Journal of Experimental Botany, Vol. 61, No. 3, pp. 629–633, 2010
doi:10.1093/jxb/erp336 Advance Access publication 18 November, 2009

Journal of Experimental Botany
www.jxb.oxfordjournals.org

OPINION PAPER

Determinants of ozone fluxes and metrics for ozone risk assessment in plants

Silvano Fares[1,2,*], Allen Goldstein[2] and Francesco Loreto[3]

[1] CNR (National Research Council)–Istituto di Biologia Agroambientale e Forestale, Via Salaria km. 29,300, I-00016 Monterotondo Scalo (Rome), Italy
[2] Department of Environmental Science, Policy, and Management, University of California, 137 Mulford Hall, Berkeley, CA 94720, USA
[3] CNR (National Research Council)–Istituto per la Protezione delle Piante, Sesto Fiorentino (Florence), Italy

* To whom correspondence should be addressed: E-mail: silvano.fares@ibaf.cnr.it

Received 11 September 2009; Revised 21 October 2009; Accepted 27 October 2009

Abstract

Tropospheric ozone concentration is increasing and represents a threat to single plants and whole ecosystems. The deleterious ozone effects mainly occur when (i) ozone concentration in the air builds up; (ii) the pollutant enters the leaf through stomatal uptake, and (iii) ozone-produced reactive oxygen species are not efficiently scavenged by leaf antioxidants and then oxidize leaf tissues. The sensitivity of plants to ozone is species-specific, and a correct risk assessment should be based on a metric that correctly takes into account the ambient concentration of ozone, the physiological control on stomatal apertures, and the efficiency of leaf antioxidant system. Current methodologies have been analysed to evaluate ozone risk assessment, and, by phasing-in and phasing out sources and sinks of ozone, elements of improvements for the current metrics have been suggested.

Key words: Antioxidants, ozone, risk assessment, volatile isoprenoids.

Ozone impact on plants

Tropospheric ozone is considered to be one of the most dangerous oxidant molecules for plants and its concentration has been increasing in the troposphere, particularly in northern mid-latitudes (Brasseur *et al.*, 1998; UNECE, 2004; Parrish *et al.*, 2009). Plants may experience injuries when chronically exposed to ozone and injuries are often severe when acute ozone exposures occur. Ozone causes biochemical and physiological changes leading to the inhibition of photosynthesis and a consequent decrease in plant growth (Guderian *et al.*, 1985), often associated with visible injuries (Bussotti *et al.*, 2003; Vollenweider and Gunthardt-Georg, 2005).

Plants act as a sink for ozone, through stomatal and non-stomatal processes (Fig. 1). Stomatal conductance to ozone is the inverse of the sum of an array of resistances that ozone meets in specific locations along the path from the outside of the leaf to the reaction site inside the apoplast (Fares *et al.*, 2008). After entering the stomata, ozone reacts with the liquid components of the apoplast to create reactive oxygen species (ROS, mainly H_2O_2, but also OH radicals; Kangasjarvi *et al.*, 2005) that can oxidize the cell walls to start a cascade of reactions which lead, at the final stage, to cellular death. Ozone and ROS can also react with a multitude of apoplastic antioxidants, with ascorbic acid being the most representative (Dizengremel *et al.*, 2008). Another class of antioxidants is the volatile isoprenoids. These compounds were shown to contribute to ozone removal in the intercellular spaces and to protect plant tissues from oxidation, probably reacting with the very reactive oxygen species (e.g. $O^{\cdot}$, O^{-}) (Velikova *et al.*, 2004; Loreto and Fares, 2007), and with reactive nitrogen species generated by the first interaction of ozone with plant tissues (Velikova *et al.*, 2005, 2008; reviewed in Vickers *et al.*, 2009). Volatile isoprenoids may also contribute to gas phase chemical losses of ozone, reacting with this molecule in the leaf boundary layer. This phenomenon was found to be the main source of non-stomatal uptake, and in warm seasons was the highest source of ozone removal in a Mediterranean

