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Ozone damage, detoxification and the role of isoprenoids - new impetus for integrated models

Supriya Tiwari[A*], Rüdiger Grote[B*], Galina Churkina[C], Tim Butler[C]

[A] Department of Botany, Banaras Hindu University, Varanasi-221003, India

[B] Karlsruhe Institute of Technology, Institute of Meteorology and Climate Research, Garmisch-Partenkirchen, Germany

[C] Institute of Advanced Sustainability Studies, Potsdam, Germany

* these authors contributed equally to the manuscript

Corresponding author: Rüdiger Grote, Email: Ruediger.Grote@kit.edu

Abstract

High concentrations of ozone (O_3) can have large impacts on the health and productivity of agricultural and forest ecosystems, leading to significant economic losses. In order to estimate this impact under a wide range of environmental conditions, the mechanisms of O_3 impacts on physiological and biochemical processes have been intensively investigated. This includes the impact on stomatal conductance, the formation of reactive oxygen species and their effects on enzymes and membranes, as well as a number of induced and constitutive defense responses. This review summarizes these processes, discusses their importance for O_3 damage scenarios and assesses to which degree this knowledge is currently used in ecosystem models which are applied for impact analyses. We find that even in highly sophisticated models, feedbacks affecting regulation, detoxification capacity, and vulnerability are generally not considered. This implies that O_3 inflicted alterations in carbon and water balances cannot be sufficiently well described to cover immediate plant responses under changing environmental conditions. Therefore, we suggest conceptual models that link the depicted feedbacks to available process-based descriptions of stomatal conductance, photosynthesis and isoprenoid formation. Particularly the linkage to isoprenoid models opens up new options for describing biosphere-atmosphere interactions.

Keywords

oxidative damage, physiological defence, biogenic volatile organic compounds, reactive oxygen species, impact modelling

1 Introduction

The existence of ozone (O_3) in the atmosphere can be traced back to the first formation of oxygen by prokaryotic organisms 2-3 billion years ago, when the first stratospheric ozone layer formed. Here, its presence has been beneficial to life on earth as it prevents harmful ultraviolet radiation from reaching the earth's surface. Tropospheric O_3 in high concentrations, however, poses serious threats to human health and plant productivity. In North America and Europe, daily peak O_3 concentrations as high as 200 - 400 ppb episodically occur during the late afternoon hours in summers (Royal Society 2008). Peak O_3 concentrations are tending to decline in North America and Europe (Ashmore 2005) but are increasing in and around many cities in these regions (Paoletti *et al.* 2014). In Asia, O_3 - concentration are still soaring to new records (see Feng et al. (2015) and references therein).

High O_3 concentrations threaten human health and decrease agricultural as well as forest productivity. The general global estimates of agricultural and forest yield losses associated with high O_3 concentrations are 3 - 16 percent (Avnery *et al.* 2011) but may be more than 30 percent depending on species and metric used (Ainsworth *et al.* 2012). Ozone damages have remarkable economic importance: Chameides et al. (1994) estimated that 9 - 35% of the World's cereal crops are exposed to O_3 concentrations that possess the potential of causing yield reductions. In Europe, crop losses from O_3 damage for 23 crops in 47 countries were estimated to be €6.7 billion per year ($9.6 billion) (Holland *et al.* 2006).

In order to derive O_3 impacts on biomass and yield, a number of indices have been developed to account for some non-linear features. For example, a critical level can be defined as a "concentration, cumulative exposure or cumulative stomatal flux of atmospheric pollutants above which direct adverse effects on sensitive vegetation may occur according to present knowledge" (UNECE 2004). This concept describes a threshold concentration approach, the most prominent example of which is the AOT40 (**A**ccumulated **O**zone Exposure over a **T**hreshold of **40**ppb) index. AOT40 is calculated as the cumulative sum of differences between hourly O_3 concentrations at daylight above a threshold concentration of 40 ppb. This

index is also the most common basis for estimating the potential risk of plants to be damaged by O_3 exposure and for setting environmental quality objectives within the European Union (EU) and the United Nation Economic Commission for Europe (UN/ECE) (Directive 2002/3/EC; UN/ECE, 2004). Despite substantial criticism regarding further modifying impacts that are neglected by the method, concentration-based critical levels are commonly used because they only require O_3 measurements or estimates but no climatic or other environmental data (UNECE 2004).

The actual damage, however, does not depend on concentrations, but on the amount of O_3 that enters the plant, the capability of the plant's defense system, and the plant's sensitivity to oxidative stress (Sharma *et al.* 2012). The transport into the foliage occurs almost exclusively through stomata, which also regulate CO_2 uptake while preventing desiccation. Dry conditions may thus prevent damages even if O_3 concentrations are high. Therefore, flux-based approaches for O_3 risk assessment are preferred particularly if changing environmental conditions shall be evaluated. Such approaches are for example the '**P**hytotoxic **O**$_3$ **D**ose above a flux threshold of **Y** nmol m^{-2} projected leaf area s^{-1}', PODY (Mills *et al.* 2011), or the '**C**umulative **U**ptake index of **O**zone', CUO, used with or without threshold uptake rates (Pleijel *et al.* 2004). Flux based indices have shown to be superior to the concentration-based index AOT40 in explaining yield reductions for wheat and potato (De Andres *et al.* 2012; Pleijel *et al.* 2004) as well as biomass reductions and visible leaf injuries for trees (Karlsson *et al.* 2007). However, the regional calculations of O_3 uptake often neglect the limiting effect of soil moisture on O_3 uptake and thus tend to overestimate the actual damage (UNECE 2004). Physiological pre-disposition or detoxification capacity is also not considered in impact studies which caused Tausz et al. (2007) to propose a flux-concept weighted by the defense capacity of plants.

