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Synopsis

Monitoring Ozone Effects on Vegetation: A Review

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Abstract. One of the most phytotoxic air pollutants is ozone, which can cause considerable damage to vegetation: visible leaf injury, reduction in yield quantity and quality and reduction in photosynthesis, alterations to carbon allocation, and in the sensitivity to biotic and abiotic stresses. Ozone is a secondary pollutant and prevailed at high concentrations over rural regions. Moreover, ozone concentrations are expected to increase in the future and will continue to be a serious threat to crop productivity. This paper presents a comprehensive review the undertaken studies of ozone's impacts on crops and natural ecosystems.

Keywords: Ozone, Monitoring, Plants, Crops.

Introduction

Ozone is a naturally occurring chemical that can be found in both the stratosphere (in the 'ozone layer', 10 – 40 km above the earth) and the troposphere (0 – 10 km above the earth). Ground level ozone (O_3) is the atmospheric pollutant that is most likely to threaten food production across the globe due to its phytotoxicity and prevalence over important regions of Europe, North America and Asia (Fuhrer & Booker, 2003; Royal Society, 2008). There is always a background concentration of ozone at ground level resulting from natural sources of the precursors and stratospheric incursions. The formation of ozone is due to a large number of photochemical reactions taking place in the atmosphere and depends on the temperature, humidity and solar radiation as well as the primary emissions of nitrogen oxides and volatile organic compounds – VOCs (Ashmore, 2005; Bytnerowicz *et al.*, 2007). Tropospheric (ground-level) O_3 is a secondary pollutant, which is not directly emitted into the atmosphere, but is formed from chemical reactions in the presence of sunlight, and natural and anthropogenic precursor gases (mainly NOx and VOCs). Tropospheric O3 is characterised by complex formation mechanisms based on the photo-oxidation of VOCs in the presence of NOx, following non-linear formation pathways: NOx are involved in O_3 formation but also removal through titration (the reaction of O_3 with NO to form NO_2 and O_2) (Monks *et al.*, 2015). Together with the non-linear

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relationships between the primary emissions and the ozone formation, these effects complicates the abatement strategies for ground-level ozone and makes photochemical models crucial in addition to the monitoring data (EMEP, 2016). Additional photochemical reactions involving NOx, carbon monoxide and non-methane volatile organic compounds (NMVOCs) released due to anthropogenic emissions increase the concentration of ozone in the troposphere (Jenkin & Clemitshaw, 2000). Ozone is the most pervasive phytotoxic air pollutant affecting natural ecosystems, forest health and an important air pollutant that affects both vegetation and human health (U.S. EPA, 1997, Skarby *et al.*, 1998, Finlayson-Pitts & Pitts, 2000, Ainsworth *et al.*, 2012).

Additional tropospheric ozone is formed from complex photochemical reactions from fossil fuel burning in industrial and transport activities. These emissions have caused a steady rise in the background ozone concentrations in Europe and the USA since the 1950s (Royal Society, 2008). Ozone episodes can cause short-term responses in plants such as the development of visible leaf injury (fine bronze or pale yellow specks on the upper surface of leaves) or reductions in photosynthesis (Finlayson-Pitts & Pitts, 2000). Frequent episodes can occur longer-term responses such as reductions in growth and yield and early die-back (Emberson *et al.*, 2000; Pleijel *et al.*, 2007). An important sink for the greenhouse gases carbon dioxide (CO_2) and ozone is terrestrial vegetation. The air pollutant ozone has a negative impact on cell metabolism and growth of ozone-sensitive plant species. Hence, this will result in a positive feed-back to global warming as less CO_2 and ozone will be sequestered by vegetation, resulting in a further rise of their concentrations in the atmosphere (Sitch *et al.*, 2007).

The definitions ''injury'' and ''damage'' are important in evaluating the vegetation response to a pollutant (Guderian, 1977). Injury can be damage, or the consequences of injury can become damage if the injury can cause a subsequent impairment of the intended use of the plant. Damage to vegetation from pollutants can occur without visible symptoms of injury, but damage cannot occur without some form of injury preceding damage (Guderian, 1977). There is an urgent need to be able to assess the current and future risks from O3 exposure to crops and natural ecosystems.

Ozone impacts on crops and natural ecosystems

Ecosystems are complex systems comprising animal, plant and microorganism communities, together with the non-living environment (Millennium Ecosystem Assessment, 2005), these systems are dynamic whilst maintaining some essential resilience to natural disturbances. Earth's ecosystems provide an array of services upon which humans depend for food, fresh water, disease management, climate regulation, aesthetic enjoyment and spiritual fulfilment (Millennium Ecosystem Assessment, 2005). The role of biodiversity in ecosystem services is often rather unclearly stated – biodiversity is sometimes considered as a separate service and yet is implicit in most ecosystem services.