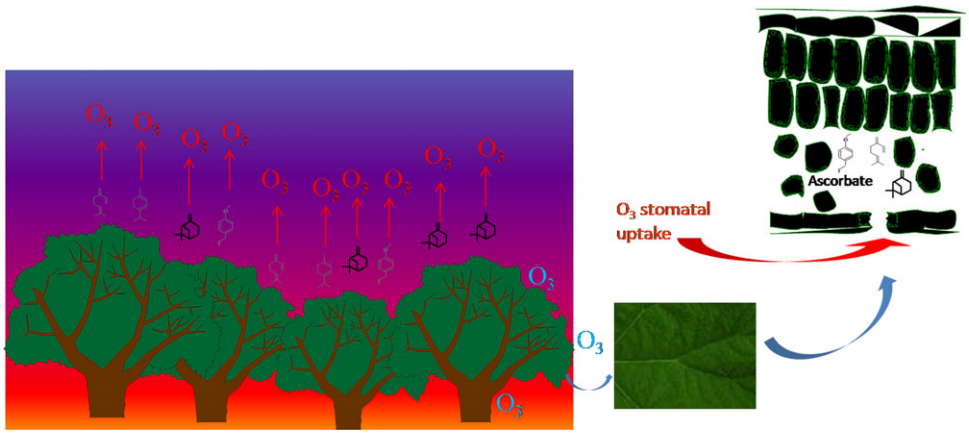

Fig. 1. Conceptual dynamic of ozone fluxes in a plant ecosystem: ozone reacts in the gas phase with BVOC (here drawn as molecules) contributing to the non-stomatal ozone sink. The blue ozone molecules represent the ozone deposition on the plant surfaces. The gradient from blue to red is the progressive decrease of tropospheric ozone concentration due to gas-phase reactions and leaf stomatal uptake. At a progressively smaller scale, detail of a leaf portion is shown and a vertical leaf section representing the cell tissue, with palisade and spongy cells, and with the apoplastic space rich in BVOC and ascorbate which detoxify ozone entering through the stomatal aperture.

ponderosa pine ecosystem (Kurpius and Goldstein, 2003) and in a Mediterranean holm oak forest (Gerosa *et al.*, 2005). Other sources of non-stomatal uptake are ozone deposition to soil, stems, and cuticles. These sources are considered of minor importance unless in conditions of surface wetness (Altimir *et al.*, 2004).

Metrics for ozone risk assessment

Several metrics have been developed for ozone risk assessment and plant damage (Musselman *et al.*, 2006; Paoletti and Manning, 2007). One class of metrics is based on the accumulated daytime ozone concentration. The European directives (UNECE, 2004) suggest the use of the AOT40 (Accumulated Ozone over a Threshold concentration of 40 ppb) for forest ecosystems. This metric only considers ozone concentrations in air that are above the 40 ppb threshold and which occur during daylight hours and over a specific solar radiation intensity as potentially damaging (Karenlampi and Skarby, 1996). However, ozone concentration in air is not always correlated to ozone flux into leaves (Kurpius *et al.*, 2002). The AOT40 index does not account for the physiological and ecological control of stomatal apertures, and for the effective amount of ozone entering leaves and oxidizing the apoplast.

A second class of ozone-risk metrics is based on accumulated stomatal fluxes above a phytotoxic threshold (Karlsson *et al.*, 2004). This is, in principle, a more tenable metric because it takes into account the effective amount of ozone responsible for plant injuries after entering stomata. Stomatal ozone fluxes are the product of the stomatal conductance (modelled in a multiplicative algorithm which takes into account the maximal stomatal conductance and all the phenological and environmental parameters affecting it; Emberson *et al.*, 2000) multiplied by the ozone concentration at the height of the top of the canopy. This value can be calculated with a deposition model which considers the ozone concentration at a certain height above the canopy and the resistances to ozone deposition on the canopy (UNECE, 2004). A flux-based method implies that a more species-specific approach is required than for an exposure-based method (Tuovinen *et al.*, 2007). Incorrect calculation of stomatal conductance would introduce a source of uncertainty, as would the application of canopy ozone concentrations if some deposition mechanisms are unexplained.

