Bioindication of troposheric ozone by native vegetation: the potential of *Viburnum lantana* for large-scale surveys

Elena GOTTARDINI[1], Antonella CRISTOFORI[1,*], Fabiana CRISTOFOLINI[1] & Marco FERRETTI[2]

[1] IASMA Research and Innovation Centre, Fondazione Edmund Mach - Environment and Natural Resources Area, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[2] TerraData environmetrics, Dipartimento via L. Bardelloni 19, 58025 Monterotondo M.mo, Grosseto, Italy.

* Corresponding author: antonella.cristofori@fmach.it

Introduction

Because its oxidative power, tropospheric ozone is considered, on a large scale, the most harmful pollutant to vegetation (Ashmore, 2005). The exceedances of critical levels set to protect vegetation are quite high and widespread, so that large parts of crops and forests in Europe are exposed to potentially harmful levels of ozone (EEA, 2009). The impact of ozone on vegetation is the result of multiple factors such as the concentration in the atmosphere, the stomatal uptake - which depends on environmental and physiological factors - and the detoxification potential of plants. The complexity of these factors and their interactions can make it difficult to establish a clear relationship between ozone and plant response under field conditions, and therefore to evaluate whether an impact is actually occurring. The use of plants as bioindicators may be a solution because ozone-specific symptom expression reflects and summarizes all processes that occur between ozone exposure and the actual response of the plant (Sawidis et al., 2011).

The aim of this research is to explore the potential of the shrub species *Viburnum lantana* L. as an *in situ* bioindicator to assess the effects of ozone on native vegetation. This species is known to be sensitive to ozone (Novak et al., 2008; Orendovici et al., 2003; VanderHeyden et al., 2001), has a specific response (visible foliar injuries consisting in red stippling and general leaf reddening on the upper leaf surface of older leaves) (Innes et al., 2001), and a wide spatial distribution (Kollmann and Grubb, 2002). However the actual responsiveness to ozone of native plants and the relationship between the intensity of responses and the levels of exposure to the pollutant under field conditions remain to be evaluated (Doley, 2010). For these purposes, two field studies were carried out, at local (Gottardini et al., 2010) and large scale.

Material and methods

The study was carried out in Trentino, a sub-alpine region of North Italy.

Local scale study: time development of ozone symptom frequency

Two 1x1 km quadrates, 3 km apart, and characterized by different ozone levels (high and low), were considered. A completely randomized experimental design was utilized to select replicates and plants within each area. Plants were monitored for the development of ozone-specific foliar symptoms, the chlorophyll content (SPAD) and the fluorescence of chlorophyll *a* during the entire growing season (May-September 2009). Air temperature (T), relative humidity (RH) and ozone concentrations were measured over the whole study period.

Large scale study: relationship between ozone exposure levels and response intensity

For the second field study, the spatial domain was the entire surface of the Trento province (6,200 km^2). A stratified random sampling design (elevation x ozone) was adopted to select 30 1x1 km quadrates. The assessment of symptomatic plants was carried out within each quadrate.

Results and discussion

Local scale study: time development of ozone symptom frequency

Ozone symptoms occurred with higher frequency at the high ozone site. Analysing data in terms of cumulated differences between the two sites (high ozone site – low ozone site), the increase in differences of frequency of symptomatic *V. lantana* plants was consistent with the increasing differences in ozone exposure and RH (positive values), and with the increase in temperature cumulated differences (negative values) (Figure 1a). These findings are coherent with the hypothesis of ozone as causal agent.

