LIFE15 ENV/IT/000183

MOTTLES guidelines

How to assess the effectiveness of air pollution control strategies for ecosystem protection

1. POLLUTION, CLIMATE CHANGE AND FORESTS

Climate change and air pollution are interlinked challenges, since they influence each other through complex interactions in the atmosphere. Thus, air pollutants control strategies have direct effects on climate change and *vice-versa*. **Tropospheric ozone (O_3) is the third most important greenhouse gas in terms of radiative forcing**, and one of main air pollutants regulated in the European Union. **Tropospheric O_3 is a secondary pollutant, meaning that it is not emitted directly, but forms when sunlight triggers reactions between natural and human-caused chemical emissions, known as O_3 precursors.** Emissions from vehicles, power plants, industrial operations, and other human activities are a primary cause of surface O_3. As O_3 concentrations are highly dependent on environmental conditions, including air temperature, its concentrations are expected to increase with climate change.

Tropospheric O_3 is one of the most serious air pollutants for human health in Europe today: high levels of O_3 can affect the respiratory system and increases mortality. **In addition, O_3 seriously threatens plants by producing biochemical and physiological changes with inhibition of carbon assimilation by photosynthesis especially when it penetrates to the intercellular spaces through stomata (small pores on the leaf surface).** Once penetrated inside leaves, O_3 causes cellular damage: reducing stomatal conductance, decreasing CO_2 assimilation rates and producing visible leaf injury.

These effects often accelerate senescence, diminish leaf area and biomass, and reduce photosynthetic capacity productivity. Such damage may be exacerbated by environmental stress. Hence, O_3 pollution has large impacts on plant function, and consequently on forest ecosystem services.

Key message: ozone is the most relevant air pollutant affecting forest vegetation, thus control is a key tool to protect vegetation and at the same time mitigate climate change effects.

THE MONITORING OF FORESTS

2.

Forest monitoring is a key step in the protection of forests from different threats related to air pollution and climate change. The monitoring of air pollution deposition to forest trees is indeed recommended in the Intensive Monitoring Programme of the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP forests) operating under the UNECE (United Nations Economic Commision for Europe) Convention on Long-range Transboundary Air Pollution (CLRTAP).

Monitoring air quality at forest sites together with forest characteristics, e.g. indicators of forest health, allows:

i) knowing the pollutant concentrations in the atmosphere;
ii) quantifying the adverse effects to forest trees and forest ecosystems;
iii) evaluating the effectiveness of the legislative precautions adopted.

At European level, O_3 is regulated with directives that limit emissions based on AOT40, i.e. a concentration index, calculated as sum of the hourly exceedances above the concentration of 40 ppb, for daylight hours (8am-8pm) during the growing season. Over these limits, national and regional regulatory agencies must provide countermeasures.

Thanks to monitoring and scientific studies, it was found that exceedances of these limits do not match the pollutant effects recorded as forest health indicators, therefore AOT40 does not seem to be an optimal standard for the protection of forests against O_3, at least in Mediterranean region during warm seasons and high ozone concentrations when stomata close in response to drought stress.

Key message: the standard to protect forests against negative impacts of ozone cannot be based on ozone concentrations only, because of the inconsistency between ozone concentration and occurred damage on trees.

3.

THE MOTTLES PROJECT

MOTTLES (LIFE15 ENV/IT/000183) was funded by the LIFE programme under the Environment and Resource efficiency sub-programme. **MOTTLES set the objective of establishing a new European forest monitoring aimed at defining new legislative standards biologically significant**, e.g. with real correspondence with the plant responses detected in forest. Hence, MOTTLES equipped a set of sites belonging to existing networks (ICP-forests, MERA) with **new instrumentation in order to obtain for the first time reliable data for the quantification of the Phytotoxic O_3 Dose (PODY).** PODY is a new and more effective metric proposed as legislative standard rather than AOT40 which is based on estimation of amount of O_3 entering stomata during a given time period.

This novel monitoring strategy is based on active monitoring, that means O_3 concentrations are recorded at forest sites by active sensors in real time (1-hour), instead of the traditional passive monitoring (where passive samplers are collected and analysed every two weeks). Real time O_3 concentrations are then combined with meteorological parameters and forest health indicators for O_3 on vegetation (visible O_3 foliar injury, crown defoliation and hourly radial growth) in order to estimate new scientifically-sound critical levels for O_3.

The new derived critical levels are proposed to be used as new legislative standards in Europe.

