ANNALI DI BOTANICA
Ann. Bot. (Roma), 2015, 5: 31–37
Journal homepage: http://annalidibotanica.uniroma1.it

FIELD SURVEYS OF OZONE SYMPTOMS IN EUROPE. PROBLEMS, RELIABILITY AND SIGNIFICANCE FOR ECOSYSTEMS

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(RECEIVED 17 FEBRUARY 2015; ACCEPTED 19 MARCH 2015)

ABSTRACT – The ICP-Forest program for the monitoring of forest conditions includes the assessment of ozone symptoms in the European forests. This contribute to discussion points out the problems related to the recognition of such symptoms, with a special focus on the difficulties to extend the results obtained in experimental conditions to woody plant species growing in the field. Non specific symptoms (such as reddening, yellowing, early senescence and leaf loss), and the concurrent action of modifying factors (high light, drought, nutrient deficiency, pest attack and fungi) make the recognition elusive. In these cases, the action of ozone cannot be proven or excluded with "ad hoc" experiments. Apparently "good" bioindicators (*Rubus* sp. *Cornus* sp. pl., *Prunus* sp. pl., *Viburnum* sp. pl. etc.) are not suitable to assess the impact of ozone on vegetation. Symptoms are not necessarily related to the ozone dose taken up by stomata, and don't are reliable indicator for biomass and productivity losses. Symptoms can be considered an epiphenomenon of more complex ecosystem processes.

KEYWORDS: CONTROLLED EXPERIMENTS; FIELD SURVEYS; ICP-FORESTS; MICROSCOPIC VALIDATION; MODIFYING FACTORS; OXIDATIVE PRESSURE

INTRODUCTION

"A theory which is not refutable by any conceivable event is nonscientific. Irrefutability is not a virtue of a theory (as people often think) but a vice" (Karl Popper, 1957).

There are no doubts that the current concentrations of tropospheric ozone [O_3] are sufficient to induce foliar symptoms in many sensitive European woody plant species (trees and shrubs), at least in experiments carried out in controlled and semi-controlled conditions (Skelly et al., 1999; VanderHeyden et al., 2001; Novak et al., 2003, 2005; Gravano et al., 2003, 2004; Bussotti et al., 2007; Gerosa et al., 2008, 2009; Marzuoli et al., 2009; Calatayud et al., 2007, 2010, 2011). Experiments allowed the researchers to identify the most sensitive species and their morphological, anatomical and ultrastructural responses. According to Innes et al. (2001) and Schaub et al. (2002) typical symptoms in broadleaved trees consist in interveinal stipples (reddish or brownish) visible on the upper (adaxial) leaf surface (Fig. 1). At microscopical level, stipples corresponds to the collapse and death of groups of cells in the upper parenchyma, i.e. the palisade tissue (Vollenweider et al., 2003; Gravano et al., 2003, 2004; Bussotti et al., 2005). The symptomatic leaves are located in the basal part of the branches (the first leaves emitted in the growing season are mostly affected), and in the lowest part of the crown. In conifers, ozone symptoms consist in chlorotic mottles on the oldest needles, with a correspondent degeneration of mesophyll cells (Soda et al., 2000; Kivimäenpää et al., 2010). Visible manifestations with a lower degree of specificity, including leaf reddening, bronzing, bleaching, yellowing, early senescence and premature leaf loss, have also been described by Innes et al. (2001).