Indirect drivers of ecosystem change are associated with demographic, economic, socio-political and cultural or religious changes, and advancements in science and technology. Stressed or degraded ecosystems do not have the resilience or re-bound capacity of pristine/unstressed systems (Rapport & Maffi, 2009).

Human influence extends into even the remotest landscapes and more often than not has a pervasive influence on the ecosystems they support, frequently irreversibly changing biodiversity. Extinction rates of species are now estimated to be 1,000 times greater than historical background levels (Millennium Ecosystem Assessment, 2005; Mantyka-Pringle *et al.*, 2012), Recent studies have identified linkages between changes in ecosystem functioning and biodiversity, highlighting the importance of adopting a multi-sectoral approach to policy and decision making (MAESTRE *et al.,* 2012; MACE *et al.,* 2012). Ecosystem services can be classified into provisioning, regulating, supporting and cultural services (MACE *et al.,* 2012). Meta-analyses of published data on effects of species loss on the key ecosystem processes of productivity and decomposition have shown how important species loss is in ecosystem service delivery (HOOPER *et al.,* 2012). Species losses of 21 – 40% reduced plant productivity by 5 – 10%, an equivalent amount of reduction as that estimated for effects of UV light and global warming. The study also indicated that species losses of 41 – 60%, as projected for global extinctions by the end of this century, is predicted to result in a 13% biomass loss, a similar amount to that predicted for ozone effects alone. In a similar study, MANTYKA-PRINGLE *et al.* (2012), investigated the synergies between climate change and habitat loss for explaining biodiversity loss.

Ozone reduces whole plant photosynthesis by directly impacting on the photosynthetic machinery (Rubisco and chlorophyll content), reducing leaf area by promoting early senescence and leaf abscission, diverting carbon (C) use into detoxification and/or repair metabolism, changing stomatal conductance (both increases and decreases have been noted, and altering C allocation in favour of the above ground parts rather than below ground parts. Carbon flux to and from the soil is also altered by changes in leaf litter quality, altered rhizodeposition of C, changes in soil microbial community composition, and altered soil processes (AINSWORTH, 2017). Tropospheric ozone has the capacity to impact on nutrient cycling by both direct and indirect mechanisms: All of these have the capacity either, independently or in concert, to ultimately reduce the long-term sustainability of ecosystems (LINDROTH *et al.,* 2001, SUN *et al.,* 2012). Tropospheric ozone is known to alter stomatal responses to environmental and in the short term (at higher concentrations) can cause stomata (leaf pores) to close, however, under prolonged chronic exposure (at lower concentrations) many reports document ozone-induced stomatal opening or loss of stomatal sensitivity to closing stimuli, such as drought, light and humidity (MILLS *et al.,* 2013). Ozone damages crop plants by, for example, reducing photosynthesis, causing a yellowing of leaves and leaf loss, decreased seed production and reduced root growth, in turn resulting in reduced yield quantity and/ or quality and reduced resilience to other stress such as drought. As a consequence, the key components of the food system that ozone interferes with are the productivity of crops, the nutritional value and the stability of food supplies as ozone concentrations and therefore impacts vary from year to year. Some of the world's most important food crops are sensitive (wheat, soybean and other pulses) or moderately sensitive (maize, rice, potato) to ozone and effects on the yield of these crops are of global significance (MILLS & HARMENS, 2011). A recent analysis has suggested that the increase in ozone since the industrial revolution has been responsible for a reduction in photosynthesis of approximately 11% in trees (WITTIG *et al.,* 2007), which may have reduced tree productivity by approximately 7% (WITTIG *et al,* 2009). In general, deciduous trees tend to be more sensitive to ozone than coniferous trees, with ozone sensitive species present across most of Europe (WITTIG ***et al.,*** 2009). If ozone concentrations are high enough to reduce photosynthesis and/or above-ground plant growth, then less CO_2 and ozone will be absorbed by the leaves of vegetation, leading to a positive feedback to atmospheric CO_2 and ozone concentrations and therefore more global warming (SITCH *et al.,* 2007). It has been estimated that ozone deposition to vegetation reduces tropospheric ozone concentrations by as much 20% (ROYAL SOCIETY, 2008). This is an especially significant function of vegetation given that ozone is the third most important greenhouse gas causing global warming (IPCC, 2007).