Despite some approaches that incorporate the water balance into O_3 uptake calculations (Ewert and Porter 2000; Van Oijen *et al.* 2004) and to consider the carbon cost of detoxification (Deckmyn *et al.* 2007; Plöchl *et al.* 2000), a mechanistic description of induced defenses is generally absent in O_3 impact models. Moreover, linkages to other physiological processes such as photosynthesis and the production of antioxidants, including the emission of biogenic volatile organic compounds (BVOC), have not yet been established in ecosystem models. Therefore, we review the current state of knowledge about O_3 impacts on plants with particular emphasis on how this knowledge can be used to describe plant response to O_3 more realistically and to incorporate it into ecosystem models. In the following, we discuss the mechanisms and environmental dependencies of O_3 uptake, subsequent generation of reactive

oxygen species (ROS) and their effects on plants, as well as the defense mechanisms adopted by plants to cope with O_3 stress. Finally, we summarize these findings into conceptual models that might be useful for further developments. The analysis is limited to stomatal and biochemical responses and does not consider long-term effects such as necrosis development and enhanced senescence of leaves.

2 Ozone damage

2.1 Stomatal regulation

Ozone enters the plant through stomatal openings of the leaves. A higher stomata density and wider stomatal aperture thus enables higher O_3 flux into the plant. Because stomata stay open under conditions that are favorable for photosynthesis, O_3 uptake is increased under high light and optimum temperature, but decreases under drought stress (Fares *et al.* 2010). Elevated [CO_2] tends to trigger stomatal closure increasing water use efficiency of the plants and thus tend to decrease O_3 influx (see Fig. 1). In general, any genetic or environmental factor that decreases stomatal conductance is decreasing the susceptibility against O_3 damage. This can be seen as the reason for the lower sensitivity to O_3 pollution found in gymnosperms compared to angiosperms (e.g. Wittig *et al.* 2007) and in C4- compared to C3 plants (e.g. Li *et al.* 2008).

Chronic O_3 exposure causes stomatal dysfunction (Fig. 1). This has been described as a decreased sensitivity of stomata to environmental conditions with the effect of de-coupling stomata responses and photosynthesis (Lombardozzi *et al.* 2012). The effect leads to hysteresis also described as stomatal sluggishness (Dumont *et al.* 2013; Hoshika *et al.* 2014), and finally causes stomata failure. This phenomenon has been widely observed and it has been proven to decrease water use efficiency and to increase O_3 uptake rate (VanLoocke *et al.* 2012; Wittig *et al.* 2007). Based on observations of increased fluxes of potassium ions into guard cells, as well as elevated cytosolic calcium concentration within these cells during O_3 exposure, alterations in membrane permeability of the stomatal cells have been suggested as a possible mechanism (Dumont *et al.* 2013). This is consistent with the detailed membrane processes of anion channel regulation due to ROS that are reviewed in Van Breusegem et al. (2008). The stomata sensitivity to O_3 seems to be smaller in young leaves (Bernacchi *et al.* 2006) and varies widely between varieties of the same crop (e.g. Morgan *et al.* 2003), as well as within a specific plant species when grown at different locations (Pyakurel and Wang 2014). Additionally, the number, size and responsiveness of stomata are known to also vary

within a canopy due to different photosynthesis activities and environmental gradients, highlighting the importance of considering various physiological and micro-meteorological feedbacks (Tarvainen *et al.* 2013; Van Wittenberghe *et al.* 2012).

2.2 Increased ROS concentration

After entering through the stomata, O_3 reacts with the liquid phase components of the apoplast which generates ROS and increases the level of the oxidative species that are already constitutively present or produced by means of other stress impacts such as biotic stress, salinity, drought or high light intensity (Das and Roychoudhury 2014). Because the destructive potential but also the mobility of ROS across membranes vary, different molecules should be distinguished: O_2 can be excited to singlet oxygen (O_2^1) or transformed to superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) or a hydroxyl radical (OH^-), by the transfer of one, two or three electrons to O_2, respectively. The first product of the oxidative degradation of O_3 in the apoplast is the O_2^- anion which appears in the cells as its conjugate acid, hydroperoxyl radical ($HO_2^{\cdot}$). $O_2^{\cdot-}$ is the most important ROS produced endogenously but does cross membranes only slowly while H_2O_2, which is a relatively long-lived neutral molecule and also $HO_2^{\cdot}$, can diffuse in the symplastic space through membrane aquaporins (see Fig. 1, Bienert *et al.* 2007). H_2O_2 then triggers the formation of OH^- radicals which are extremely reactive and are damaging to lipids, pigments, proteins, and nuclear acids but are also considered indispensable for signaling purposes in response to various kinds of stress (Dickinson and Chang 2011; Kangasjärvi and Kangasjärvi 2014).

Membrane lipids are the first targets of ROS (Sharma *et al.* 2012). When ROS concentration reaches a certain level, lipid peroxidation of membranes occurs. ROS attack particularly the unsaturated bond between carbon atoms of the phospholipid molecules and the ester linkage between glycerol and fatty acid, leading to membrane leakage and shifts in transduction properties of membranes (Sharma *et al.* 2012). With respect to the photosynthetic apparatus, ROS inhibit the membrane-bound reaction centers in the electron transport chain, inactivating photosynthesis and activating respiration (Foyer and Noctor 2011). Excessive ROS production also modifies amino acids, fragments the peptide chain, aggregates reaction products, alters the electric charge, and increases the susceptibility of proteins to proteolysis (Moller and Kristensen 2004). Proteins differ in their degree of sensitivity getting more delicate with increasing content of thiol groups and sulfur (Stohs and Bagchi 1995). Thus, ROS also have a significant detrimental effect on carbon assimilation because many enzymes

of the Calvin cycle possess thiol groups. Overall, protein content in O_3 stressed plants tend to decline (Tiwari and Agrawal 2011). ROS finally induce damages to the nuclear material of the cell (mitochondrial, chloroplastic and cytosolic DNA). Oxidative stress results in deoxyribose strand breakage, removal of nucleotides, modifications in the organic bases of nucleotides, and DNA-protein crosslinks facilitating mutations (Imlay and Linn 1988). Mitochondrial and chloroplast DNA are more susceptible to oxidative damage than nuclear DNA due to a lack of protective proteins, histones, and because of their location close to the ROS producing systems (Richter 1992).