Based on these experimental evidences, field surveys to

doi: 10.4462/annbotrm-13060

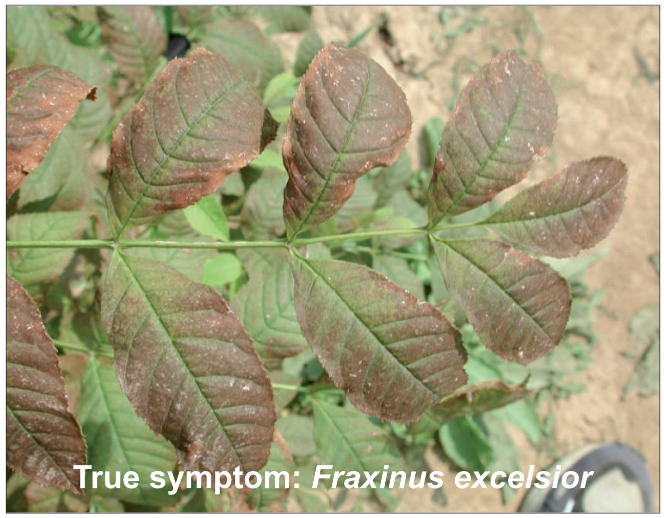

Fig.1. Examples of typical ozone symptoms on *Acer pseudoplatanus* and *Fraxinus excelsior*, with interveinal stipples on the upper leaf blade. Stipples affect the leaves in basal position on the branches.

assess the spread and intensity of ozone symptoms on the natural vegetation have been carried out. In previous experiences in North America, surveyors take in account only few selected species displaying unambiguous symptoms, and that were well tested in experimental conditions (Coulston et al., 2003; Smith et al., 2003). In Europe the field surveys are currently carried out at the Level II plots belonging to the ICP-Forests program, and consider all the woody species falling within a specific sampling design (Schaub et al., 2010). Since symptoms were not reproduced experimentally for all species, a criterion of "ozone-like" symptoms was adopted. This criterion was based on the similarity between the symptoms observed in a variety of field grown species and those experimentally reproduced on a limited number of species.

The notation "ozone-like" was extensively used in the first decade of the present century to remark the possibility, but not the certainty, that a given manifestation may be caused by ozone (Manning et al., 2002; Manning and Godzik, 2004; Bussotti et al., 2003a; 2006a; Ferretti et al., 2007; Bussotti and Ferretti, 2009), although the first results suggest no (or very weak) correlations between ozone levels and the diffusion of foliar injuries. Field experiences have been recently reviewed by Mills et al. (2011), but a serious reflection on the limits of this first generation of foliar symptoms assessment surveys is still lacking. In the first period the concept "ozone-like" was useful to promote field surveys and experiments, but it is now necessary to exit this ambiguity and decide what it is really attributable to ozone and what must be discharged, reconsidering the current strategies of field assessment.

Uncertainties and errors in recognizing ozone symptoms

Among the factors that reduce the reliability of the field surveys, the first issue concerns the accordance among different surveyors in evaluating the symptoms in the same leaf sample (Bussotti et al., 2003b, 2006b). In general, experienced surveyors use more prudential criteria than non experienced ones. In many cases the source of errors relies in the uncertainties to assess non specific responses like early senescence, yellowing and reddening (see Figs. 2-3). When these symptoms appear in experimental conditions it is easy to relate them to the action of ozone, but the same manifestations in the field can be induced by a variety of uncontrolled factors. In some *taxa* leaves become red as early response to many environmental stress, with a pattern similar to that induced by ozone (for ex., species belonging to the genus *Rubus*, *Cornus*, *Viburnum*, *Prunus* etc.). These species are apparently good bioindicators, but may induce errors in evaluating the results of a survey. In recent years a number of local surveys on field assessment of foliar symptoms were reported for Europe and Asian far East countries (Hunova et al., 2011; Calderon Guerrero et al., 2013; Wan et al., 2013, 2014). There are several reasons because in most cases the symptoms described in literature are not suitable indicators of ozone stress in field surveys. In particular:

- The symptoms observed in the field can be explicated with causes different from ozone (high sun radiations, lack of nutrients, senescence etc.), or are different from those reproduced experimentally on the same species;