Key message: ozone standard to protect forests based on uptake into the leaf should be applied. To calculate such standard active monitoring in forests is needed.

THE MOTTLES MONITORING METHOD TO PROTECT FORESTS FROM O_3 POLLUTION

The MOTTLES method is developed by the following steps:

- **monitoring climatic variables and real time O_3 concentrations** at forest sites;
- **monitoring forest health indicators** by permanent sensors and field surveys;
- **calculating O_3 related metrics** (AOT40 and PODY);
- **processing correlations** between pollutant metrics and forest health indicators;
- **defining the critical levels** above which damage to plants occurs;
- **verifying where this limit is currently exceeded** in Europe.

The long-term monitoring network established by MOTTLES is active in three European countries at high and medium O_3 risk: Italy, France and Romania.

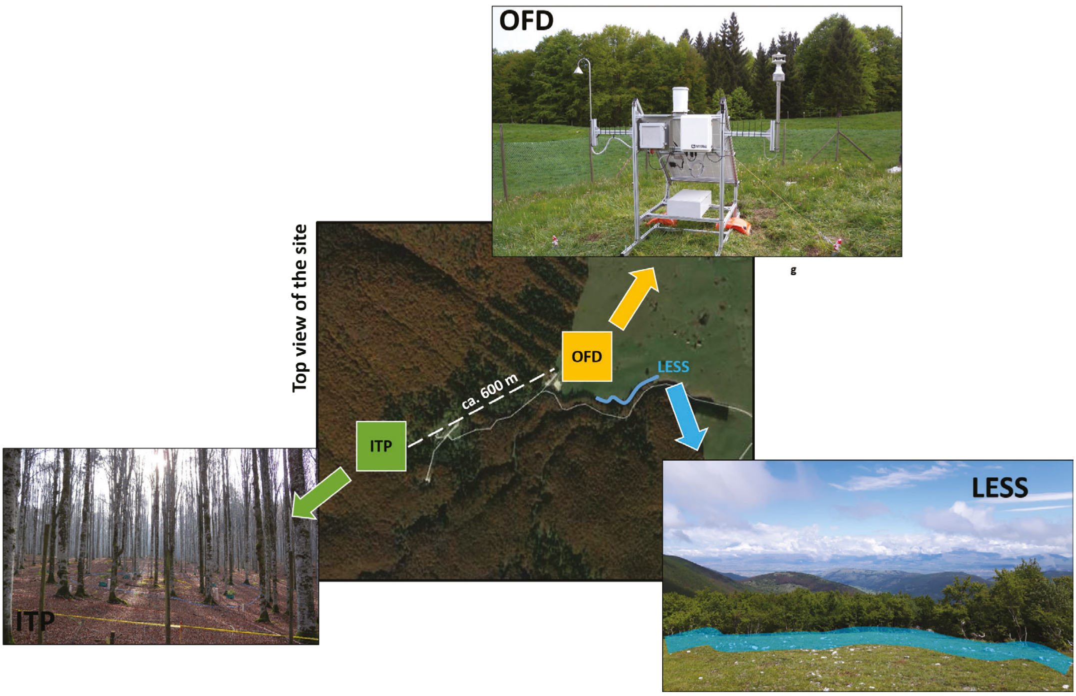
Key message: critical levels over Europe based on ozone fluxes should be calculated by an active measurement network.

5. MOTTLES MONITORING STATION SET UP

The selection of the forest site:

Monitoring for forest protection from O_3 can be conducted by focusing on a specific target that may be represented by a single forest species or forest type or biogeographical region. In Europe there are 9 biogeographical regions (Atlantic, Continental, Alpine, Mediterranean, Boreal, Macaronesica, Pannonica, Steppica and the Black Sea region), and more than 70 forest types, as defined by the European Environment Agency with the adoption of the 2000 Habitat directive.

MOTTLES selected 17 sites to represent a number of forest species, forest types, biogeographical areas and exposures to a range of O_3 pollution. The network covers large soil and climatic gradients, 4 biogeographical areas (Atlantic, Alpine, Continental and Mediterranean) and 9 major forest types of Europe, extending from the schlerophyll forests of the Mediterranean area at Castelporziano (Italy) to the mountainous beech forests of the Alpine region of Romania. The target



species are the most abundant tree species at each site, representing the forest type. The network hosts 11 target tree species (7 broadleaved and 4 coniferous). The mean annual temperatures range from 2°C in Fundata (Romania) to 17°C in Castelporziano (Italy).