Typical effects of ozone on sensitive species include: accelerated aging and changes in biomass, resource allocation and/ or seed production. Each of these can impact on the vitality of component species of plant communities, potentially altering plant biodiversity as well as that of the animals, fungi, bacteria and insects that live in close association with plants or in nearby soils. Ozone-induced changes in species diversity or shifts in species balance will impact on many ecological processes, thereby impacting on ecosystem services, flows, goods and values. Effects on species balance have been widely reported from controlled exposure experiments, but a less clear picture emerges from field-based studies with long established communities and from field surveys. Although more studies are needed, it is clear that impacts of ozone are of particular concern for global biodiversity hotspots (MILLS *et al.,* 2013). Importantly, field validation of effects observed under experimental conditions is still lacking for many species and plant communities. Indirect effects remain mostly unknown, despite the fact that they are probably of great importance in terms of assessing ozone effects on ecosystem biodiversity.

Ozone biomonitoring

Ozone biomonitoring is a detection and monitoring technique that includes documenting ozone-induced visible injury to known ozone-sensitive species under conditions of ambient exposure. Ozone is routinely monitored throughout the world and data are mostly recorded as hourly or half-hourly averages.

The Swiss chemist Schönbein in the mid-1800s made the earliest measurements of ground-level ozone, using passive exposure of ʻʻtest papersʼʼ impregnated with potassium iodide (cited in LONDON, 1985).

Tropospheric ozone can be monitored with mechanical monitors, or passive, cumulative, total exposure samplers (MANNING *et al.,* 1996; BYTNEROWICZ *et al.,* 2002; HUNOVA *et al.,* 2003). Mechanical monitors are expensive and require electricity and a safe climate-controlled shelter for effective operation. Passive samplers are easy to use, inexpensive and require no electricity and have been used in forested, wilderness and remote region, especially in Europe, to identify locations and areas where ambient ozone concentrations exceed normal background levels and resulting pollution levels of ozone may injure sensitive plants. They can be co-located with mechanical monitors. Combining cumulative monitoring data with GIS techniques allows accurate depiction of air quality for large geographic regions (BYTNEROWICZ *et al.,* 2002).

The dose–response relationships have been used to assess risk, either by quantifying yield losses for economic crop loss estimates (ADAMS *et al.,* 1989) or by mapping critical levels exceedance (SIMPSON *et al.,* 2007). Dose–response relationships are central to risk assessments since they provide the link between a pollutant dose and a plant response of concern (EMBERSON *et al.,* 2003). Such relationships would be derived from co-ordinated standardised experimental campaigns assessing crop response to a range of pollutant concentrations (UNSWORTH & GEISSLER, 1992). China, India, Japan and Pakistan have investigated a wide range of crop species and cultivars using a variety of experimental methods and design (EMBERSON *et al.,* 2001, 2003; MAUZERALL & WANG, 2001).

Open-top chamber have been used in multi-year studies to estimate the long-term impacts of ozone on growth of tree seedlings or saplings. (HEAGLE *et al.,* 1973). Dose/response studies can be done by adding ozone to ambient air and results have been used to determine air quality standards for ambient ozone (US EPA, 1996). Typically, cylindrical open-top chambers are placed over field plots of soil-grown plants and supplied with filtered air, non-filtered air, or non-filtered air with ozone added. Such chambers provide climatic conditions that are similar, but not identical, to those outside (Colls *et al.*, 1993), and thus some reservations about extrapolation to field conditions remain. This would suggest that open-top chamber data would tend to overestimate the adverse effects of a given ozone concentration (Pleijel *et al.*, 1994; Fuhrer, 1994).

Ozone phytomonitoring

Ozone pollution has been shown to have an adverse effect on tree growth and alter tree succession, species composition, and pest interactions (Forest Health and Ozone, 1987; Miller & Millecan, 1971; Smith, 1974). In addition, we know that ozone causes direct foliar injury to many species (Skelly *et al.*, 1987; Treshow & Stewart, 1973). We can use this visible injury response to detect and monitor ozone stress in the forest environment. Bioindicators are plants that exhibit typical ozone injury symptoms in the field. Many have been identified and verified and good methodology is available for field surveys in conjunction with active ozone monitors or passive ozone samplers (Manning *et al.*, 1990, 2002; Krupa *et al.*, 1998; Innes *et al.*, 2001). A useful bioindicator plant may be a tree, a woody shrub, or a nonwoody herb species. The essential characteristic is that the species respond to ambient levels of ozone pollution with distinct visible foliar symptoms that are easy to diagnose. Field studies and experiments have identified ozone sensitive species and characterized the ozone specific foliar response for bioindicators (Davis & Umbach 1981; Duchelle & Skelly 1981; Krupa & Manning, 1988; Mavity *et al.*, 1995; Brace, 1996).

