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Defense and avoidance of ozone under global change

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Modeling of ozone effects on plants should include a measure for the plant defense capacity.

Abstract

The level II approach of the critical loads concept adopted by the UNECE aims at a flux based evaluation and takes into account environmental factors governing stomatal conductance. These factors will probably be affected by global change. The flux concept predicts that a decrease in stomatal conductance would protect trees from air pollution effects by decreasing uptake. However, experimental evidence is inconclusive. Numerous results suggest that pollutants and factors subject to global change (drought, CO_2) may interact and even exacerbate effects, probably because antioxidative defense systems are involved in both, defense against pollutant effects and protection from natural stress. An effective pollutant dose, which is weighted by physiological defense capacity, would better predict such effects. In this review paper we argue that the flux-based approach is imperfect, because global change effects may also modify the physiological susceptibility to ozone. Instead, a flux concept weighted by defense capacity should be tested.

Keywords: Antioxidative defense; Drought; Elevated CO_2; Global change; Ozone flux

1. Introduction

While it is well known that increased tropospheric ozone (O_3) concentrations can be toxic to plants in general and to forest trees in particular (Reich, 1987; Ashmore, 2005), establishing clear cause and effect relationships for forest trees under ambient conditions is difficult (Manning, 2005). After decades of research it is perhaps surprising that the process base of ozone damage to plants is still not fully clarified (Matyssek et al., 2005). In addition to scaling problems from small seedlings typically subjected to experimental exposures to mature forest trees (Manning, 2005), interactive effects with other environmental factors come into play in natural stands. In particular, climate change and the increase in ozone concentrations have common causes in the burning of fossil fuels and have to be viewed concurrently. As a consequence, risk assessments at natural stands are difficult and ambiguous and often do not reflect the observations made in the field (Matyssek and Innes, 1999; Loibl et al., 2004).

The approach of ''Critical Levels for Ozone'' introduced by the UNECE originally defined exposure-based threshold values of the AOT40 (accumulated dose over the threshold of 40 nl l^{-1}). Exceedance of this threshold would indicate a risk of biomass loss of more than 10% (LRTAB, 2004). In a level II approach of the Critical Levels concept a flux based evaluation is tested (Emberson et al., 2000; Ashmore et al., 2004). In such a model the external exposure – defined as the AOT40 – is translated to the dose actually taken up into the plants. For this purpose, environmental meteorological factors (e.g. ambient vapor pressure deficit, solar radiation) governing stomatal conductance can be taken into account, or a simple stomatal model can even improve the performance

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of the model (Karlsson et al., 2004). It is widely anticipated that these factors controlling stomatal behavior will vary in the future due to global change. In the view of the flux based concept, a decrease of stomatal conductance (e.g. due to increasing drought or elevated CO_2 as anticipated by many global change scenarios) would protect trees from certain air pollution effects through avoidance of uptake.

However, the experimental evidence does not always support this hypothesis. There are numerous results suggesting that pollutants and factors subject to global change (drought, CO_2) may interact in different ways and even exacerbate effects. The stress physiological basis thereof is that the antioxidative defense system comes into play in both, defense against pollutant effects, and protection from natural stress effects (e.g. drought). It has been suggested that an ''effective pollutant dose'', which is weighted by physiological defense capacity, would be more appropriate to predict such effects.

In this opinion paper (which is not meant to be an exhaustive review), we argue that that a flux-based approach alone is imperfect, because global change effects may also modify the physiological susceptibility to ozone. Instead, a flux concept weighted by defense capacity should be widely tested.

2. From ozone exposure to plant responses

Ozone effects on plants are the result of a three-step chain of events: exposure, uptake, and biological effect (Fig. 1). Only the amount of ozone arriving at the cell membrane or, perhaps, at the cell wall (apoplast), can have a biological effect on the tissue.