Plant sensitivity to ROS damages varies with abiotic conditions such as drought, salinity and soil acidity, high or low temperatures, excess light, or inadequate mineral nutrient supply (Das and Roychoudhury 2014). For example, plants suffering from K deficiency are extremely sensitive to elevated atmospheric O_3 concentrations while O_3 susceptibility is decreased in plants grown at excess nitrogen, phosphorous, or potassium (Singh *et al.* 2010). The specific causes for these reactions are yet unclear but they all have in common that photosynthesis is impaired which on the one hand leads to increased internal [CO_2] and thus stomatal closure (see above), and on the other decreases the level of available chemical energy with likely effects on aquaporin regulation (Maurel *et al.* 2015). This mechanism has also been shown responsible for the fast increase of mesophyll resistance to CO_2 transport (Miyazawa *et al.* 2008). Aquaporins are also directly regulated by ROS enabling e.g. optimal water flow related to light intensity (see also Fig. 1, Kim and Steudle 2009) and are thus likely to be affected by ozone stress which has however, not directly been demonstrated.

3 Defense responses

3.1 Stomatal closure

From what has been said before it is clear that stomata pose a primary defense barrier to O_3 damages. In the presence of high O_3, stomata decrease their conductance within minutes, probably in response to ROS accumulation, but can fully recover within the following hour (Vahisalu *et al.* 2010). During recovery, oscillations of stomata opening occurs depending on the strength of the initial O_3 pulse which indicates the induction of a counteracting mechanism (Moldau *et al.* 2011). The initial dynamic can be followed by a transient decrease of conductance until equilibrium is reached if the O_3 exposure prolongs. For example, a meta-analysis of 53 peer reviewed studies to assess the response of soybean to an average chronic O_3 exposure of 70 ppb, showed a conductivity reduction of 17% (Morgan *et al.* 2003) and free

air fumigation, aimed to double the ambient O_3 concentration, lead to 10-20% decrease in beech trees (Hoshika *et al.* 2015). The phenomenon is generally explained by an increase of internal [CO_2] triggered by a decreased photosynthesis and/or increased internal respiration and is similar to the response under increased ambient CO_2 levels (see Fig. 1).

3.2 ROS scavenging

Plants have highly efficient non-enzymatic and enzymatic defense mechanisms capable of detoxifying a substantial amount of ROS (see reviews Das and Roychoudhury 2014; Karuppanapandian *et al.* 2011; Sharma *et al.* 2012). The major enzymatic components of the antioxidative defense system are Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Catalase (CAT) and enzymes of the Ascorbate-Glutathione (AA-GSH) cycle. Non-enzymatic antioxidants are tocopherol, carotenoids, and phenolic compounds, which are omnipresent in plant physiological processes and can be viewed as a constitutive defense. Ascorbate and glutathione (GSH) form a system which interacts between apoplast and symplast and which can be increased in response to increased O_3 exposure, thus acting as an induced defense mechanism (Kumari *et al.* 2015). Since constitutive and induced elements of detoxification are multiple and operate mutually in different subcellular compartments and on different time scales, a particular constitutive antioxidant level cannot be determined (De Temmerman *et al.* 2002a).

The antioxidants present in the apoplast scavenge O_3 and the derived products thereby serve as the first detoxifying barrier of the cells, protecting the membranes from O_3 injury. Apoplastic ascorbate (AA) is the most important antioxidant that is utilized by the plant's defense machinery to protect against O_3 damage by reacting with O_3, O_2^- anions, and H_2O_2 (see Das and Roychoudhury (2014) and references therein). In fact, the level of AA, is considered to be a good indicator of O_3 tolerance and has been depicted as 'the heart of the redox hub' (Foyer and Noctor 2011). In addition to ascorbate, SOD and APX are two important antioxidative enzymes present in the apoplastic spaces of leaves. The enzyme SOD catalyzes specifically the dismutation of O_2^- to O_2 and H_2O_2 which is particularly important because the plasma membranes are very little permeable to charged O_2^- anions. Therefore, SOD is intrinsically produced in the apoplast. In contrast, APX is produced only in the cytosol and is then transported to the apoplast using two molecules of AA during the process of scavenging H_2O_2 (Patterson and Poulos 1995).

When the production of ROS from the breakdown of O_3 in the apoplast exceeds the apoplastic antioxidative capacity, an endogenous, self-propagating ROS generation process is induced that continues even after O_3 exposure has stopped (Castagna and Ranieri 2009): GSH is synthesized in the cytosol and chloroplasts of plant cells and acts as a proton donor in presence of ROS, yielding GSH disulphide. The most important function of GSH in antioxidative defense is the regeneration of AA through the AA-GSH cycle as described above but it also reacts directly with O_2^-, OH and H_2O_2 and can therefore function as an additional free radical scavenger. Apart from GSH, thioredoxin and peroxiredoxins detoxify peroxides and regulate redox homeostasis by maintaining the NADPH/$NADP^+$ balance. In addition, peroxidases are known to oxidize various substrates utilizing H_2O_2 or organic hydroperoxides (see Bela *et al.* 2015 and references therein).

To select the appropriate modelling strategy it is important to remember that some ROS, i.e. H_2O_2, can diffuse through aquaporins and thus ROS produced at a specific cellular site (e.g. chloroplast) during stress can affect other cellular compartments as well. Therefore, a differentiation between compartments within the symplast seems inappropriate if the model does not consider transfer between cell organelles. This is independent of the fact that indeed some ROS scavenging mechanisms exist in separate cell structures as for example CAT, which is found only in peroxisomes. Other investigations have shown that antioxidant enzymes can partly replace each other although the sensitivity to stressors might not be identical. For example plants with suppressed APX production induce SOD and CAT, whereas plants with suppressed CAT production induce APX and GPX (Willekens *et al.* 1997).

3.3 Formation of volatile organic compounds

During the last 15 years, it became apparent that a number of biogenic volatile organic compounds (BVOCs) play an important role in providing antioxidative defense to the plants against O_3 stress (Loreto *et al.* 2004; Possell and Loreto 2013; Velikova *et al.* 2012). O_3 can induce the production of all kinds of BVOCs (see review of Calfapietra et al. (2013) and references therein as well as various other publications such as Bourtsoukidis et al. (2012)). The experimental results are not yet fully conclusive since some studies have found no induction of BVOC emissions (Hartikainen *et al.* 2009) or even a negative impact of O_3 exposure on isoprene (Calfapietra *et al.* 2008) or terpene emission (Llusia *et al.* 2014). Velikova et al. (2005) states that isoprene is only stimulated if O_3 stress affects

photosynthesis. This indicates that the degree of induction depends on O_3 concentrations, species and the effectiveness of constitutive defense mechanisms.