During the years 1994, 1995 and 1996 participants of the ICP Vegetation conducted studies in ambient air using the ozone-protectant ethylenediurea (EDU) at experimental sites and/or in commercial fields (Ball *et al.*, 1998). Species tested in this way included subterranean clover (*Trifolium subterranean),* bean (*Phaseolus vulgaris),* radish (*Raphanus sativus),* white clover (*Trifolium repens),* red clover (*Trifolium pratense),* tomato (*Lycopersicon esculentum),* soybean (*Glycine max),* watermelon (*Citrullus lanatus)* and tobacco (*Nicotiana tabacum)*. Between 1996 and 2006, the ICP Vegetation biomonitoring programme has involved exposure of an ozone sensitive biotype of white clover (*Trifolium repens* Regal, NC-S) to ambient air (Hayes *et al.*, 2007; Mills *et al.*, 2011). Since 2008, participants of the ICP Vegetation have been conducting biomonitoring campaigns using ozone-sensitive (S156) and ozone-resistant (R123) genotypes of *Phaseolus vulgaris* (Bush bean, French Dwarf bean). The bean genotypes were develop (Reinert & Eason, 2000) and tested as a bioindicator system (Burkey *et al.*, 2005) in the USA. In 2012, experiments were conducted with ozone-sensitive and ozone-resistant bean at nine sites across Europe and one in the USA.

Sixteen species of native detector plants for ambient ozone have been identified for use in Central and Eastern Europe. They include the forbs *Alchemilla sp., Astrantia major, Centuarea nigra, Centauria scabiosa, Impatiens parviflora, Lapsana communis, Rumex acetosa* and *Senecio subalpinus;* the shrubs *Corylus avellana, Cornus sanguinea* and *Sambucus racemosa;* the trees *Alnus incana, Pinus cembra* and *Sorbus aucuparia;.* and the vines *Humulus lupulus* and *Parthenocissus quinquefolia* (Manning *et al.*, 2002).

Some of the most important native (or naturalized) tree and shrub species that showed ozone-like symptoms in Mediterranean countries (Spain, Italy, Israel) are *Abies cephalonica, Acer platanoides, Acer pseudoplatanus, Ailanthus altissima, Arbutus unedo, Alnus incana, Betula pendula, Carpinus betulus, Cornus spp., Corylus avellana, Crataegus spp., Fagus sylvatica, Frangula alnus, Fraxinus excelsior, Fraxinus ornus, Juglans regia, Morus spp, Myrtus communis, Pinus halepensis, Pinus pinea, Pistacia lentiscus,* *Pistacia terebintus, Populus spp., Prunus amygdalus, Prunus avium, Prunus serotine, Prunus spinose, Robinia pseudoacacia, Rhamnus spp., Rosa spp., Salix spp., Sambucus spp., Tilia spp., Ulmus glabra,* and *Viburnum spp.* (BUSSOTTI & GEROSA, 2002).

Similar studies were conducted in Asia and North America for wheat (*Triticum aestivum L.*) and rice (*Oryza sativa L.),* and group of crop species (legumes: soybean (Glycine max L.) and mung bean (*Vigna radiate (L.*) R. Wilczek) (EMBERSON, 2009) and *Fagus sylvatica* in Switzerland (CLARK *et al.*, 2000). Maize (*Zea mays L.)* is the receptor of interest in the main maize producing countries, i.e. South Africa, Zambia and Zimbabwe (VAN TIENHOVEN *et al.*, 2006). Phytotoxic ozone effect at urban and rural sites in Bulgaria were assessed with seedlings of ozone-sensitive (*Quercus robur* L.), (*Fraxinus excelsior* L.,) and ozone-tolerant (*Quercus rubra* Michx.) (PARVANOVA *et al*, 2003; 2009).

Conclusions

Based on the reviewed literature data we conclude that:

A combination of scientific experiments, mathematical models and predictions of pollutant emissions revealed that ambient ozone concentrations are sufficient to cause impact on vegetation.

Planting ozone sensitive species is a useful tool for demonstrating the occurrence of visible leaf injury in ambient conditions.

Trees, shrubs, forbs and vines could be apply to assess the symptoms of probable ozone injury in the vicinity of passive ozone samplers or active ozone monitors in forest condition networks in mostly mountainous regions.

Ozone-sensitive plant species include crops such as wheat, soybean, potato, tomato beans and pulses; trees such as beech, birch, Norway spruce, poplar, oak; and (semi) natural vegetation (represented by *Trifolium* spp. (clover family) and provisionally *Viola* spp.

Ozone concentrations in forest areas appear to be high enough and of sufficient duration to cause foliar injury on a wide variety of native plants.

Ozone remains an important phytotoxic air pollutant. Economic impacts are also possible if growth rates of commercially important tree species are reduced. There is a need to quantify the effects of anthropogenically induced ozone on forest growth in order to establish the economic consequences for the wood industry.

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