Recent studies have demonstrated that, in plant ecosystems emitting isoprenoids, especially monoterpenes and sesquiterpenes, two classes of biogenic volatile organic compounds that react quickly with ozone, non-stomatal ozone deposition can be the dominant process (Kurpius and Goldstein, 2003; Bouvier-Brown *et al.*, 2009). This implies that the ozone concentration at the canopy level can be less than the ozone concentration at the measuring height above the canopy. This ozone gradient increases under conditions of low vertical mixing. If this reduction in ozone concentration is not properly taken into account in ecosystems emitting isoprenoids, the stomatal fluxes would be overestimated.

Ozone sources and sinks are (often) out-phased over the day

Previous research (Massman *et al.*, 2000; Musselman *et al.*, 2006) introduced the importance of plant defence mechanisms for detoxifying ozone and suggested their inclusion in flux-based metrics for ozone-risk assessment. In a recent article, Heath *et al.* (2009) highlight how the ambient ozone concentration, stomatal conductance, and apoplastic ascorbate follow different diurnal trends (Fig. 2) causing a temporal decoupling of ozone exposure and ozone flux.

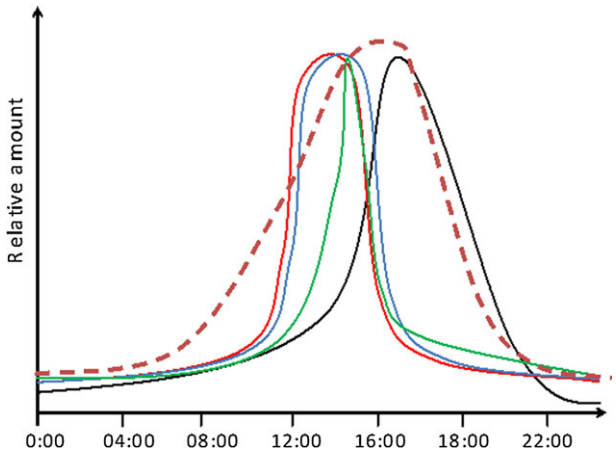

Fig. 2. Diurnal trends of stomatal conductance (red line), apoplastic reduced ascorbate concentration (blue line), isoprenoid emission (green line), tropospheric ozone concentration (black line), and isoprenoid intercellular concentration (brown broken line). All trends of emissions or concentrations are estimated on the basis of measurements performed in a *Pinus ponderosa* ecosystem in the Sierra Nevada mountains of California (Kurpius and Goldstein, 2003) and in a dune ecosystems dominated by holm oak in central Italy (Fares *et al.*, 2009). Data were collected under unstressed conditions and normalized to peak values during the day.

Ambient ozone concentration generally peaks in the afternoon (~16.00–18.00 h; Fig. 2) whereas maximal stomatal opening often occurs earlier (~13.00–15.00 h) (Kurpius and Goldstein, 2003; Massman, 2004; Fares *et al.*, 2009). In addition, plants in Mediterranean environments often experience a midday reduction in stomatal conductance in response to high temperatures and drought stress in the warmest central hours of the day (Raschke and Resemann, 1986). This causes the maximum stomatal conductance to occur earlier in the day (~10.00–11.00 h), and further decouples the diurnal cycles of stomatal opening and atmospheric ozone concentrations (Cieslik, 2004), thus reducing the potential oxidative ozone damage.

The capacity for the regeneration of antioxidants within the cell is driven by an 'oxidative signalling' process and is linked to appropriate changes in reducing power (NADPH), which depends on carbon metabolism (Dizengremel *et al.*, 2008). For this reason, the maximum level of apoplastic antioxidants, primarily ascorbate, and the activity of the enzymes involved in the ascorbate cycle peaks in a temporal window close to that of maximal stomatal conductance and photosynthesis, preceding the daily peak in ambient ozone concentration by several hours (Pelzer and Polle, 2000; Cheng and Ma, 2004).

Phenolic compounds, such as chlorogenic acid and quercetin, are also important antioxidants, and may protect plants against ozone (Yamaji *et al.*, 2003; Kontunen-Soppela *et al.*, 2007). The production of these compounds does not necessarily follow a diurnal cycle but is often temporally coincident with the antioxidant defences mentioned above and associated with structural acclimation of the leaves in response to high atmospheric ozone (Oksanen *et al.*, 2005).