The location of the forest stand must be suitable from an ecological and a logistic point of view i.e. it must be homogeneous for age, structure, density and tree composition, but also easily accessible. In addition, **the site must be suitable for the monitoring design i.e. meteorological and O_3 parameters must be recorded in open areas** (open field–OFD), while soil moisture and forest–health indicators must be recorded into the forest (in the plot–ITP). Therefore, a clearing must be present in the vicinity of the site. In the MOTTLES network, the average distance between OFD and ITP is 600 m.

MOTTLES Open Field Station:

Each OFD station must be equipped with sensors for rainfall, air temperature, relative humidity, air pressure, solar radiation, wind speed and direction while tropospheric O_3 must be measured by an active monitor providing a continuous real-time measurement of O_3 concentrations. All sensors must be installed at 2 meters above ground to avoid disturbances by the surrounding vegetation. Data acquisition interval can be in the order of seconds to minutes, and an average hourly value can be stored directly by a data-logger to facilitate post-processing routines. If a GPR connection is available, data can be transmitted to a central server via a GPRS modem to minimize personnel access to the site. In case of transmission failure, the data–logger memory ensures the integrity of acquired data so that the acquisition continues without interruption. Where GPRS is not available, data must be manually downloaded. Where the electrical system is not available, power supply can be assured by solar panels with backup batteries.

The real time data acquisition system for all data from field sensors, including O_3 concentrations, is the main novelty of this monitoring system. This allows i) avoiding frequent travels to sites, and ii) calculating the metrics AOT40 and PODY by using real data, instead of modelled data.

MOTTLES In The Plot Station:

Each ITP station must be equipped with soil moisture and soil temperature sensors placed at 10 cm depth, by avoiding areas around tree stems (1m) or other disturbances. Moreover, in the ITP a minimum of 4 dendrometers are installed on dominant or co–dominant trees selected among individuals of the target tree species. **This real time tree growth measure is used as one of the forest health indicators**, as explained below. Also in this case, data are stored by a data-logger every hour; data are transmitted by a GPR connection to a central server and where mains is not available, power supply is assured by solar panels with backup batteries.

Light-exposed sampling site (LESS)

The definition of the LESS must follow the ICP-forests manual, e.g. a 30-200 m long light-exposed forest edge (within a maximum radius of 500 m) centred around the OFD station. **In MOTTLES, the length of a LESS is 50 m**. After determining the start and end point of the LESS, the number of possible 2 x 1 m non-overlapping quadrates fitting into the selected forest edge must be determined. From this total number of non-overlapping quadrates, the size of the subset to be sampled is determined considering 10% error and their position along the LESS is randomly chosen, e.g. if the length of the LESS is 50 m, the total number of non-overlapping quadrats is 25, then the adjusted sample size is 25.

Key message: the selection of the appropriated site to derive critical levels for forest protection should follow a list of requirements established and revised in the MOTTLES project.

FIELD SURVEY OF FOREST - HEALTH INDICATORS

6.

In addition to sensors installed for environmental variables, the novel monitoring system must include annual field surveys and sensors to assess forest response indicators to O_3. The measured forest health indicators are:

• The visible foliar O_3 injury

Many plant species respond to ambient O_3 pollution with O_3-specific visible foliar injury that can be diagnosed in the field. **Visible foliar O_3 injury is considered as an important plant–health indicator in forest monitoring because it is specific of O_3 injury and is not caused by other co-occurring factors.** Conifers develop yellowish spots (chlorotic mottle), which in the most severe cases can degenerate into necrosis. Broadleaves develop a wide range of symptoms especially on the adaxial surface of the leaf, affecting the interveinal areas. Acute injury includes bleaching (small unpigmented necrotic spots), flecking (small necrotic areas), stippling (tiny punctuate spots were a few palisade cells are dead or injured, and may be white, black, red or red purple). Chronic injury consists in pigmentation (leaves turn red-brown to brown as pigments accumulate), chlorosis (due to chlorophyll breakdown) and premature senescence.
Surveys for visible foliar injury must be carried out by the same two people in each country from August to early–mid September, i.e. the time when

O_3 symptoms are more likely to be observed in the MOTTLES countries. The sampling is conducted at ITP on the dominant tree species and at the LESS on all woody species. The field protocols for the assessment of visible foliar O_3 injury by MOTTLES surveyors followed ICP–forests methodologies, slightly adapted to the MOTTLES specific conditions and it is available at the MOTTLES website https://mottles-project.wixsite.com/life. Annual inter–comparison exercises for survey teams are highly recommended.

