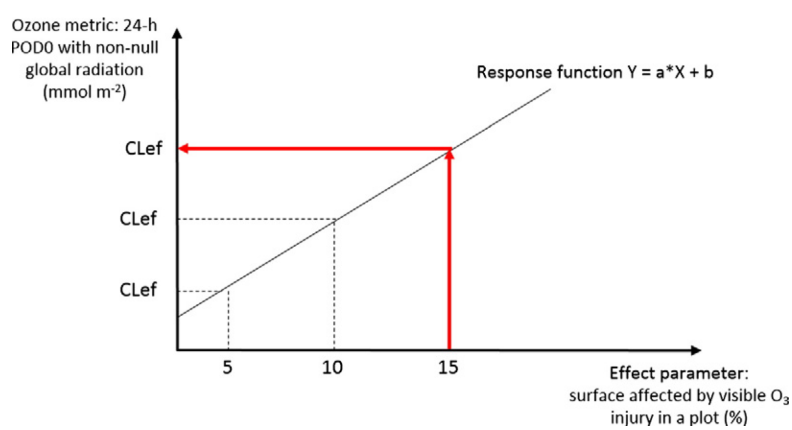
Pierre Sicard [a,*], Alessandra De Marco [b], Laurence Dalstein-Richier [c], Francesco Tagliaferro [d], Camille Renou [a], Elena Paoletti [e]

[a] ACRI-HE, 260 route du Pin Montard, BP 234, 06904 Sophia Antipolis cedex, France
[b] ENEA (Italian National Agency for New Technologies, Energy and Sustainable Economic Development), 76, Lungotevere Thaon de Revel, Rome, Italy
[c] GIEFS (Groupe International d'Etudes des Forêts Sud-européennes), 60, Avenue des Hespérides, 06300 Nice, France
[d] IPLA (Istituto per le Piante da Legno e l'Ambiente), Corso Casale 476, 10132 Turin, Italy
[e] IPSP-CNR (Consiglio Nazionale delle Ricerche — Istituto per la Protezione Sostenibile delle Piante), Via Madonna del Piano 10, 50019 Sesto Fiorentino (Florence), Italy

## HIGHLIGHTS

- We develop new flux-based critical levels CLef for forest protection against visible $O_3$ injury.
- We recommend the use of POD0 calculated for hours with a non-null global radiation.
- We propose CLef = 19 & 32 mmol $m^{-2}$ for high & moderate $O_3$ sensitive conifers respectively.
- We propose CLef = 19 & 25 mmol $m^{-2}$ for high & moderate $O_3$ sensitive broadleaves respectively.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Southern forests are at the highest ozone ($O_3$) risk in Europe where ground-level $O_3$ is a pressing sanitary problem for ecosystem health. Exposure-based standards for protecting vegetation are not representative of actual field conditions. A biologically-sound stomatal flux-based standard has been proposed, although critical levels for protection still need to be validated. This innovative epidemiological assessment of forest responses to $O_3$ was carried out in 54 plots in Southeastern France and Northwestern Italy in 2012 and 2013. Three $O_3$ indices, namely the accumulated exposure AOT40, and the accumulated stomatal flux with and without an hourly threshold of uptake (POD1 and POD0) were compared. Stomatal $O_3$ fluxes were modeled (DO3SE) and correlated to measured forest-response indicators, *i.e.* crown defoliation, crown discoloration and visible foliar $O_3$ injury. Soil water content, a key variable affecting the severity of visible foliar $O_3$ injury, was included in DO3SE. Based on flux–effect relationships, we developed species-specific flux-based critical levels (CLef) for forest protection against visible $O_3$ injury. For $O_3$ sensitive conifers, CLef of 19 mmol $m^{-2}$ for *Pinus cembra* (high $O_3$ sensitivity)

* Corresponding author.
*E-mail address:* pierre.sicard@acri-he.fr (P. Sicard).

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and 32 mmol m$^{-2}$ for *Pinus halepensis* (moderate $O_3$ sensitivity) were calculated. For broadleaved species, we obtained a CLef of 25 mmol m$^{-2}$ for *Fagus sylvatica* (moderate $O_3$ sensitivity) and of 19 mmol m$^{-2}$ for *Fraxinus excelsior* (high $O_3$ sensitivity). We showed that an assessment based on PODY and on real plant symptoms is more appropriated than the concentration-based method. Indeed, POD0 was better correlated with visible foliar $O_3$ injury than AOT40, whereas AOT40 was better correlated with crown discoloration and defoliation (aspecific indicators). To avoid an underestimation of the real $O_3$ uptake, we recommend the use of POD0 calculated for hours with a non-null global radiation over the 24-h $O_3$ accumulation window.



## 1. Introduction

Forests have significant functions for economic and recreational purposes (Kohut, 2005), environmental protection and nature conservation (Lorenz and Fischer, 2013). Surface ozone ($O_3$) is an important atmospheric pollutant, with harmful effects on crops and forests (Paoletti, 2006; De Marco et al., 2010; Mills et al., 2011; Sicard et al., 2011). Current levels are high enough to negatively affect trees by inducing visible foliar injury, decreased chlorophyll content in leaves, decreased photosynthesis, altered allocation of carbon and decreased fitness (Bytnerowicz et al., 2003; Dalstein et al., 2005; Karnosky et al., 2007a, b; Paoletti et al., 2009a; Mills et al., 2011; Büker et al., 2012; Fares et al., 2013). Our results confirm that surface $O_3$ levels in the South are higher than in the rest of Europe (Jonson et al., 2001; Konovalov et al., 2008; Vestreng et al., 2009; Sicard et al., 2013), and represent a potential threat to human well-being (WHO, 2008) and vegetation (Sanz et al., 2000; Paoletti, 2006, 2009a). Surprisingly, the detrimental impact of $O_3$ on Southern European forest ecosystems remains largely under-investigated (Paoletti, 2006).