Volatile isoprenoid emissions are well known to depend on light and temperature, and on the availability of photosynthetic intermediates, and thus also follow a similar daily pattern (Fig. 2) (Niinemets *et al.*, 2004). Thus both non-stomatal ozone deposition (in which isoprenoids are also involved; Fares *et al.*, 2008) and leaf antioxidant capacity trigger leaf protection during the phase of higher stomatal conductance in the central hours of the day. Whereas, during the afternoon hours, the stomatal-driven depression of photosynthesis may reduce carbon metabolism and allocation to antioxidant defences, thus triggering ozone injuries even under limited stomatal aperture.

It is noted, however, that volatile isoprenoids may accumulate inside leaves when stomata close, reaching concentrations much higher than those in the atmosphere (Loreto *et al.*, 1996). In fact, stomatal closure may dramatically increase leaf temperature, decoupling it from the outside air temperature (Singsaas *et al.*, 1999). Isoprenoid production increases exponentially with temperature (Loreto and Sharkey, 1990), and thus isoprenoid accumulation inside leaves is often enhanced in the afternoon, coincident with the depression of stomatal opening and high ozone concentrations in the atmosphere. Indeed, in Fig. 2, the concentration of isoprenoids, calculated from emission rates and stomatal conductances during summer days in a live oak ecosystem in the Mediterranean area (Fares *et al.*, 2008) and in a *Pinus ponderosa* ecosystem in the Sierra Nevada mountains of California (Kurpius and Goldstein, 2003) was the only process in phase with ozone accumulation in the air. Production of volatile isoprenoids inside leaves may, therefore, be a key process for detoxifying ozone, possibly by one of the mechanisms reviewed in Vickers *et al.* (2009), during daily peaks of ozone.

In summary, the mismatch between ozone concentrations and stomatal aperture generally makes exposure-based metrics less suitable than flux-based metrics. The works reviewed by Heath *et al.* (2009) correctly suggest that a stomatal-driven flux-based approach may establish a phytotoxic threshold of accumulated ozone fluxes, with emphasis on when stomatal conductance is high. We argue that this approach, however, does not take into account the temporal variability of defence mechanisms, and, in particular, the reduction of photosynthetically-generated antioxidants when photosynthesis is inhibited by stomatal closure. In these circumstances however, atmospheric ozone concentrations are very high and even a minor amount of ozone entering stomata may cause serious injuries if the antioxidant capacity is low.

Whereas the flux-based metric is the most suitable for ozone-risk assessment, apoplastic ascorbate may not be the only antioxidant to take into consideration in parameterizing ozone fluxes. Antioxidants such as phenolic compounds and volatile isoprenoids may also be important during peak episodes of tropospheric ozone. Phenolic compounds may play an important role during the night, especially under conditions when atmospheric ozone concentration remains

high and stomata do not totally close, for example in mountain areas characterized by strong valley breezes. The production of volatile isoprenoids is generally phased-in with peak ozone episodes during the day, and may importantly contribute to removing ozone inside the mesophyll. Given the complexity and interspecific differences in antioxidant systems, future research should investigate the relationship between oxidative leaf injury and antioxidant dynamics, as this could improve the predicting capacity of flux-based metrics.

References

Altimir N, Tuovinen JP, Vesala T, Kulmala M, Hari P. 2004. Measurements of ozone removal by Scots pine shoots: calibration of a stomatal uptake model including the non-stomatal component. *Atmospheric Environment* **38,** 2387–2398.

Bouvier-Brown NC, Goldstein AH, Gilman JB, Kuster WC, de Gouw JA. 2009. *In situ* ambient quantification of monoterpenes, sesquiterpenes, and related oxygenated compounds during BEARPEX 2007: implications for gas and particle-phase chemistry. *Atmospheric Chemistry and Physics Discussion* **9,** 10235–10269.

Brasseur GP, Kiehl JT, Müller JF, Schneider T, Granier C, Tie XX, Hauglustaine D. 1998. Past and future changes in global tropospheric ozone: impact on radiative forcing. *Geophysical Research Letters* **25,** 3807–3810.