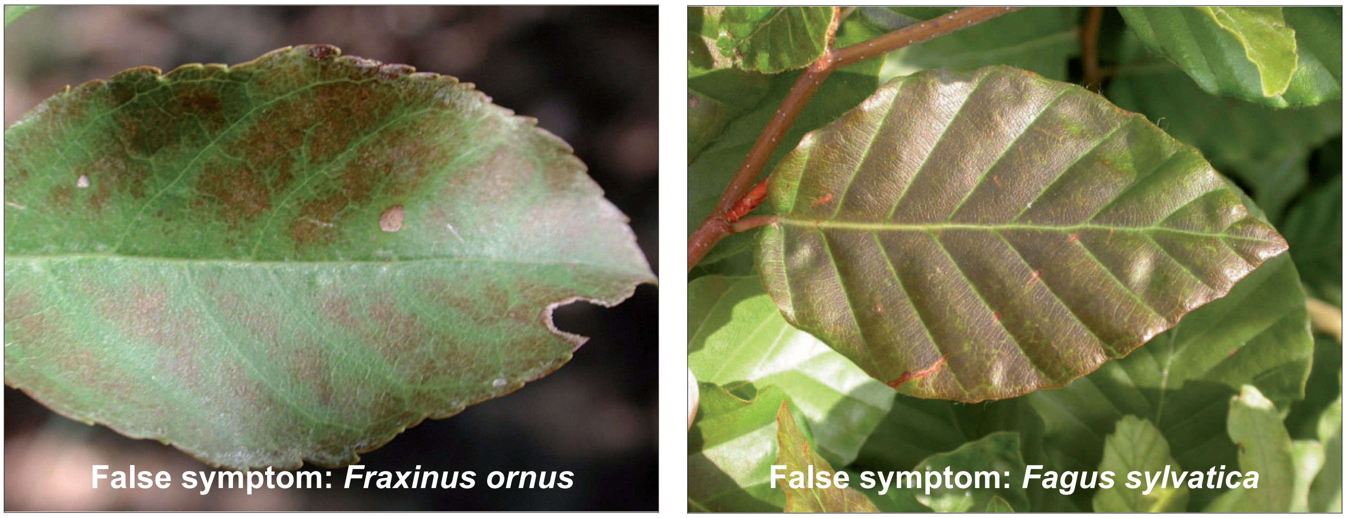

Fig. 2. False or ambiguous symptoms. Leaf bronzing on *Fraxinus ornus* and *Fagus sylvatica*. The pattern of the symptoms interests the outer leaves, in the apical position on the branches and directly exposed to light. This behavior is opposite to the typical ozone symptoms and can be attributed directly to high light. Leaf bronzing on *F. ornus* was not reproduced with ozone treatment in experimental conditions (Paoletti et al., 2009). Ozone can enhance bronzing on *F. sylvatica*, but this symptoms was observed also at low ozone concentration (Cascio et al., 2010).