Forests have many important functions for economic activity, nature conservation, environmental protection, and carbon sinks. Forests and woodlands of the Mediterranean region cover about 9% of the region's land area and constitute a unique world natural heritage in terms of biological diversity (FAO, 2013). The Mediterranean area is sensitive to climate change because it represents a transition zone between arid and humid regions of the world (Scarascia-Mugnozza and Matteucci, 2012). In addition, Southern Europe is representative of water-limited environments (dry and semi-dry habitats), that cover about 41% of Earth's land surface (Reynolds et al., 2007).

In Europe, forest monitoring has concentrated on crown defoliation and discoloration as indicators of forest health and vitality (Rossini et al., 2006; Fischer and Lorenz, 2011), while visible foliar $O_3$ injury is usually the first unequivocal marker of the presence of phytotoxic levels of $O_3$ (Grulke, 2003; Matoušková et al., 2010). Visible injury can be diagnosed by examination of leaves or needles (Grulke, 2003) and is useful for biomonitoring of $O_3$ pollution (Bergmann et al., 1999). In Europe, $O_3$-induced chlorotic mottles, light-green spots, stippling and leaf necrosis have been reported for forest trees (Sanz et al., 2000; Innes et al., 2001; Calatayud and Cerveró, 2007; Novak et al., 2008; Paoletti et al., 2009b) and confirmed by microscopical analysis (Wieser, 1999; Günthardt-Goerg et al., 2000; Sanz et al., 2000, 2001; Dalstein et al., 2005; Wieser et al., 2006; Paoletti et al., 2009c; Sicard et al., 2010). Injury occurrence depends on various parameters and interactions (Kohut, 2005). The plant species must be (i) genetically predisposed to be $O_3$ sensitive, (ii) under optimal environmental conditions for $O_3$ uptake (temperature, humidity, solar radiation, soil water content) and (iii) exposed to ambient levels of $O_3$ that exceed the threshold required for injury occurrence (Grulke, 2003; Emberson et al., 2007; Schaub et al., 2010; Büker et al., 2011). Responses to $O_3$ vary by species, genotype, plant phenology, leaf age, position in the canopy (Wieser et al., 2000; Lee et al., 2002; Schaub et al., 2005; Karnosky et al., 2007a,b) and nutrient availability (Tjoelker and Luxmoore, 1991).

Ozone-exposure critical levels (CLec) were proposed for the protection of vegetation under the framework of the Convention on Long-Range Transboundary Air Pollution (CLRTAP) of the United Nations Economic Commission for Europe (UNECE) and are the base of the Ambient Air Quality Directive 2008/50/EC of the European Union. Critical levels are defined as the "concentration, cumulative exposure or cumulative stomatal flux of atmospheric pollutants above which direct adverse effects on sensitive vegetation may occur according to present knowledge" (UNECE, 2010). To protect vegetation, current European standards use the $O_3$ exposure index AOT40, *i.e.* the cumulative exposure to $O_3$ hourly concentrations exceeding 40 ppb over the daylight hours of the growing season (Paoletti and Manning, 2007). Ozone effects on vegetation depend not only on the atmospheric concentrations but also on $O_3$ uptake through the stomata. The stomatal flux-based approach estimates the amount of $O_3$ that is absorbed into the leaves or needles through stomata and integrates the effects of multiple climatic factors, vegetation characteristics and local and phenological inputs on $O_3$ uptake or flux (Paoletti and Manning, 2007). Phytotoxic Ozone Dose above a threshold Y of uptake (PODY) is the accumulated stomatal $O_3$ flux over the growing season and can be modeled using the Deposition of Ozone and Stomatal Exchange model (DO3SE; UNECE, 2010). Currently, PODY is under discussion as new European legislative standard and a validation of the threshold Y under field conditions is still missing.

To date, most experiments to establish biologically relevant plant responses to $O_3$, such as visible foliar injury, have been performed on seedlings under controlled conditions that are not representative of actual field conditions, so that the results may not help developing realistic standards (Paoletti and Manning, 2007; Matoušková et al., 2010; González-Fernández et al., 2013). Epidemiological studies, where large-scale biological responses are compared with field data, may provide useful information for establishing the best standards and thresholds for forest protection from $O_3$ (Paoletti and Manning, 2007) and deriving new stomatal flux-based critical levels (CLef) for forest protection against visible $O_3$ injury.

In this study, stomatal $O_3$ fluxes were modeled (DO3SE) and correlated (Spearman test) to real-world forest-response indicators, *i.e.* crown defoliation, crown discoloration and visible foliar $O_3$ injury, as assessed during field surveys carried out at 54 plots in 2012 and 2013. Three $O_3$ indices, namely the accumulated exposure AOT40, and the accumulated stomatal flux with and without an hourly threshold of uptake (POD1 and POD0, respectively) were compared in South-eastern France and North-western Italy. From the statistically significant flux–effect relationships, new species-specific CLef were proposed for forest protection against visible $O_3$ injury.

The aims of this study were (i) to evaluate the performance of $O_3$ risk metrics, *i.e.* POD0, POD1 and AOT40, by a large-scale field investigation in Southern Europe, (ii) to define which threshold Y is the most biologically-based; (iii) to determine the best time-window of PODY accumulation; (iv) to assess the most important environmental variables that affect crown defoliation, discoloration and visible foliar $O_3$ injury of adult trees under field conditions; (v) to suggest new epidemiologically-based $O_3$ critical levels for forest protection against visible $O_3$ injury, and (vi) to rank forest species on the basis of their sensitivity to ambient $O_3$ in mature trees.