Ozone exposure measurement is a physical rather than biological problem and reliable data can be measured or modeled in many instances. Much has been written about the preferred metric describing ozone exposure and the scientific community has generally adopted the AOT40 concept as a suitable descriptor (LRTAB, 2004), although it e.g. neglects night time exposure which may be significant in some cases (Wieser and Havranek, 1995; Matyssek et al., 1995; Grulke et al., 2004). While it may be difficult to condense the exposure characteristic, which is composed of time courses of ambient concentrations, into one representative value, we can safely accept that it is presently in principle possible to characterize the exposure in detail. However, a characterization of exposure alone is obviously not enough to conduct a meaningful risk assessment for biological systems.

The uptake of ozone is largely controlled by the stomata, the main entry pathway for ozone. Stomatal conductance (g_s) is the metric required to calculate the ozone flux into the leaf at a given external concentration. Current models describing ozone uptake into plant foliage usually build in environmental factors that control stomatal opening (e.g. a metric that allows for soil drought or air humidity) or use models for stomatal conductance (Karlsson et al., 2004).

Once ozone has entered the substomatal cavity it reacts quickly with molecules in the adjacent cell walls or with constituents of the outer cell membrane. The intercellular concentration of ozone is assumed to be close to zero because of the high reaction velocity of such chemical reactions (Laisk et al., 1989). However, the reaction products of ozone – reactive oxygen species (ROS) or oxidation products of biomolecules originating from the interaction of ozone with cellular redox systems – initiate or mediate reactions within the living tissues leading to known ozone effects such as decreases in photosynthetic rates, discoloration, and cell death. There is evidence that suggests than ozone interferes with a cell-death related signaling pathway, which uses ROS as trigger substances (Matyssek and Sandermann, 2003; Baier et al., 2005). More generally, ozone effects on living cells are related to

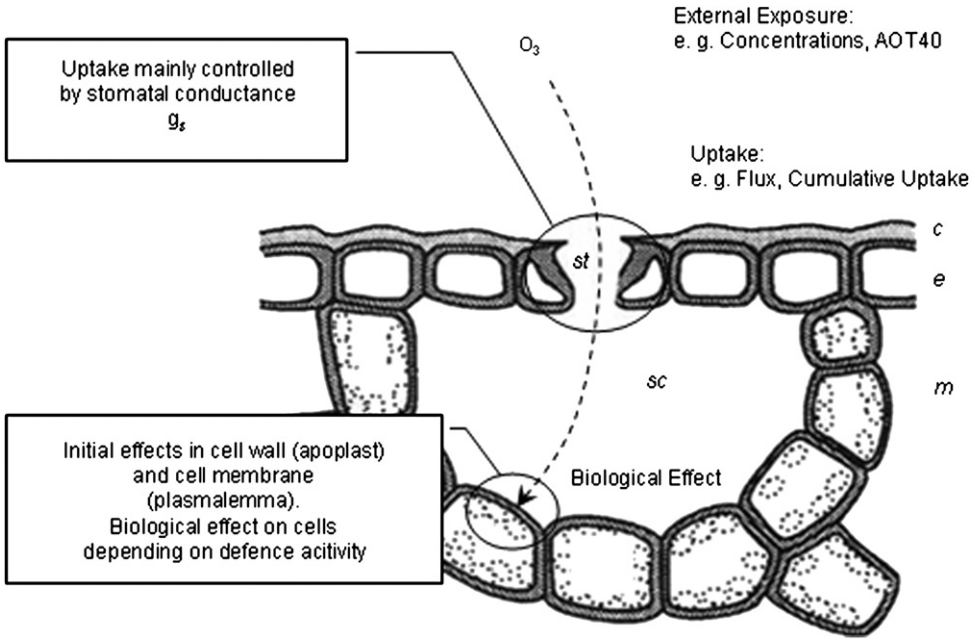

Fig. 1. Scheme of O_3 uptake and biological effect on leaves. st stoma, sc substomatal cavity, c cuticle, e epidermis, m mesophyll, g_s stomatal conductance.

the action of ROS and the interaction with antioxidative defense systems (see below).

Each of these steps of ozone effects on plants is likely to be interactively modified by other environmental factors, which will be altered by global change.