Isoprenoid and monoterpene biosynthesis mainly occurs in mature chloroplasts through the methylerythritol 4-phosphate (MEP) pathway. Monoterpenes and other terpenes are also produced in the cytosol from precursors produced in mitochondria and peroxisomes (Vickers *et al.* 2014). The formation of terpenoids can be stimulated by jasmonates, which in turn can originate from ROS degeneration (Spinelli *et al.* 2011). It has also been suggested to explain the formation of isoprenoids by increases in phosphoenolpyruvate production (Dizengremel *et al.* 2012). Phosphoenolpyruvate serves as substrate for the MEP pathway and is amplified by O_3 exposure (Valkama *et al.* 2007). Increased emissions of oxygenated defense related volatile oxylipins can be explained by the stimulation of the lipoxygenase pathway due to the peroxidation of membrane lipids by O_3 or other ROS (e.g. Porta and Rocha-Sosa 2002).

The beneficial impact of BVOCs on the sensitivity of plants to O_3 stress has been shown in a number of experiments. It starts already in the gas phase outside the leaves when O_3 is destroyed by reactions with isoprenoids in low NOx atmospheres (Fares *et al.* 2008). Exposure to high O_3 led to less cellular damage and less damage to the photosynthetic processes when isoprene was provided simultaneously, and higher damage occurred when isoprene emission was suppressed (Behnke *et al.* 2009). Furthermore, experiments using transgenic tobacco plants confirmed that isoprene-emitting plants show increased resistance to ozone-induced oxidative stress (Vickers *et al.* 2009). The latest evidence that the protective role of isoprene originates at least partly from the capability to quench ROS has been provided by the finding of isoprene oxidation products within the leaves (Jardine *et al.* 2012). Although some of the oxidation products are actually toxic, the enhanced detoxification rate leads to a reduction of visible, physiological, anatomical, and ultrastructural (chloroplast) damage when exposed to O_3 (Possell and Loreto 2013; Velikova *et al.* 2005).

Apart from direct detoxification, a stabilizing impact of isoprenoids on membranes is supposed to play a key role in their protective impact against oxidative stress (Velikova *et al.* 2014; Velikova *et al.* 2015). For example, the synthesis of zeaxanthin, an isoprenoid which is derived from *β*-carotene and known to increase the rigidity of membranes, is preferred under stressful conditions (Brunetti *et al.* 2014). Very recently, however, Harvey et al. (2015) showed that even in high emitter species internal isoprene concentration might not be sufficient to affect membrane lipids but instead suggested that thylakoid embedded proteins are modulated, indicating a more direct link to membrane conductance than previously

thought. In addition, the isoprenoid impact on thylakoid membranes seems to reduce the formation of ROS (Velikova *et al.* 2012). Since isoprene is very volatile, it can be assumed that this effect is confined to the membranes closest to its origin, which are those of the chloroplasts.

4 Considering feedbacks for modelling

In contrast to leaf-scale approaches, most simulations to estimate O_3 damage at global and regional scales are based on concentration-response functions, where productivity losses depend on O_3 concentrations or accumulated O_3 exposure as independent variables (e.g. Avnery *et al.* 2011). Only in few cases, stomatal conductance has been accounted for to calculate the actual amount of damaging agents that reach the sensitive leaf structures (Lombardozzi *et al.* 2015; Sitch *et al.* 2007) but induced defense mechanisms that may provide important feedbacks have not yet been considered (see Fig. 2). To provide accurate estimates of agricultural and forest production responses to high O_3 concentrations, it seems essential to consider plant vulnerability that varies with abiotic or biotic conditions. Such differentiated impacts can only be simulated if direct and indirect defense mechanisms such as stomatal closure, ROS scavenging and dynamic changes in membrane susceptibility are accounted for. In the following sections, these mechanisms will be separately discussed.

4.1 Modelling stomatal conductance

Since stomatal conductance has been considered a main influencing factor to O_3 impacts, models have been developed to describe this process (e.g. Emberson *et al.* 2000). This is based on the concept that conductance for O_3 is similar to that of CO_2 (Van Oijen *et al.* 2004), which has formerly been derived directly from environmental conditions (Jarvis and McNaughton 1986) or indirectly from photosynthesis (Ball *et al.* 1987). Thus, uptake is calculated from air concentrations of O_3 at the leaf surface using the implicit assumption that O_3 is almost instantly removed from the stomata cavities (Laisk *et al.* 1989). However, 1) the assumption of instant O_3 removal might not actually be valid (Moldau and Bichele 2002) and 2) O_3 also affects stomatal responses as discussed above, leading to non-linear developments of conductivity with time and exposure.

The first issue links O_3 uptake to oxidation capacity within the cells and thus to terpenoid production and is not considered in models yet (Loreto and Fares 2007). The second issue is

only considered in model approaches that account for long-term (cumulative) O_3 impacts. The latest elaboration of this approach has been presented by Kinose et al. (2014), who improved stomata responses for various tree species considering also short-term impacts of O_3 concentration:

$$gs = g_{max} \times \min(f_{phen}, f_{O3c}) \times f_{light} \times \max[f_{min}, (f_{temp} \times f_{VPD} \times f_{SWC} \times f_{O3i} \times f_{transp})] \quad (1)$$

where g_{max} is the maximum stomatal conductance to water vapor. The limiting functions (f_{phen}, f_{O3}, f_{light}, f_{temp}, f_{VPD}, f_{SWC}, etc.) are scaled from 0 to 1 as a proportion of g_s to g_{max}. The following limitations on stomatal conductance are represented: f_{phen} - leaf phenological changes with aging, f_{O3c} - cumulative stomatal uptake of O_3, f_{light} - photosynthetic photon flux density, f_{min} - minimum stomatal conductance, f_{temp}- air temperature, f_{VPD} - vapor pressure deficit, f_{SWC} - soil water content, f_{O3i} - acute effect of O_3, f_{transp} – leaf water loss; for detailed equations to derive these factors see Kinose et al. (2014).