• The crown defoliation

This is an important indicator for forest health, since O_3 may have a negative impact on crown defoliation, because of earlier leaf shedding. **The crown defoliation, estimated visually, expresses the loss of leaves/needles in the crown of a tree compared to a reference tree of the same species, with full foliage, in the immediate vicinity of the sample site or a photo image applicable to a tree species.** The assessable crown includes only those parts that are not influenced by other crowns i.e. by shading. Following ICP-forests, crown condition assessments are mandatory at least once a year.

The time of the assessment should be between the end of the first flush of foliage (when leaves and needles are fully developed) and the beginning of autumnal senescence. **For most species, the most suitable time for the assessment is from mid–July until end of August.** The assessments must be done during the same period and under similar conditions each year. In regions with scarce water availability or subjected to severe drought which may damage plants, the assessment may be shifted to early summer. Within each MOTTLES site, 20 predominant, dominant and codominant trees were selected for crown assessment. The intensity of each type of defoliation was recorded by 5% steps according to the ICP-forests protocol.

The forest growth

The forest growth, in term of tree radial growth, can be a forest health indicator for O_3, as O_3 can impair the photosynthetic carbon assimilation. Variations in stem radius must be continuously monitored. This is possible by automatic point dendrometers, installed on a minimum of 4 trees per site of the target species.

In MOTTLES we used linear variable transducers that measure the linear displacement of a stainless-steel sensing rod, which is pressed against the bark. The transducer was mounted on a frame attached to the stem by two titanium rods at 130 cm above the ground (breast height). As the stem expands or contracts, the rod transmits the signal to the transducer. The sensor output is converted into a numerical value (length of sensor in millimeters) using a linear calibration regression equation. In this case raw data must be averaged every hour. Daily dendrometer data are used to estimate seasonal stem radial growth, resulting by the sum of daily increments.

Key message: specific ozone damage (visible injury) should be assessed at forest sites to derive critical levels for ozone flux, in order to select indicators most properly linked to ozone pollution and not to confounding environmental factors.

7. THE STOMATAL OZONE FLUX

The O_3 effects on vegetation depend not only on the atmospheric concentrations, explicit in the present O_3 metric recommended by the air quality Directive 2008/50/EC i.e. AOT40, but **also result from the O_3 uptake (or flux) through the stomata into the plants.** The exposure index AOT40 cannot consider differences between tree species, forest types, plant genotypes, site conditions and does not take into consideration the physical, biological, and meteorological processes controlling the transfer of O_3 from the atmosphere into the leaf or needle.

Therefore, **a new metric has been proposed to protect vegetation, i.e. the Phytotoxic Ozone Dose, defined as the accumulated O_3 flux entering into the leaves via the stomata, over a detoxification threshold Y (PODY), integrating the effects of multiple climatic factors, vegetation characteristics, and local and phenological inputs on O_3 uptake or flux.**

For damage occurrence, the vegetation must be a) genetically predisposed to be O_3 sensitive; b) under optimal environmental conditions for O_3 uptake (temperature, relative humidity, solar radiation, soil water content) and c) exposed to ambient O_3 levels exceeding the threshold required for injury occurrence.

PODY (mmol m-2) is calculated from hourly data, by extrapolating the values measured at 2 m a.g.l. up to the actual crown height, as:

$$\text{PODY} = \int_{i=1}^{n} \left[\left(\left(g_{sto} \times [O_3]\right) - \text{Y}\right), 0\right] dt$$

where gsto represents hourly values of stomatal conductance estimated by the DO3SE model as recommended by the Convention on Long Range Transboundary Air Pollution manual (CLRTAP, 2017), $[O_3]$ is hourly O_3 concentrations (ppb) and dt = 1h. PODY is accumulated over the period recommended by the EC air quality Directive (i.e. 1st April to 30th September, 8-20h CET). Within MOTTLES, we evaluate any possible Y threshold (nmol O_3 m-2 PLA s-1) for effects on forest-health indicators. Here, however, we present only the results of Y = 1.

Key message: Phytotoxic Ozone Dose (POD) is an appropriated standard to estimate ozone damage to vegetation and evaluate effective protection measures for forests.