3. Ozone and antioxidative defense systems

Oxidative stress in green plant cells results mainly from the production of reactive oxygen species (ROS) in electron transport chains such as in the photosynthetic apparatus (photo-oxidative stress), mitochondria, or from the direct action of oxidizing pollutants (such as ozone, in itself an ROS). ROS, such as the superoxide anion free radical (O_2^-), the hydroxyl free radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), or singlet oxygen ($^1O_2$), are highly toxic molecules able to modify or destroy cell constituents such as proteins, lipids, pigments, nucleic acids etc. Since the occurrence of photo-oxidative stress is an inescapable feature of oxygenic photosynthesis, plants have evolved a suite of protective systems – the antioxidative defense systems – to keep ROS under control.

These antioxidative defense systems of plant cells consist of low-molecular weight antioxidants – the central compounds in plants are ascorbic acid (Smirnoff, 2000) and glutathione (DeKok and Tausz, 2001) – and enzymes related to redox reactions and regeneration of antioxidants (Fig. 2). In a recent paper, D'Haese et al. (2005) showed that ascorbate and homoglutathione (a glutathione-analogue replacing glutathione in some Fabaceae) account for 60–70% of the total antioxidative capacity in symplast extracts of *Trifolium repens* leaves, which underlines the major importance of these compounds. Some of the antioxidative components – e.g. ascorbate – are also present in considerable concentrations in the apoplast (Polle et al., 1995).

While ROS were long viewed as simply toxic to living cells, it is now clear that they can also act as messengers in cell responses to the environment. For example, H_2O_2 is produced in response to pathogen attack and is involved in the triggering of the hypersensitive reaction characterized by programmed death of cells. In such a view, the antioxidative defense systems are not only a protection against ROS, but have the potential to mediate and control signal transduction, and to play a role in environmental redox sensing in cells (Foyer and Noctor, 2005).

Oxidative stress related to ozone impact is very likely also transduced by ROS, which are formed as products of ozone reactions in the apoplast and at the cell membranes. Hence, ozone-induced oxidative stress is mediated and controlled by the antioxidative defense systems. Of the numerous studies reporting effects of ozone on the antioxidative defense systems, and a majority observed increased concentrations of antioxidants or elevated defense enzyme activities upon ozone exposure (e.g. Herbinger et al., 2005). In *Populus tremuloides*, free-air ozone exposure led to changes in the expression of a multitude of genes. A number of antioxidative defense- and signaling-related genes were upregulated (Gupta et al., 2005). Ozone sensitivity of plants seems to be related to the antioxidant status, in particular the ascorbate status (Chen and Gallie, 2005). Ascorbate is also present in considerable concentrations in the apoplast (the cell walls) and is thought to form a first line of defense against ozone (Polle et al., 1995). Acute ozone exposure can even lead to a depletion (oxidation) of the apoplastic ascorbate pool (Luwe, 1996), because the regeneration of ascorbate from dehydroascorbate requires retranslocation into the symplast. Some observations suggest that this glutathione-dependent regeneration may be the rate limiting step (Luwe, 1996).

Functional evidence for a direct relation of single antioxidative compounds – such as ascorbate – with ozone sensitivity, however, remains rather ambiguous. For example, transgenic tobacco plants with overexpressed dehydroascorbate-reductase – increasing endogenous ascorbate concentrations – were more