Despite the fact that the described approach, which is based on a formulation originally developed by Jarvis and McNaughton (1986), is very widespread in ecological modelling, it is prone to two basic criticisms: First, a multiplicative function neglects the interactions between the specific influences which are more likely the more impacts are considered. Second, the response functions are empirically derived on a phenomenal level, requiring a new, experiment-based parameterization for every species and each new influencing factor. In complex ecosystems and scenario simulations that go beyond currently experienced environmental conditions, a mechanistic approach that inherently considers physiological interactions and can be parameterized with inherent species-specific properties is preferable, although it might not be tractable on a regional scale (Gustafson 2013).

Such a mechanistic approach has been investigated by Lombardozzi et al. (2012) who have investigated stomata responses under cumulative O_3 exposure and simulated it with either an empirical approach or the coupled Farquhar/ Ball-Berry model, which assumes that conductance is linked to photosynthesis via internal [CO_2]. The overall response could only be explained by considering direct as well as indirect (due to photosynthesis reduction) O_3 effects. This, however, doesn't take the sluggishness or hysteresis effect into account that results in more O_3 uptake after sufficient cumulative exposure. A model to include this feedback impacts has been suggested by Hoshika et al. (2014; 2012) who found that ozone-induced impairment of stomatal control was better explained by O_3 flux per net

photosynthesis than by flux only. For more information on the determination of surface O_3 and the scaling from leaf to canopy, we refer to other publications and references therein (see Bryan and Steiner 2013; Karnosky *et al.* 2005).

4.2 Representation of physiological responses

4.2.1 Oxidative damage and detoxification

O_3 impacts are generally simulated in a lumped fashion, assuming a linear decrease of net primary production with O_3 uptake above a defined threshold (De Andres *et al.* 2012; Ewert and Porter 2000; Sitch *et al.* 2007; Van Oijen *et al.* 2004). The approach implicitly assumes that defense is only constitutive so that it is immediate, all costs for defense formation are already considered with the standard rate of maintenance respiration, and that the magnitude of the effect is independent of environment or physiological state. However, the concept neglects that, despite the presence of one or more constitutive detoxification systems, additional antioxidative agents or enzymes are induced when plants are exposed to O_3. Modelling might or might not consider that the antioxidant defense may be temporally set, with enzymatic antioxidants and isoprenoids acting in different periods of the day (Fares *et al.* 2010). Thus, ROS degradation is increased compared to the scavenging of a constitutive defense system alone (Iriti and Faoro 2009). Such a mechanism can increase the threshold of O_3 uptake without any visible damage but decreases net primary productivity. Recent measurements from soybean cultivars support this mechanistic view (Betzelberger *et al.* 2012). It might be sensible to differentiate between three inductive systems of defense: apoplastic (e.g. ascorbate), symplastic (e.g. SOD) and isoprenoid related. Functionally, the first system increases the scavenging capacities while the second and third may additionally increase membrane stability. Only when the defense capacity or regeneration speed is insufficient, such a detoxification strategy cannot prevent damage to photosynthesis. Thus, either very intense stress or prolonged exposure may lead to a shortage of chemical energy and a breakdown of defense. The result is a decrease in enzyme integrity and a destruction of structural cell components.

The most detailed biochemical model which is intended as a potential module within an ecosystem model explicitly describes the reaction of O_3 with ascorbate as detoxification process and takes into account the regeneration of this agent in dependence of various cell properties (Plöchl *et al.* 2000). A less detailed approach has been used within an ecosystem

model by Deckmyn et al. (2007) who proposed to account for a reduction in carboxylation rate (Vcmax) as well as for detoxification costs due to enhanced respiration rates (Rrep):

$$Vcmax_{OZ} = Vcmax - (SOZ \times feff \times Vcmax/100\%) \quad (2)$$

$$Rrep = CREP \times [(Vcmax - Vcmax_{OZ}) / Vcmax] \times 100\% \quad (3)$$

where $Vcmax_{OZ}$ denoting the maximal carboxylation rate under the experienced O_3 regime. SOZ is a species-specific parameter describing reduction in Vcmax per unit daily effective O_3 flux (%). *f*eff is the effective stomatal O_3 flux per unit leaf area which is calculated in dependence on stomatal conductance. CREP is the carbon necessary to repair a certain amount of damage to Vcmax (for more comprehensive explanations see Deckmyn et al. (2007)). The linear response of Vcmax on O_3 is in accordance with observations (e.g. Betzelberger *et al.* 2012) although also exponential decreases have been measured (Goumenaki *et al.* 2010). Both, the Plöchl as well as the Deckmyn model however fail to describe an increased detoxification capacity in dependence on stress as outlined by Heath et al. (2008).

4.2.2 BVOC formation and membrane stabilization

Dependencies of plant isoprenoid emissions to environmental factors have been described already more than 30 years ago (Tingey 1979). However, O_3 is not yet among them which is surprising, given the presented findings making isoprenoid production a likely candidate for a defense system that could be mechanistically linked to models of stomata- and photosynthesis processes (Pinto *et al.* 2010). Only few suggestions have been made to represent BVOC emissions in dependence of O_3 uptake or concentration so far. Calfapietra et al. (2009) suggested an empirical linkage between BVOC emission and O_3 which stimulates emission at low and inhibits it at high doses. For sesquiterpene emission of spruces, Bourtsoukidis et al. (2012) proposed an exponential emission response to increasing ambient O_3 concentrations. These approaches follow the general logic of most emission models, which view the emission process as an independent physiological process that does not account for activity changes throughout the year and is supposed to have only negligible respiration costs.