8. THE OZONE METRIC RECOMMENDED BY MOTTLES: PODY

The biologically-sound stomatal flux-based standard (PODY) is under discussion as new European legislative standard although critical levels for vegetation protection still need to be validated. The critical level approach was developed within the UNECE CLRTAP for assessing the risk of air pollution impacts to ecosystems and was applied for emission reduction strategies under the 1999 Protocol to Abate Acidification, Eutrophication and Ground-level O_3. 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"*. Since O_3 background concentrations are increasing, it is important to define appropriate and realistic critical levels, representative of actual field conditions, to **a)** protect vegetation; **b)** improve understanding and monitoring of O_3 effects on ecosystems; **c)** scientifically assess the effectiveness of air pollution control strategies and **d)** undertake measures for abatement of O_3 precursors emissions.
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. Indeed, **a standard for forest protection is biologically relevant when it translates into real-world forest impacts.** Epidemiological studies, where large-scale biological responses are compared with field data, may provide useful information for establishing the best standards and deriving new PODY-based critical levels (CL_{ef}) for forest protection against O_3 injury. We correlated PODY to real-world forest impacts in terms of different effect parameters, namely visible foliar O_3 injury, crown defoliation and radial growth.

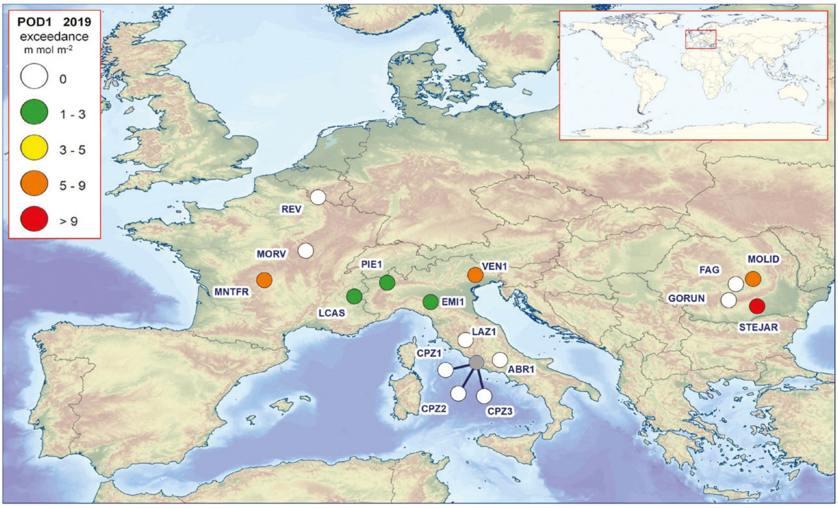
New CLef were derived by joining data from all plots and years. Based on these results, MOTTLES recommends visible foliar O_3 injury as effect parameter and POD1 as ozone metric.

Key message: MOTTLES results indicated visible ozone injury as suitable parameter to describe ozone impacts on vegetation, and POD1 as the ozone metric to be adopted to derive Critical Levels.

THE MAP OF CRITICAL LEVEL EXCEEDANCES

9.

Based on PODY-effect relationships, we developed species-specific flux-based critical levels for forest protection against visible O_3 injury. The suggestion of new critical levels for the protection of vegetation against O_3 will serve as a decision-support tool for European authorities. This tool is commonly used to anticipate negative effects of air pollution and, therefore, to protect ecosystems before the changes become irreversible. Over these limits, national and regional regulatory agencies must provide countermeasures. We obtained a CLef of 12 mmol m-2 for broadleaved species (that dominate the MOTTLES forest sites of MORV, FAG, GORUN, STEJAR, ABR1, CPZ1, CPZ2, EMI1, LAZ1, PIE1, VEN1) and of 5 mmol m-2 for conifers (at LCAS, MNTFR, REV, MOLID, CPZ3, TRE1) based on visible injury over the LESS and POD1.The map shows areas where such critical levels were exceeded in 2019, i.e. where air pollution control strategies for ecosystem protection were not effective, e.g. in 2019: LCAS and MNTFR in France; MOLID and STEJAR in Romania; EMI1, PIE1 and VEN1 in Italy.



Key message: Critical Levels maps give an overview of the potential damage of ozone on forests in specific years.

SUMMARY

MOTTLES deployed national networks of sites where long-term details on the best species-specific critical level, O_3 metric and Y threshold can be tested with the intent to estimate the new ozone (O_3) metric PODY, i.e. the stomatal uptake of O_3 above a threshold Y, based on actual measurements at field stations.

The National Emission Ceilings (NEC) Directive recommends calculating the exceedances of PODY-based critical levels for evaluating the impacts of O_3 pollution on forest ecosystems in Europe.

These guidelines summarize the main project outputs for establishing MOTTLES-type sites and networks, and for deriving O_3 pollution control strategies for forest ecosystem protection.

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