Bussotti F, Cozzi A, Bettini D. 2003. Ozone like visibile foliar symptoms at the permanent monitoring plots of the CONECOFOR programme in Italy. *Ozone and forest management in Italy.* Second report of the Task Force on integrated and Combined (I&C) evaluation of the CONECOFOR programme. Annali Istituto Sperimentale per la Selvicoltura, Special Issue, Arezzo, 99–106.

Cheng L, Ma F. 2004. Diurnal operation of the xanthophyll cycle and the antioxidant system in apple peel. *Journal of American Society of Horticultural Sciences* **129,** 313–320.

Cieslik SA. 2004. *Ozone uptake by various surface types: a comparison between dose and exposure*. New York: Pergamon-Elsevier Science Ltd., 2409–2420.

Dizengremel P, Le Thiec D, Bagard M, Jolivet Y. 2008. Ozone risk assessment for plants: central role of metabolism-dependent changes in reducing power. *Environmental Pollution* **156,** 11–15.

Emberson LD, Ashmore MR, Cambridge HM, Simpson D, Tuovinen J. 2000. Modelling stomatal ozone flux across Europe. *Environmental Pollution* **109,** 403–413.

Fares S, Loreto F, Kleist E, Wildt J. 2008. Stomatal uptake and stomatal deposition of ozone in isoprene and monoterpene emitting plants. *Plant Biology* **10,** 44–54.

Fares S, Mereu S, Mugnozza GS, Vitale M, Manes F, Frattoni M, Ciccioli P, Gerosa G, Loreto F. 2009. The ACCENT-VOCBAS field campaign on biosphere-atmosphere interactions in a Mediterranean ecosystem of Castelporziano (Rome): site characteristics, climatic and meteorological conditions, and eco-physiology of vegetation. *Biogeosciences* **6**, 1043–1058.

Gerosa G, Vitale M, Finco A, Manes F, Ballarin Denti A, Cieslik S. 2005. Ozone uptake by an evergreen Mediterranean forest (*Quercus ilex*). I. Micrometeorological flux measurements and flux partitioning. *Atmospheric Environment* **39,** 3255–3266.

Guderian R, Tingey DT, Rabe R. 1985. Effects of photochemical oxidants on plants. In: Guderian R, ed. *Air pollution by photochemical oxidants. Formation, transport, control and effects on plants*. Berlin: Springer-Verlag, 129–334.

Heath RL, Lefohn AS, Musselman RC. 2009. Temporal processes that contribute to nonlinearity in vegetation responses to ozone exposure and dose. *Atmospheric Environment* **43,** 2919–2928.

Kangasjarvi J, Jaspers P, Kollist H. 2005. Signalling and cell death in ozone-exposed plants. *Plant, Cell and Environment* **28,** 1021–1036.

Kärenlampi L, Skärby L, eds. 1996. *Critical levels for ozone in Europe: testing and finalising the concepts*. Finland: University of Kuopio.

Karlsson PE, Uddling J, Braun S, *et al*. 2004. New critical levels of ozone impact on trees based on AOT40 and leaf accumulated uptake of ozone. *Atmospheric Environment* **15,** 2283–2294.

Kontunen-Soppela S, Ossipov V, Ossipova S, Oksanen E. 2007. Shift in birch leaf metabolome and carbon allocation during long-term open-field ozone exposure. *Global Change Biology* **13,** 1053–1067.

Kurpius MR, Goldstein AH. 2003. Gas-phase chemistry dominates O_3 loss to a forest, implying a source of aerosols and hydroxyl radicals to the atmosphere. *Geophysical Research Letters* **30,** 1371.

Kurpius MR, McKay M, Goldstein AH. 2002. Annual ozone deposition to a Sierra Nevada ponderosa pine plantation. *Atmospheric Environment* **36,** 4503–4515.

Loreto F, Ciccioli P, Brancaleoni E, Cecinato A, Frattoni M, Sharkey TD. 1996. Different sources of reduced carbon contribute to form three classes of terpenoid emitted by *Quercus ilex* L. leaves. *Proceedings of the National Academy of Sciences, USA* **93,** 9966–9969.