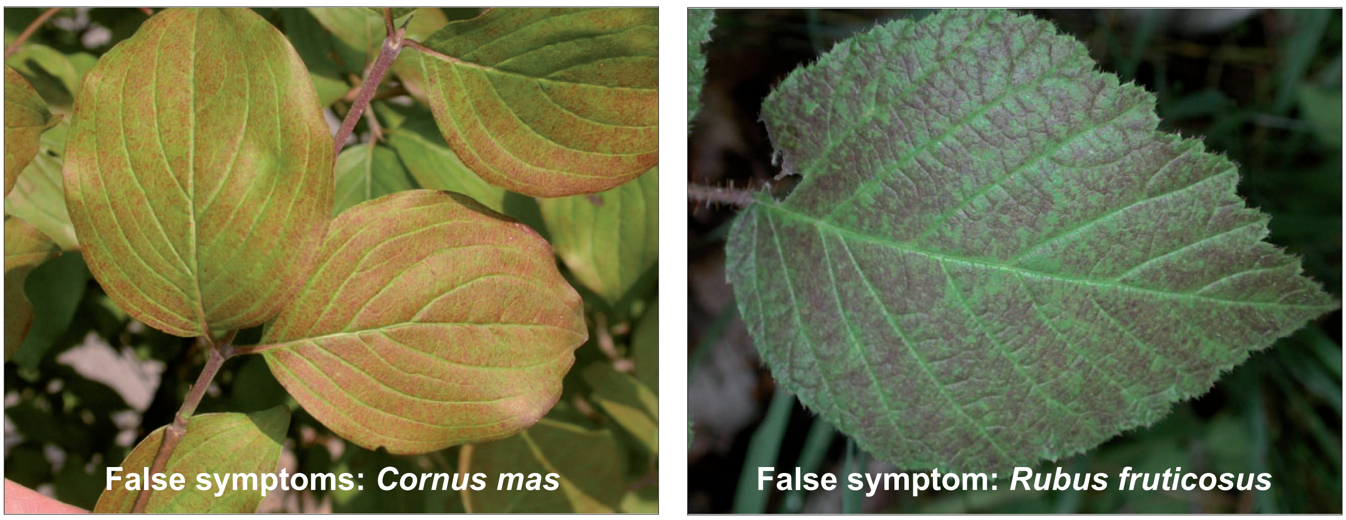

Fig. 3. False or ambiguous symptoms. Leaf reddening in leaves of *Cornus mas* and *Rubus fruticosus*. These species are very common in the forest understory of Mediterranean and Central Europe, and have been considered as bioindicator. Leaf reddening, however, can be induced by a variety of environmental stress factors including hifg light, drought, senescence. Their evaluation is therefore problematic.

- Symptoms occur on species considered ozone resistant, and were not reproduced in experimental conditions.

In the European program an important role is attributed to the so-called "microscopic validation", i.e. the confirmation of the actual role of ozone as causal agent by means of the observation of the microscopic alteration in the mesophyll (Vollenweider et al., 2003a). In our opinion, because of the low degree of specificity of the responses, the microscopic analysis is more reliable in excluding rather than in confirming the action of ozone.

We underline that a supposed ozone symptom can be considered as such only when it has been reproduced unequivocally in experimental condition, without the possibility of any confounding factor. The possibility that the "ozone like" manifestations recognized in the field can be attributable or not to ozone only by visual and/or microscopic observations, without an effective experimental validation, is not testable (always there is the possibility that an environmental factor not considered may have, or not have, the ability to induce the same symptom, with or without a possible synergistic effect with ozone), and consequently not refutable. According to the general laws of epistemology (Popper, 1957) a theory with these characteristics must be rejected.

Occurrence of symptoms and absorbed ozone

There is a general (tacit) agreement that the appearance and spread of symptoms depend from the absorbed dose of ozone (Marzuoli et al., 2009), but comparing data from different open-top chamber experiments (see literature cited in Introduction) such assumption appears weak. In semi-controlled and field conditions the response of the plant depends by the interaction of different factors that can promote or depress the onset of symptoms, and not always ozone plays a primary role. High light is perhaps the major driver for ozone and ozone like symptoms (Davison et al., 2003). Light intensity, especially in Southern Europe conditions, is always exceeding the saturation requirements of photosynthesis. High light produces an over-excitation usually disposed of by photochemical and non-photochemical de-excitation processes, but a fraction of radiations exceeds the de-excitation capacity and is involved in ROS formation and accumulation. This process may have more deleterious consequence when the Calvin cycle is suppressed, as consequence of the inactivation of Rubisco by the action of high light itself (Jagtap et al., 1998), drought (Flexas and Medrano, 2002) or ozone (Fontaine et al., 2003), so compromising the photochemical de-excitation pathways. Such situation provokes the accumulation of reactive oxygen species (ROS), inducing metabolic and ultrastructural changes having the same nature of those usually attributed to ozone. In a long period the photosynthetic apparatus can be damaged from the action of the ROS, and senescence processes, including the breakdown of chlorophyll, are triggered.