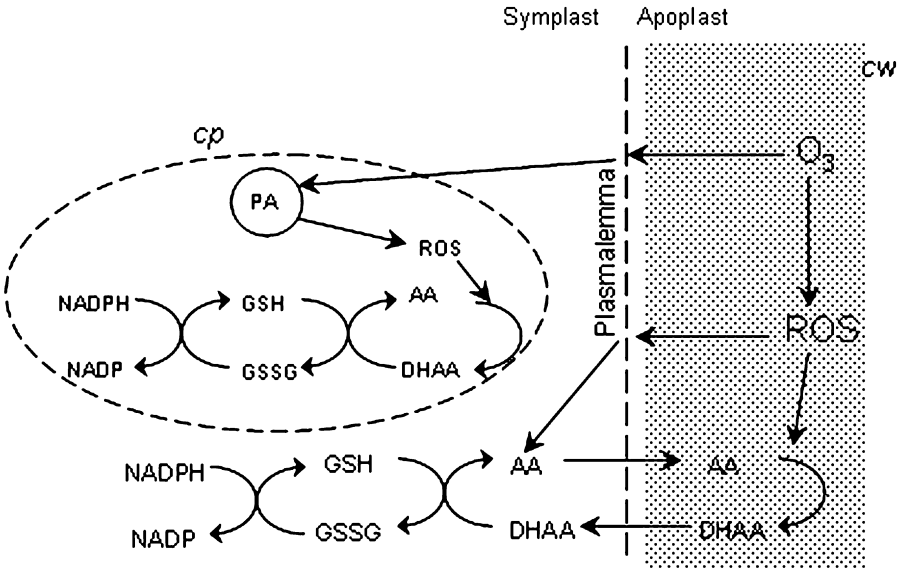

Fig. 2. Simplified scheme of antioxidative defense systems in green cells, their compartmentation, and their interaction with O_3. ROS reactive oxygen species, AA ascorbic acid, DHAA dehydroascorbic acid, GSH glutathione, GSSG oxidised glutathione, PA photosynthetic apparatus, cp chloroplast, cw cell wall.

tolerant to O_3 despite higher uptake rates (Chen and Gallie, 2005). In contrast, apoplastic ascorbate alone could not explain differential ozone tolerance of *Trifolium* clones (D'Haese et al., 2005).

From these findings we can conclude that the antioxidative defense systems play an important role in conferring ozone tolerance to plants, but that the exact mechanisms are yet to be elucidated. In any case, the antioxidant status of the tissue (concentrations, redox states) would be crucial in determining the biological effect of ozone.

4. Global change factors (drought and elevated CO_2) and the antioxidative defense systems

Drought induces stomatal closure, which will not only limit water loss through transpiration, but also CO_2 uptake for photosynthesis. Under high light conditions, this will result in excess excitation energy in the chloroplasts and photo-oxidative stress in green tissues (Smirnoff, 1993). The antioxidative and photoprotective systems are crucial in plant defense against adverse drought effects (Flexas and Medrano, 2002). A multitude of studies revealed drought effects on the antioxidative systems of tree foliage, mostly showing increased antioxidant concentrations or changes in the redox state (e.g. Šircelj et al., 2005). This led to some confusion whether drought exerts pressure on the antioxidative systems thereby weakening the antioxidative defense, or if it elicits an increase in antioxidant concentration and thereby potentially strengthens defense capacities. A time-course analysis of progressing drought showed that antioxidants follow a stress-response concept with an initial signaling phase followed by increasing antioxidative capacity, which then breaks down immediately before the tissues experience irreparable damage (Tausz et al., 2004a).

The effect of elevated CO_2 on the antioxidative defense system of plants is less clear, despite numerous completed studies. Some authors argued that elevated CO_2 would increase antioxidative defense capacity by increasing the assimilate availability. This may be particularly true for ascorbate, which is closely linked to carbohydrate metabolism (Smirnoff, 2000). Accordingly, some studies found increased concentrations of ascorbate upon exposure to elevated CO_2 and concluded that the oxidative risk was diminished (Marabottini et al., 2001). In other studies, there was no indication that elevated CO_2 would strengthen the antioxidative defense, because no effects on antioxidants were observed (for example in *Pinus ponderosa*, Tausz et al., 2004b). Gene expression studies on aspen trees exposed to free-air elevated CO_2 even indicated decreased expression of antioxidative genes (Karnosky et al., 2003). Presently it seems inconclusive as to whether elevated CO_2 enhances plant protection from oxidative stress by increasing antioxidative defense.

5. Interactive effects of elevated CO_2 and O_3

A number of studies have directly addressed potential interactions between elevated CO_2 and O_3, including a large scale free-air exposure study on trees (Karnosky et al., 2003). Most studies indicated that elevated CO_2 may ameliorate the adverse effects of O_3 on trees, but the mechanisms of such an effect remain unclear.