At the same time of the onset and spread of foliar symptoms, a decrease in chlorophyll content (Chl SPAD) and photosynthetic performance (PItot) occurred (Figure 1b). In particular, the analysis of the fluorescence transient of chlorophyll *a* showed an early response to ozone of the I-P phase, which started to decrease at the high ozone site four weeks before the onset of symptoms. The I-P phase represents the efficiency of electron transport to reduce the final acceptors of the electron transport chain, i.e., ferredoxin and NADP.
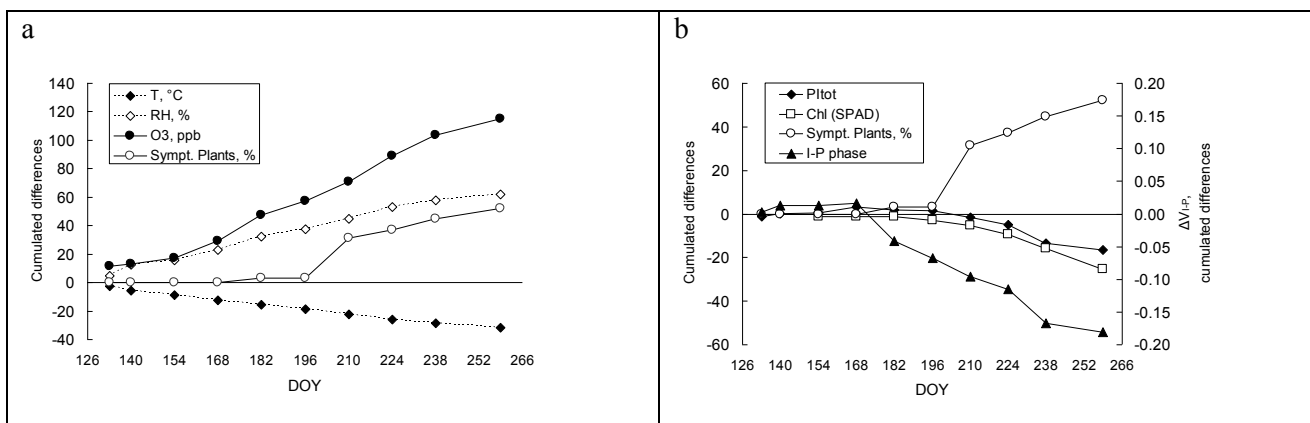

Fig. 1. Time development of the cumulated differences between the two sites (high ozone site – low ozone site) for frequency of symptomatic plants vs: (a) temperature, relative humidity, ozone concentrations; (b) chlorophyll content (Chl SPAD) and chlorophyll *a* fluorescence (PItot, I-P phase).

Large scale study: relationship between ozone exposure levels and response intensity

An higher frequency of symptomatic plants was observed at higher ozone levels (Figure 2).
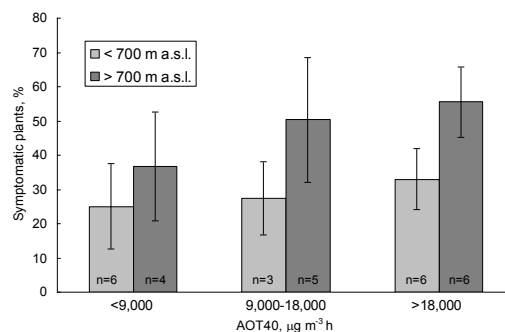
Fig. 2. Percentage of symptomatic plants per elevation and AOT40 stratum. Bars represent the standard error. The number of quadrates in each stratum is reported (n).

Interestingly, when comparing similar ranges of ozone exposures, symptomatic plants were always more frequent at higher altitudes (above 700 m a.s.l.); this may be due to the additional oxidative stress affecting plants at higher altitudes (e.g. due to solar radiation), and/or to an higher ozone uptake attributable to more favourable environmental conditions (high relative humidity and low temperature). However, when analysing the 30 individual values, frequency of symptoms was not significantly correlated to the level of ozone exposure.

Conclusion

Sensitivity and specificity of response of *V. lantana* to ozone was tested and verified under real field conditions. *V. lantana* seems suitable as *in situ* bioindicator to assess, on a qualitative basis, the potential impact of ozone on native vegetation. Failure in obtaining a statistical dose-effect relationship reflects the expected complexity of ozone-plant interaction and the inherent large variability of biological response. With these limitations, however, foliar symptoms on this species can be considered a valid response indicator of ozone, suitable for large-scale surveys and in remote areas.

References

Ashmore, M.R., 2005. Assessing the future global impacts of ozone on vegetation. Plant Cell and Environment 28, 949-964.

Doley, D., 2010. Rapid quantitative assessment of visible injury to vegetation and visual amenity effects of fluoride air pollution. Environmental Monitoring and Assessment 160, 181-198.

EEA, 2009. Assessment of ground-level ozone in EEA member countries, with a focus on long-term trends. European Environment Agency Report no 7/2009. ISSN 1725-2237. European Environment Agency Report no 7/2009. ISSN 1725-2237.

Gottardini, E., Cristofori, A., Cristofolini, F., Bussotti, F., Ferretti, M., 2010. Responsiveness of Viburnum lantana L. to tropospheric ozone: field evidence under contrasting site conditions in Trentino, northern Italy. Journal of Environmental Monitoring 12, 2237-2243.

Innes, J.L., Skelly, J.M., Schaub, M., 2001. Ozone and broadleaved species. A guide to the identification of ozone-induced foliar injury. Ozon, Laubholz- und Krautpflanzen. Ein Führer zum Bestimmen von Ozonsymptomen. Birmensdorf, Eidgenössische Forschungsanstalt WSL, Bern, Stuttgart, Wien.