Loreto F, Fares S. 2007. Is ozone flux inside leaves only a damage indicator? Clues from volatile isoprenoid studies. *Plant Physiology* **143,** 1096–1100.

Loreto F, Sharkey TD. 1990. Looking for the causes of isoprene emission by plants: a survey in red oak leaves. *Plant Physiology* **93,** 126.

Massman WJ. 2004. Toward an ozone standard to protect vegetation based on effective dose: a review of deposition resistances and a possible metric. *Atmospheric Environment* **38,** 2323–2337.

Massman WJ, Musselman RC, Lefohn AS. 2000. A conceptual ozone dose–response model to develop a standard to protect vegetation. *Atmospheric Environment* **34,** 745–759.

Musselman RC, Lefohn AS, Massman WJ, Heath RL. 2006. A critical review and analysis of the use of exposure- and flux-based ozone indices for predicting vegetation effects. *Atmospheric Environment* **40,** 1869–1888.

Niinemets U, Loreto F, Reichstein M. 2004. Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends in Plant Science* **9,** 180–186.

Oksanen E, Riikonen J, Kaakinen S, Holopainen T, Vapaavuori E. 2005. Structural characteristics and chemical composition of birch (*Betula pendula*) leaves are modified by increasing CO_2 and ozone. *Global Change Biology* **11,** 732–748.

Paoletti E, Manning WJ. 2007. Toward a biologically significant and usable standard for ozone that will also protect plants. *Environmental Pollution* **150,** 85–95.

Parrish DD, Millet DB, Goldstein AH. 2009. Increasing ozone concentrations in marine boundary layer air inflow at the west coasts of North America and Europe. *Atmospheric Chemistry and Physics* **9,** 1303–1323.

Pelzer D, Polle A. 2001. Diurnal fluctuations of antioxidative systems in leaves of field-grown beech trees (*Fagus sylvatica*): responses to light and temperature. *Physiologia Plantarum* **111,** 158–164.

Raschke K, Resemann A. 1986. The midday depression of CO_2 assimilation in leaves of *Arbutus unedo* L.: diurnal changes in photosynthetic capacity related to changes in temperature and humidity. *Planta* **168,** 546–558.

Singsaas EL, Laporte MM, Shi JZ, Monson RK, Bowling DR, Johnson K, Lerdau MT, Jasentuliytana A, Sharkey TD. 1999. Kinetics of leaf temperature fluctuation affect isoprene emission from red oak (*Quercus rubra*) leaves. *Tree Physiology* **19,** 917–924.

Tuovinen JP, Simpson D, Emberson L, Ashmore M, Gerosa G. 2007. Robustness of modelled ozone exposures and doses. *Environmental Pollution* **146,** 578–586.

UNECE. 2004. Revised manual on methodologies and criteria for mapping critical levels/loads and geographical areas where they are exceeded. www.icpmapping.org.

Velikova V, Edreva A, Loreto F. 2004. Endogenous isoprene protects *Phragmites australis* leaves against singlet oxygen. *Physiologia Plantarum* **122,** 219–225.

Velikova V, Fares S, Loreto F. 2008. Isoprene and nitric oxide reduce damages in leaves exposed to oxidative stress. *Plant, Cell and Environment* **31,** 1882–1894.

Velikova V, Pinelli P, Pasqualini S, Reale L, Ferranti F, Loreto F. 2005. Isoprene decreases the concentration of nitric oxide in leaves exposed to elevated ozone. *New Phytologist* **166,** 419–426.

Vickers CE, Gershenzon J, Lerdau MT, Loreto F. 2009. A unified mechanism of action for isoprenoids in plant abiotic stress. *Nature Chemical Biology* **5,** 283–291.

Vollenweider P, Gunthardt-Goerg MS. 2005. Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. *Environmental Pollution* **137,** 455–465.

Yamaji K, Julkunen-Tiitto R, Rousi M, Freiwald V, Oksanen E. 2003. Ozone exposure over two growing seasons alters root-to-shoot ratio and chemical composition of birch (*Betula pendula* Roth). *Global Change Biology* **9,** 1363–1377.