Drought stress is believed to delay or avoid the appearance of symptoms, but drought and ozone are not always coincident and we can find a series of interactive effects not fully explored. Summer drought doesn't avoid the ozone uptake in the first part of the season (when there is not still water deficit in the soil), so producing an overlap of oxidative stress factors (Pollastrini et al., 2014). During the summer the concomitant action of ozone and drought stress in an environment characterized by high solar radiations may further increase the oxidative pressure since the Rubisco is inactivated (mesophyll limitation, Fontaine et al., 2003; Flexas and Medrano, 2002), or because of the lack of intercellular carbon due to the stomatal closure (stomatal limitation, Flexas and Medrano, 2002; Kitao et al., 2012). In these cases the pressure of electron flow in the ETC and consequently the production of ROS is enhanced because of the lack of sinks for photochemical de-excitation. This situation can be further exacerbated by rain pulses during the summer season. In leaves subjected to strong oxidative pressure, the uptake of a moderate dose of ozone can trigger a cascade of consequences including widespread foliar symptoms. In this case, foliar symptoms are not produced by the "critical" dose absorbed by leaves, but by the "marginal" dose that allow to overcome the oxidative pressure tolerated by leaves.

Does foliar symptoms affect ecosystem functions?

Ozone impacts negatively ecosystem functions and services such as carbon sequestration and timber production (Wittig et al., 2009), but the reduction of tree growth doesn't seem to be related to foliar symptoms. In some cases field surveys (Vollenweider et al., 2003b) and open-top chamber experiments (Novak et al., 2007) allowed to find appreciable stem growth reduction connected to widespread symptoms in sensitive species (*Prunus serotina* Ehrh. and sensitive poplar clones). In other studies, the reduction of photosynthetic surface was compensate by the emission of new leaves and/or the increased efficiency of the remaining leaves (*compensatory photosynthesis*) without loss of woody biomass (Desotgiu et al., 2012, Pollastrini et al., 2013). Growth reduction may be consequence of many different physiological processes that compromise the photosynthesis and that don't imply the onset of foliar symptoms. In the Kranzberg experimental forest (Kitao et al., 2012, Matyssek et al., 2015) the reduction of photosynthetic activity and loss of growth was not connected to high ozone fluxes and the damage to photosynthetic apparatus but to the stomatal closure (*avoidance* mechanism). From an ecological point of view, we hypothesize that maintaining living leaves (not symptomatic) in a stressful environment determines metabolic costs for the tree (diversion of resources for defense and repair; increasing respiration rates) leading to the reduction of growth. In this perspective foliar injuries (HR – Hypersensitive Response) can be interpreted as a mechanism to protect the whole tree against metabolic losses, since the death of the damaged cells and the leaf loss allow to redirect the resources to the healthy cells/ leaves so maintaining high levels of photosynthesis.

Conclusions

The experiences developed during many years in controlled and semi-controlled experiments, as well in field surveys, convinced us that the ozone foliar symptoms is an epiphenomenon in the more general context of the impact of oxidative stress on forests. Ozone symptoms don't represent *per se* a threat for forest functioning and health, but may be the "signal" of ongoing impacts on the ecosystem. This signal, however, is often very confused and its interpretation is extremely difficult; moreover the links between visible manifestation and ecosystem functions and services are ambiguous. Developing a conceptual framework to link visible manifestations of oxidative stress and the reduction of forest growth and efficiency is a challenge for future researches.

References

Bussotti F., Mazzali C., Cozzi A., Ferretti M., Gravano E., Gerosa G., Ballarin-Denti A., 2003a.Ozone expositions and injury symptoms on vegetation in an Alpine valley (North Italy). In: Karnosky DF, Percy KE, Chappelka AH., Simpson CJ, Pikkarainen J. (Eds.). Air Pollution, Global Change and Forests in the New Millennium. Develop Environ Sci. 3 (Series editor, S.V. Krupa) Elsevier Science Ltd., Oxford (UK). Pp. 259-268.