Kollmann, J., Grubb, P.J., 2002. Viburnum lantana L. and Viburnum opulus L. (V-lobatum Lam., Opulus vulgaris Borkh.). Journal of Ecology 90, 1044-1070.

Novak, K., Schaub, M., Fuhrer, J., Skelly, J.M., Frey, B., Krauchi, N., 2008. Ozone effects on visible foliar injury and growth of Fagus sylvatica and Viburnum lantana seedlings grown in monoculture or in mixture. Environmental and Experimental Botany 62, 212-220.

Orendovici, T., Skelly, J.M., Ferdinand, J.A., Savage, J.E., Sanz, M.J., Smith, G.C., 2003. Response of native plants of northeastern United States and southern Spain to ozone exposures; determining exposure/response relationships. Environmental Pollution 125, 31-40.

Sawidis, T., Breuste, J., Mitrovic, M., Pavlovic, P., Tsigaridas, K., 2011. Trees as bioindicator of heavy metal pollution in three European cities. Environmental Pollution 159, 3560-3570.

VanderHeyden, D., Skelly, J., Innes, J., Hug, C., Zhang, J., Landolt, W., Bleuler, P., 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. Environmental Pollution 111, 321-331.

The response of the foliar anti-oxidant system of white willow to low-level air pollution

Tatiana Wuytack[1], Hamada AbdElgawad[2], Jeroen Staelens[3,4], Han Asard[2], Pascal Boeckx[3], Kris Verheyen[4], Roeland Samson[1]

1 Department of Bioscience Engineering, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium. E-mail: tatiana.wuytack@ua.ac.be, roeland.samson@ua.ac.be

2 Laboratory for molecular plant physiology and biotechnology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium. E-mail: hamadagh_2005@yahoo.com, han.asard@ua.ac.be

3 Isotope Bioscience Laboratory (ISOFYS), Department of Applied Analytical and Physical Chemistry, Ghent University, Coupure Links 653, B-9000 Gent, Belgium. E-mail: jeroen_staelens@yahoo.com

4 Forest & Nature Lab, Department of Forest and Water Management, Ghent University, Geraardsbergsesteenweg 267, B-9090 Gontrode (Melle), Belgium. E-mail: kris.verheyen@ugent.be

1. Introduction

Air pollutants may generate oxidative stress in plants [1], which in turn alters the intracellular redox environment [2] and generates excessive amounts of reactive oxygen species (ROS) [3]. ROS are not only comprised of free superoxide and hydroxyl radicals but also of molecules such as hydrogen peroxide (H_2O_2), singlet oxygen and ozone (O_3) [4] derived from photorespiration, the photosynthetic apparatus and mitochondrial respiration [3]. This enhanced ROS production can pose a threat to cells, giving rise to membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damage [4]. Lipid peroxidation leads to the production of malondialdehyde, which is seen as an indicator for a variety of abiotic and biotic stresses [6]. To protect cells under stressful conditions plant tissues contain enzymes for scavenging ROS (e.g., superoxide dismutase, catalase, peroxidase) and low-molecular mass anti-oxidants (e.g., flavonoids and phenols) [5]. For example, [7] found an increase of ascorbate and phenolics after O_3 exposure, which suggested the triggering of a defense mechanism to high O_3 concentrations. Scavenging of superoxide radicals is achieved by superoxide dismutase, while H_2O_2 is scavenged by catalase, ascorbate peroxidase, various other peroxidases and phenolic compounds [5]. Our knowledge about the above-mentioned biochemical adjustments of plants, caused by the exposure to air pollution, is mostly based on experiments where plants have been exposed to high concentrations of a single air pollutant during short periods, provoking acute damage under experimental conditions. Less information is gathered about the response of biochemical plant characteristics on longer-term exposure to multiple ambient air pollution sources in field conditions. Therefore, the main objective of this study was to investigate the response of biochemical leaf characteristics such as the content of anti-oxidant molecules and enzymes of white willow (*Salix alba* L.) to ambient concentrations of NO_x, O_3, SO_2 and fine particulate matter (PM_{10}).

2. Materials and methods

Sixteen air quality monitoring stations, located in Belgium, were selected as sampling locations for active biomonitoring with white willow (*Salix alba* L.). In the vicinity of each measuring station, twelve stem cuttings of white willow were individually planted in 3.5 dm3 pots with uniform potting soil (pH-H_2O 5.5). During the experimental period (April – September 2011), the plants were well watered by using a semi-automatic water supply system (see [1]). In September 2011, malondialdehyde (MDA), total antioxidant capacity (FRAP), polyphenol, flavonoid, ascorbate (ASC) and glutathione (GSH) content were measured, as