Therefore, in addition to O_3 impact on stomatal behavior, we suggest three lines of development to improve the relationship between isoprenoid emissions and O_3 stress in

process-based ecosystem models (depicted in Fig. 3). First, new modelling options might arise from more mechanistic approaches that link emission to photosynthesis such as suggested by Morfopoulos et al. (2014) and Grote et al. (2014). This model states that isoprenoid formation increases when photosynthesis is decreased (e.g. due to stress impact on Vcmax as depicted in Fig. 4 a and c) because the reducing power which is still produced by photosystem II is accumulating under these conditions and can be channeled into BVOC synthesis. The impact is sensitive to the degree of stress but responds only slightly to the kind of stress function applied (see linear and exponential stress responses depicted in Fig. 4). This approach could offer a mechanistic explanation for observed phenomena and at the same time provides an explanation for decreasing emission in response to increasing [CO_2] (which would increase Vcmax). Current research indicates that this might apply particularly to isoprenoids but other BVOCs might be affected by means of the same limitation process. Second, seasonal dynamics might be described with a more mechanistic approach that changes potential emissions (emissions observed under defined light, temperature and CO_2 conditions without further stressors) dynamically as a cumulative function of enzymatic synthesis and degradation. Such a model is described in Lehning et al. (1999) but instead of enzymatic activity cumulatively building up with temperature, effective O_3 uptake could trigger the increase in detoxification capacity, similar as has been observed by various authors (Dumont *et al.* 2014; Kumari *et al.* 2015; Rozpądek *et al.* 2013). This capacity can then serve as a threshold beyond which photosynthesis is assumed to be damaged. Based on the findings of membrane stabilization by means of isoprenoids, we finally propose that the O_3 damaging impact is decreased due to increasing isoprenoid production, which in effect also decreases its emission into the atmosphere and explains findings of decreased emission under high O_3 regimes. We are proposing this simplifying modelling procedure fully aware of the fact that the production of specific compounds may vary with species and environmental boundary conditions and that the detailed mechanisms are not fully understood yet (Brunetti *et al.* 2014).

4.3 Recovery and environmental boundary conditions

A plant's sensitivity to oxidative stress also depends upon its ability to recover from O_3 injury. Recovery is possible if damages are not severe and the rate of scavenging of ROS is larger than its formation rate. The length of the night with low O_3 concentration and impeded O_3 uptake by lacking air-mixing is important to prevent the plants from chronic injury

(Matyssek *et al.* 2015). This may be a possible reason why plants are more susceptible to O_3 damage in summer in Nordic countries (De Temmerman *et al.* 2002b). Although literature indicates that in some cases, as for example due to air mass transport, O_3 concentration can still be high during night we suggest using length of night as a proxy to describe plant's recovering ability. This view is corroborated by findings that O_3 decreases during nighttime may originate from non-stomatal deposition rather than uptake (Launiainen *et al.* 2013).

5 Conclusion

Ozone interacts with weather because warm and sunny conditions favor O_3 formation and high photosynthesis but decrease water availability and thus stomatal conductance. Therefore the role of stomatal behavior is essential, but has been only partly considered in current ecosystem model approaches. Another feedback that needs to be further examined and considered is that ROS play a major role in stimulation of the plant's defense mechanism. Although the interactions that are outlined in the text and in Fig. 2 may still be too complex to be added to an ecosystem model, it may be well possible to use a more condensed version of this mechanism. In Fig. 3 we thus suggest how a conceptual model of defense against oxidative stress which could be coupled to basic processes (photosynthesis, leaf longevity, stomatal conductance) that are generally considered in ecosystem models. The impacts of radiation, temperature, and water availability are indirectly accounted for because isoprenoid production, stomatal conductance, and detoxification processes are mechanistically described. Modelling could be strongly supported by experimental research, helping to quantify the induced generation of isoprenoids and the effectiveness of antioxidative substances in scavenging ROS. We assume that particularly isotopic techniques and related methodologies could greatly enhance our understanding of the phenomenon. We also strongly encourage model developers to consider the respiratory costs of the defense actions explicitly and introduce a two-way linkage between isoprenoid emission and photosynthetic activity. Although long-term (chronic) effects are not explicitly considered here, the description of oxidative stress mechanisms suggest that that the enzymatic activity of basic defense systems should account for a time-dependency of activation (or degradation) state. Therefore, current approaches used for describing seasonal dependencies of emission activity as described for example in Monson et al. (2012) may be useful.

A description of O_3 impacts that accounts for more internal feedbacks and thus implicitly considers a range of environmental conditions in addition to the O_3 concentration is beneficial

for two reasons. First, it would provide a more reliable estimate of productivity losses in agriculture and forestry, particularly under changed climatic conditions. Second, an integrated approach would provide consistent input of O_3 deposition and VOC emission from the biosphere into coupled climate-air chemistry models.

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Figure captions

Fig. 1: ROS impact in the apoplast and symplast. Arrow colors are selected to indicate transport (blue), decreasing impact (red), increasing impact (black), and optimum relationship (green). The optimum relationship indicates that mesophyll conductance is likely to increase with ROS concentration until aquaporins or other active transport mechanisms are damaged so that conductance is decreasing again (see section 2.2). SC and MC stand for stomatal and mesophyll conductance, respectively. Broken lines indicate less important transport routes.

Fig. 2: Pathways of ozone detoxification in the leaves. Reactive oxygen species (ROS) originate from O_3 which is taken up or endogenously produced and is detoxified in the apoplast (APO) or transported to the symplast (SYM) where it also can be generated and detoxified. Once in the symplast it enhances the detoxification capacity by increasing a) the ascorbate (AA) – glutathione (GSH) cycle (and other synthesis pathways), and b) the production capacity of isoprenoids (ISO). (Pools are presented as rectangles, valves indicate that the process is a mechanistically defined, clouds represent precursors and products that are not specifically defined, thick arrows represent matter flows, thin arrows indicate modifying impact).

Fig. 3: Conceptual model of three defense mechanisms: Stomatal conductance (red), induced defenses (green), and tissue protection (orange). Isoprenoid formation is considered as protecting tissue neglecting detoxification properties, and is induced by oxidative stress (in addition to other impacts). Damage relates to photosynthesis activity, leaf longevity or other processes. Feedbacks to stomatal conductance and isoprenoid production are depicted in Fig. 2. Wide arrows represent matter fluxes and thin arrows influences. Processes are shown in white boxes. R-COH indicates aldehydes as an example of detoxification end-products.

Fig. 4: Simulated increases in isoprenoid emission rate (B and D) with decreasing Vcmax (A and C) in response to ozone exposure (expressed as AOT40 which is introduced as ‘y’ in the equation). The JJV model was applied with a standard parameter set for photosynthesis as described in Grote et al. (2014). Vcmax has been reduced linearly (A and B, expressed with the parameter pl) or exponentially (C and D, expressed with the parameter pe) as both response types have been described in literature (equations are given in the figure, see section 4.2 for literature references).