Bussotti F., Schaub M., Cozzi A., Kräuchi N., Ferretti M., Novak K., Skelly J., 2003b. Assessment of ozone visible symptoms in the field, perspectives of quality control. Environmental Pollution 125, 81-89.

Bussotti F., Agati G., Desotgiu R., Matteini P., Tani C., 2005. Ozone foliar symptoms in woody plants assessed with ultrastructural and fluorescence analysis. New Phytologist 166, 941-955.

Bussotti F., Cozzi, A., Ferretti M., 2006a. Field surveys of ozone symptoms on spontaneous vegetation. Limitations and potentialities of the European programme. Environmental Monitoring and Assessment 115, 335-348.

Bussotti F., Schaub M., Cozzi, A., Gerosa G., Novak K., Hug C., 2006b. Sources of errors in assessing ozone visible symptoms on native vegetation. Environmental Pollution 140, 257-268.

Bussotti F., Desotgiu R., Cascio C., Strasser R.J., Gerosa G., Marzuoli R., 2007. Photosynthesis responses to ozone in young trees of 3 species with different sensitivities, in a two-year open-top chamber experiment (Curno, Italy). Physiologia Plantarum 130, 122-135.

Bussotti F., Ferretti M., 2009. Visible injury, crown condition, and growth responses of selected Italian forests in relation to ozone exposure. Environmental Pollution 157, 1427-1437.

Calatayud V., Cerveró J., Sanz M.J., 2007. Foliar, physiological and growth responses of four maple species exposed to ozone. Water Air and Soil Pollution 185, 239-254.

Calatayud V., Marco F., Cerveró J., Sánchez-Peña G., Sanz M.J., 2010. Contrasting ozone sensitivity in related evergreen and deciduous shrubs. Environmental Pollution 158, 3580-3587.

Calatayud V., Cerveró J., Calvo E., García-Breijo F.-J., Reig-Armiñana J., Sanz M.J., 2011. Responses of evergreen and deciduous *Quercus* species to enhanced ozone levels. Environmental Pollution 159, 55-63.

Calderon Guerrero C., Günthard-Goerg M.S., Vollenweider P., 2013. Foliar symptoms triggered by ozone stress in irrigated Holm oaks from the city of Madrid, Spain. PLoS ONE 8 (7), e69171.

Cascio C., Schaub M., Novak K., Desotgiu R., Bussotti F., Strasser R.J., 2010. Foliar responses to ozone of *Fagus sylvatica* L. seedlings grown in shaded and in full sunlight conditions. Environmental and Experimental Botany 68, 188-197.

Coulston J.W., Smith G.C., Smith W.D., 2003. Regional Assessment of ozone sensitive tree species using bioindicator plants. Environmental Monitoring and Assessment 83, 113-127.

Davison A.W., Neufeld I.S., Chappelka A.H., Wolff K., Finkelstein P.L., 2003. Interpreting spatial variation in ozone symptoms shown by cutleaf cone flower, *Rudbeckia laciniata* L. Environmental Pollution 125, 61-70.

Desotgiu R., Cascio C., Pollastrini M., Gerosa G., Marzuoli R., Bussotti F., 2012. Chlorophyll a fluorescence analysis along a vertical gradient of the crown in a poplar (Oxford clone) subjected to ozone and water stress. Tree Physiology 32, 976-986.

Ferretti M., Calderisi M., Bussotti F., 2007. Ozone exposure, defoliation of beech (*Fagus sylvatica* L.) and visible foliar symptoms on native plants in selected plots of South-Western Europe. Environmental Pollution 145, 644-651.

Flexas J., Medrano I., 2002. Drought-inhibition of photosynthesis in C_3 plants, stomatal and non-stomatal limitation revisited. Annals of Botany 89, 183-189.

Fontaine V., Cabane M., Dizengremel P., 2003. Regulation of phosphoenolpyruvate of carboxylase in *Pinus halepensis* needles submitted to ozone and water stress. Physiologia Plantarum 117, 445-452.