Firstly, elevated CO_2 could decrease stomatal conductance (as regulated by inter-cellular CO_2 concentration c_i) and – consequently – O_3 uptake. In a recent comprehensive review, Paoletti and Grulke (2005) discussed the interactions of CO_2 and O_3 on stomata in detail. Among numerous other gaps in current knowledge, the authors highlighted that it is as yet unclear, if and how stomatal conductance will be modified by elevated CO_2 over the long term. The authors concluded that only if "stomatal acclimation to CO_2 does not lessen over time, (this) will corroborate the protection against O_3". Several other uncertainties emerged in that review, such as e.g. the largely different behavior of different species, and the incomplete knowledge about stomatal sensing mechanisms. Further, the effects of long term elevated CO_2 on guard cell anatomy, especially stomatal density and pore size, potentially modify O_3 uptake at the level of the stomata.

Secondly, as discussed above, elevated CO_2 may increase defense capacities. Many studies failed to find evidence for this effect, or, if elevated CO_2 ameliorated O_3 effects, this was mainly ascribed to the stomatal effects. In a recent study, Booker and Fiscus (2005) showed that the same O_3 flux into leaves of soy bean had a less detrimental effect at elevated CO_2. However, this could not be explained by elevated antioxidant concentrations, which lead the authors to conclude that "increased carbon assimilation, and possibly as yet undetermined factors" contributed to the observed decrease in sensitivity. Although there is good evidence that elevated CO_2 increases a variety of defense metabolites such as phenolic glycosides (Karnosky et al., 2003, 2005), the effect of elevated CO_2 on the more ubiquitous antioxidative systems such as ascorbate and glutathione, in particular in interaction with other stress factors (such as O_3), remains unclear.

6. Interactive effects of drought and O_3

According to O_3 uptake models, the interactive effects between drought and O_3 should be straightforward: drought-induced stomatal closure will limit pollutant uptake into the leaves and hence protect trees from ozone damage. Some support for this hypothesis is provided by the evaluation of O_3 uptake models, but results in the field can often not be adequately explained by simply including stomatal uptake rates into the models (e.g. Davison et al., 2003; Loibl et al., 2004). More complicated interactive effects on stomata have been emphasized (Paoletti and Grulke, 2005). O_3 itself has an effect on stomata of some species, which may lead to slow ("sluggish") reactions to environmental stimuli or even incomplete closure (Reich and Lassoie, 1984; Grulke et al., 2005). This would exacerbate drought effects, because the plants would be less able to limit and control their water loss (compare also Karnosky et al., 2005).

On the other hand, plants employ the antioxidative defense systems to counteract both drought and O_3 stress, hence a certain cross tolerance effect may occur between both stress

factors. Some evidence for a ''hardening'' effect of drought stress to subsequent O_3 exposure was reported (Kronfuß et al., 1998). Such an effect would strongly depend on the timing, intensity, and order of exposure to the different stressors.

Some results even suggest that drought might exacerbate O_3 effects. For example, we investigated *Pinus jeffreyi* trees within the same stand, but with different access to water and, consequently, different drought exposure at their natural site. As expected, O_3 uptake of xeric trees was much less than into mesic trees, but some symptoms (e.g. the ''chlorotic mottling'' regarded as the definitive O_3 symptom for this pine species) were even more pronounced in xeric trees (Grulke et al., 2003b). These results show that either the ''O_3 symptoms'' are not perfectly specific for O_3, or that drought stressed trees are more sensitive to O_3 than non-stressed trees. A multivariate analysis of antioxidative parameters and symptoms suggested two major axes of variation, one related to O_3 uptake and the other one rather related to photo-oxidative stress, which may be the consequence of drought exposure (Grulke et al., 2003a). Chlorotic mottling was related equally to both axes of variation, which indicates that it may not be exclusively related to O_3, but a more general symptom of oxidative stress.

Such results highlight the need to enhance the current flux-based models of O_3 effects by a plant specific ''sensitivity'' component related to the stress-physiological state.