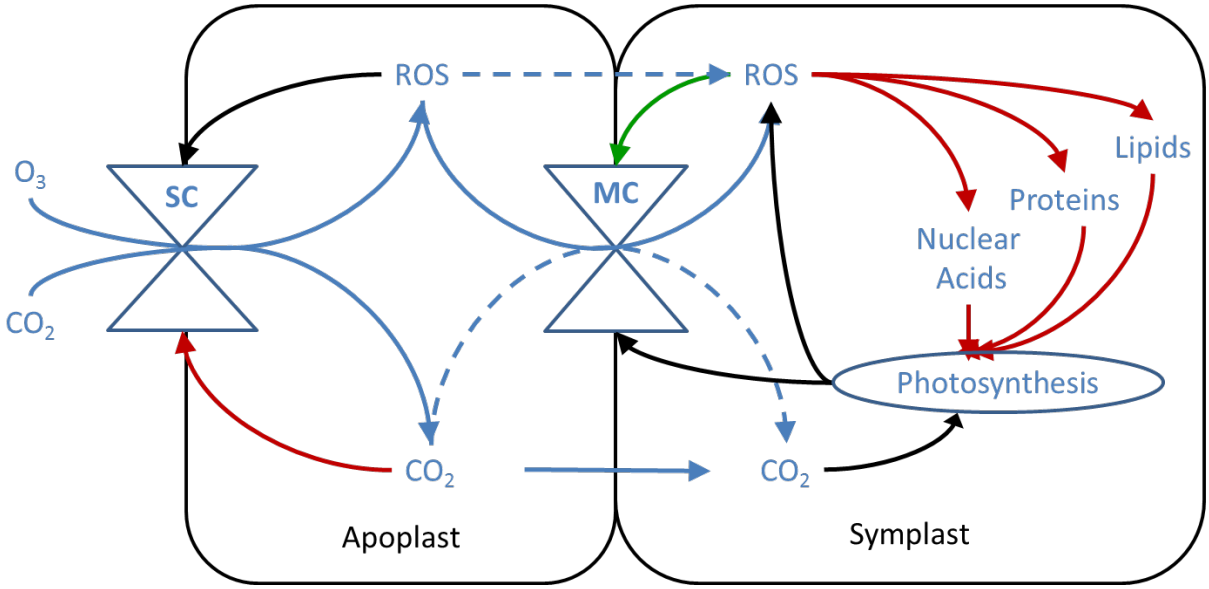

ROS
ROS
O3
SC
MC
Lipids
Proteins
Nuclear
Acids
CO2
Photosynthesis
CO2
CO2
Apoplast
Symplast

Figure1: …

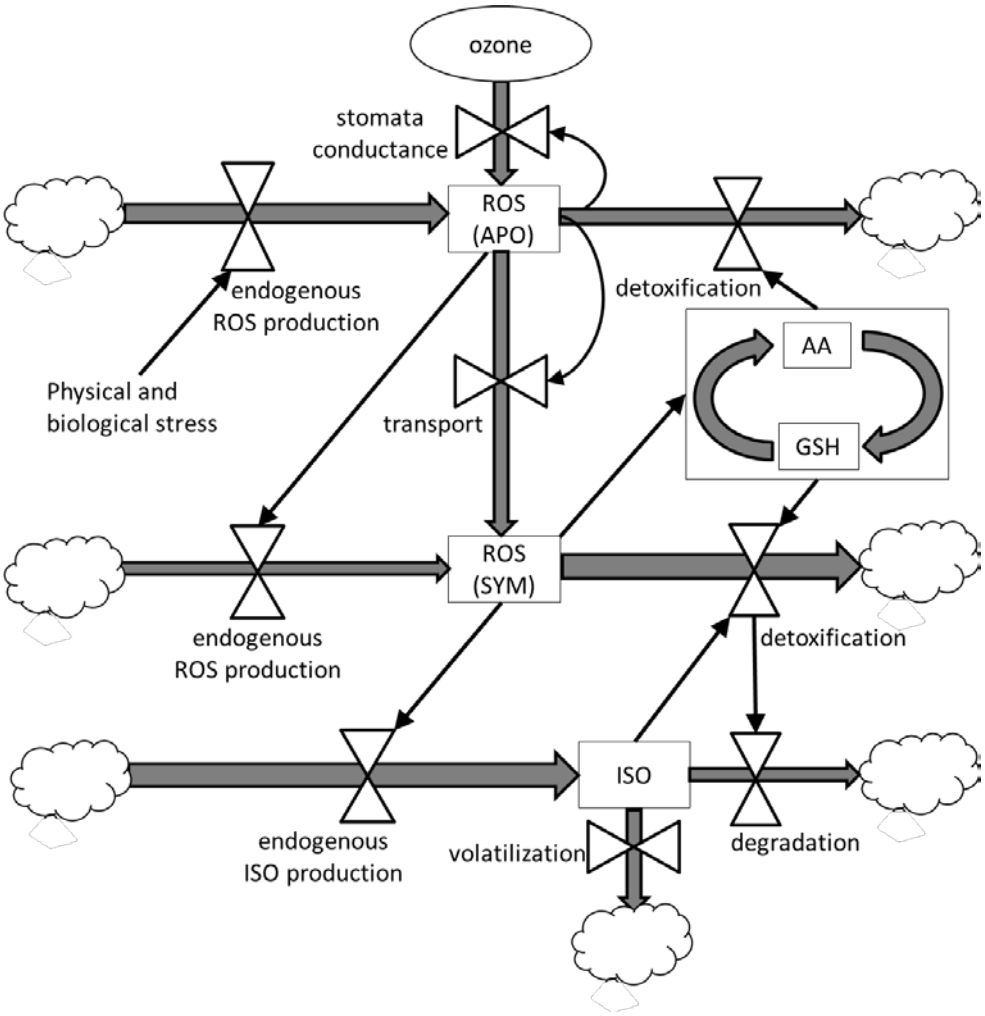
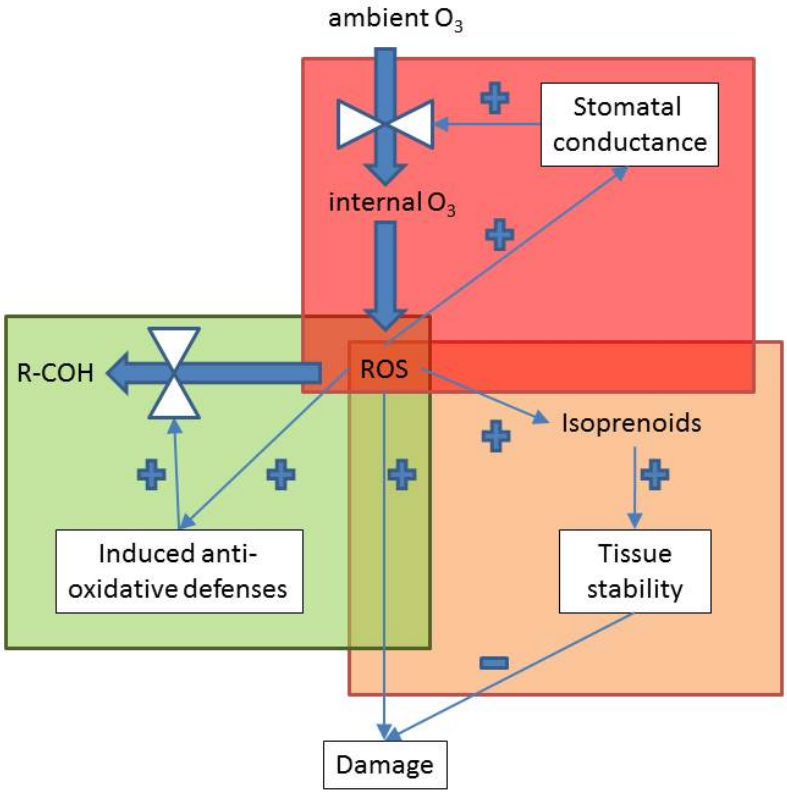