Gerosa G., Marzuoli R., Desotgiu R., Bussotti F., Ballarin-Denti A., 2008. Ozone uptake vs ozone exposure in relation to visible leaf injury in young trees of *Fagus sylvatica* L. and *Quercus robur* L. An Open-Top Chambers experiment in South Alpine environmental conditions. Environmental Pollution 152, 274-284.

Gerosa G., Marzuoli R., Desotgiu R., Bussotti F., Ballarin Denti A., 2009. Validation of the stomatal flux approach for the assessment of ozone visible injury in young forest trees. Results from the TOP (transboundary ozone pollution) experiment at Curno, Italy. Environmental Pollution 157, 1497-1505.

Gravano E., Giulietti V., Desotgiu R., Bussotti F., Grossoni P., Gerosa G., Tani C., 2003. Foliar response of an *Ailanthus altissima* clone in two sites with different levels of ozone-pollution. Environmental Pollution 121, 137-146.

Gravano E., Bussotti F., Strasser J.R., Schaub M., Novak K., Skelly J., Tani C., 2004. Ozone symptoms in leaves of woody plants in open top chambers, ultrastructural and physiological characteristics. Physiologia Plantarum 121, 620-633.

Hůnová I., Matoušková L., Srněnský R., Koželková K., 2011. Ozone influence on native vegetation in the Jizerske hory Mts. of the Czech Republic, results based on ozone exposure and ozone-induced visible symptoms. Environmental Monitoring and Assessment183, 501-515.

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. Haupt, Bern.

Jagtap V., Bhargava S, Streb P., Feierabend J., 1998. Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. Journal of Experimental Botany 49, 1715-1721.

Kitao M.J., Winkler B., Löw M., Nunn A.J., Kuptz D., Häberle, K.-H., Reiter I.M., Matyssek R., 2012. How closely does stem growth of adult beech (*Fagus sylvatica*) relate to net carbon gain under experimentally enhanced ozone stress? Environmental Pollution 166, 108-115.

Kivimäenpää M., Sutinen S, Calatayud V., Sanz M.J., 2010, Visible and microscopic needle alterations of mature Aleppo pine (*Pinus halepensis*) trees growing on an ozone gradient in eastern Spain. Tree Physiology 30, 541-554.

Manning W.J., Godzik B., 2004. Bioindicator plants for ambient ozone in Central and Eastern Europe. Environmental Pollution 139, 33-39.

Manning W.J., Godzik B., Musselman R., 2002. Potential bioindicator plant species for ambient ozone in forested mountain areas of central Europe. Environmental Pollution 119, 283-290.

Marzuoli R., Gerosa G., Desotgiu R., Bussotti F., Ballarin Denti A., 2009. Ozone fluxes and foliar injury development in a sensitive poplar clone *(Populus maximowiczii* Henry X *P*. x *berolinensis* Dippel - Oxford clone). A dose-response analysis. Tree Physiology 29, 67-76.

Matyssek R., Baumgarten M., Hummel U., Häberle K.-H., Kitao M., Wieser G., 2015. Canopy-level stomatal narrowing in adult *Fagus sylvatica* under O_3 stress - Means of preventing enhanced O_3 uptake under high O_3 exposure? Environmental Pollution 196, 518-526.

Mills G., Hayes F., Simpson D., Emberson L., Norris D., Harmens I., Büker P., 2011. Evidence of widespread effects of ozone on crops and (semi-)natural vegetation in Europe (1990–2006) in relation to AOT40- and flux-based risk maps. Global Change Biology 17, 592-613.

Novak K., Skelly JM., Schaub M., Kraeuchi N., Hug C., Landolt W., Bleuler P., 2003. Ozone air pollution and foliar injury development. Environmental Pollution 125, 41-52.

Novak K., Schaub M., Fuhrer J., Skelly JM, Hug C., Landolt W., Bleuler P., Kräuchi N., 2005. Seasonal trends in reduced leaf gas exchange and ozone-induced foliar injury in three ozone sensitive woody plants species. Environmental Pollution 136, 33-45.