7. How to weight O_3 influx physiologically?

The conceptual model of ozone injury to plants published by Massman et al. (2000) embraced the idea of a defense component offsetting the effect of the ozone flux into the leaf. The authors stated that ''at present it is impossible to model or directly parameterize'' the defense capacity (Massman et al., 2000). However, while in the last years many attempts have been made to evaluate the O_3 influx into tree foliage, only few studies attempted a direct weighting of this influx by the physiological status of the tree, which would determine its susceptibility (but compare e.g. Ashmore, 2005 and Martin et al., 2001).

Components of the antioxidative system are good candidates for such a weighting, but there is no obvious single candidate molecule. Given the dynamic nature of O_3 influx, ROS generation, scavenging, and the regeneration of antioxidants, the turnover rates of antioxidant pools may be more important than concentrations alone. Polle (2001) used a computer simulation model to estimate fluxes and turnover rates in antioxidative detoxification pathways in chloroplasts. However, many simplifications – e.g. the assumptions that all reactions occur in homogeneous aqueous phase, that there are no competing reactions, or that the enzymes in question are not regulated – were necessary. Furthermore, the model was only applied to the chloroplast. Regardless of the high value of this approach as an analytical tool to improve our understanding of the antioxidative systems, it seems presently impractical to measure antioxidant turnover rates and use them as an estimate for plant susceptibility.

Wieser et al. (2002a) suggested a simpler and practical approach to weight the O_3 influx by antioxidant concentrations present in the leaves: antioxidant concentrations based on the leaf area were related to the maximum O_3 flux, which gives an estimate of the potentially available detoxification capacity per O_3 influx (Eq. (1)).

$$AA = A/F_{O3} \tag{1}$$

AA is the potentially available antioxidant amount per unit ozone influx (in nmol Antioxidant $nmol^{-1}$ s O_3^{-1}), A is the antioxidant concentration per leaf area (nmol m^{-2} leaf surface area), and F_{O3} the maximum ozone influx (nmol O_3 m^{-2} s^{-1}). Because of the predominant role of ascorbate, Wieser et al. (2002a) tested their approach with this antioxidant. The results helped to explain differences in the susceptibility between shade and sun canopy of spruce (Wieser et al., 2002b). This model was taken up in further studies comparing ozone effects in chambers and under open air conditions (Nunn et al., 2005). However, other antioxidants such as glutathione (which is ultimately responsible for the regeneration of ascorbate) should be tested as well. This model still needs fairly involved direct measurements of antioxidant concentrations in leaves, but is potentially able to account for differences in tolerance among species, seasons, canopy positions etc. Currently, this model is being evaluated on the Kranzberg Forest site (Nunn et al., 2005), where a multiple year dataset including antioxidant concentrations (Herbinger et al., 2005) and O_3 flux data (Löw et al., in preparation) will become available. Other intensive study sites would lend themselves to the evaluation of such a model.

As an even simpler approach, Matyssek et al. (manuscript in preparation) suggest the use of specific leaf area (leaf area per g leaf dry weight, m^2 g^{-1}, SLA) as a first approximation to weigh the ozone influx by the potentially available detoxification capacity. This would e.g. account for the relative tolerance of sclerophyllous trees (low specific leaf area) compared to deciduous broadleaves. While not a strong advance in mechanistic understanding (the approach essentially assumes that the detoxification capacity per g mesophyll is constant across species and during time), such a simple approach could be evaluated on large, readily available data-sets.

We suggest a three tiered approach towards refining the flux concept: Firstly, the simple weighting by SLA as a surrogate for mesophyll detoxification capacity can be tested across large data-sets and potentially accounts for between species and between ecosystem differences in sensitivity. Secondly, components of the antioxidant system used according to the model by Wieser et al. (2002a) potentially account for differences in defense capacity among species, seasons, and related to the physiological state of the trees. Thirdly, more detailed basic research into the turnover of antioxidative systems and the mechanisms by which ozone interacts with cell metabolism may help to advance the modeling of detoxification rates in a fully mechanistic approach.

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