ozone
stomata
conductance
ROS
(APO)
endogenous
ROS production
Physical and
biological stress
transport
detoxification
AA
GSH
ROS
(SYM)
endogenous
ROS production
detoxification
ISO
endogenous
ISO production
volatilization
degradation

Figure2: …

1



2

3 Figure 3: …

4

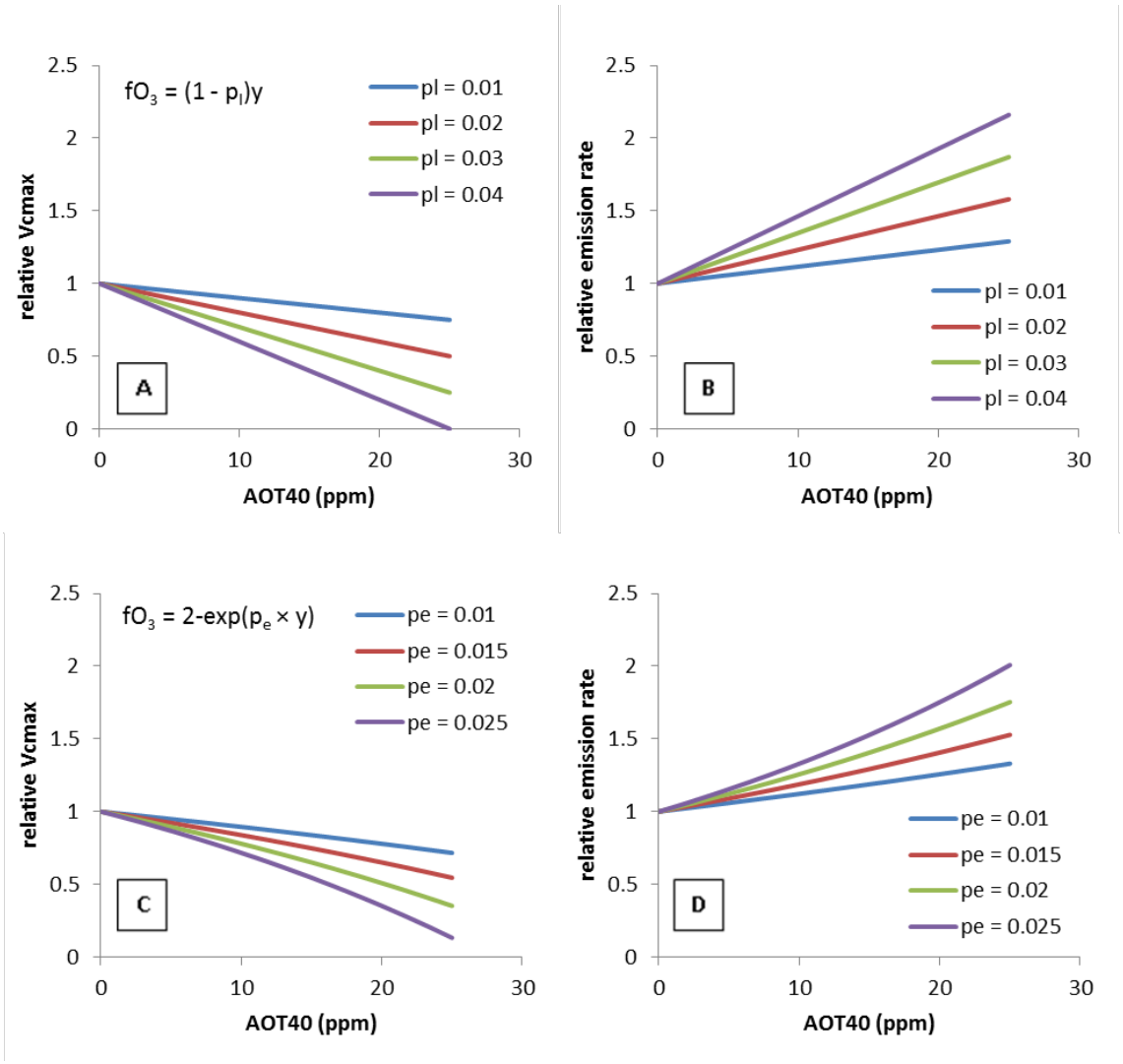

fO3 = (1 - pl)y
pl = 0.01
pl = 0.02
pl = 0.03
pl = 0.04
relative Vcmax
A
AOT40 (ppm)
relative emission rate
B
fO3 = 2-exp(pe × y)
pe = 0.01
pe = 0.015
pe = 0.02
pe = 0.025
C
D

Figure 4: ....