Novak K., Cherubini P., Saurer M., Fuhrer J., Skelly J.M., Kräuchi N., Schaub M., 2007. Ozone air pollution effects on tree-ring growth, $\delta^{13}C$, visible foliar injury and leaf gas exchange in three ozone sensitive woody plant species. Tree Physiology 27, 941-949

Paoletti E., Contran N., Bernasconi P., Günthardt-Goerg M.S., Vollenweider P., 2009. Structural and physiological responses to ozone in Manna ash (*Fraxinus ornus* L.) leaves of seedlings and mature trees under controlled and ambient conditions. Science of the Total Environment 407, 1631-1643.

Pollastrini M., Desotgiu R., Camin F., Ziller L., Marzuoli R., Gerosa G., Bussotti F., 2013. Intra-annual pattern of photosynthesis, growth and stable isotope partitioning in a poplar clone subjected to ozone and water stress. Water Air and Soil Pollution 224, 1761 (11 pp.).

Pollastrini M., Desotgiu R., Camin F., Ziller L., Gerosa G., Marzuoli R., Bussotti F., 2014. Severe drought events increase the sensitivity to ozone on poplar clone. Environmental and Experimental Botany 100, 94-104

Popper K., 1957. Science, conjectures and refutations. I n: Philosophy of Science, a Personal Report in British Philosophy in Mid-Century. C A Mace publisher (London UK.).

Schaub M., Calatayud V., Ferretti M., Brunialti G., Lövblad G., Krause G., Sanz M.J., 2010. Monitoring of Ozone Injury. Manual Part X. In, Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. UNECE ICP Forests Programme Co-ordinating Centre, Hamburg. [http,//www.icp-forests.org/pdf/FINAL_AAQsympt.pdf]

Schaub M., Jakob P., Bernhard L., Innes J.L., Skelly J.M., Kräuchi N., 2002. Ozone injury database. Swiss Federal Research Institute WSL, BirmensdorF., CH. [http,//www.ozone.wsl.ch]

Skelly J.M., Innes J.L., Savage J.E., Snyder K.R., Vanderheyden D., Zhang J., Sanz M.J., 1999. Observation and confirmation of foliar ozone symptoms of native plant species of Switzerland and Southern Spain. Water Air and Soil Pollution 116, 227-234.

Smith G., Coulston J., Jepsen E., Prichard T., 2003. A national biomonitoring program – results from field surveys of ozone sensitive plants in Northeastern forests (1994-2000). Environmental Monitoring and Assessment 87, 271-291.

Soda C., Bussotti F., Grossoni P., Barnes J., Mori B., Tani C., 2000. Impacts of urban levels of ozone on *Pinus halepensis* foliage. Environmental and Experimental Botany 44, 69-82.

VanderHeyden D.J., Skelly J.M., Innes J.L., 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.

Vollenweider P., Ottiger M., Günthard-Goerg M.S., 2003a. Validation of leaf ozone symptoms in natural vegetation using microscopical methods. Environmental Pollution 124, 101-118.

Vollenweider P., Woodcock I., Kelty M.J., Hofer R.-M., 2003b. Reduction of stem growth and site dependency of leaf injury in Massachusetts black cherries exhibiting ozone symptoms. Environmental Pollution 125, 467-480.

Wan W., Xia Y., Zhang I., Wang J., Wang X., 2013. The ambient ozone pollution and foliar injury of the sensitive woody plants in Beijing exurban region. Acta Ecologica Sinica 33, 1098-1105.

Wan W., Manning W.J., Wang X., Zhang I., Sun X., Zhang Q. 2014. Ozone and ozone injury on plants in and around Beijing, China. Environmental Pollution 191, 215-222.

Wittig V.E., Ainsworth E.A., Naidu S.L., Karnosky D.F., Long SP., 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry, a quantitative meta-analysis. Global Change Biology 15, 396–424.