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Ozone effects on trees, where uptake and detoxification meet

Abstract: Ozone is the most important air pollutant and its concentration in ambient air is still rising. Ozone concentrations measured at reference height (50 m is EMEP ozone modelling height), do not reflect the real concentration at the top of the vegetative canopy and do not provide sufficient information about the ozone flux entering the leaves. Modelling stomatal conductance is leading to estimations of cumulative ozone uptake and enables much better to evaluate the impact of ozone on trees.
The negative impact of ozone exposure has a measurable effect on physiological processes such as stomatal conductance, photosynthesis and respiration. Disturbance of the basic physiological processes is leading to growth and wood production losses.
There have been several attempts to establish critical levels (CL) for ozone effects on forest trees. Average concentrations and cumulative exposure indices are satisfactory to some extent, but do not fully describe the potential impact of ozone exposure. Much more promising is an evaluation based on the effective ozone flux, which is a function of the absorbed ozone flux and the defensive response.
Ozone uptake takes place primarily through the stomata and reactions of ozone with hydrocarbons released by the plant cells and transformations of dissolved ozone in the apoplastic fluid create many reactive oxygen species of which free radicals are able to initiate membrane lipid peroxidation and destruction of cell membranes.
The defence of a plant against absorbed ozone starts in the apoplastic fluid. Ascorbate is believed to be a very important radical scavenger avoiding detrimental effects of reactive oxygen species to the membranes. Other important antioxidants are phenolics. The defensive response can be linked to the abundance of ascorbate or the ability of the plants to regenerate (reduce) ascorbate from monodehydroascorbate and dehydroascorbate. The reduction of dehydroascorbate takes place in the symplast where ascorbate can be transported back through the plasma membrane into the apoplast.
Ozone exposure also causes oxidative stress of the plant cell interior by the formation of reactive oxygen species. Plants can cope with those toxic substances in the symplast by using antioxidants such as ascorbate, α-tocopherol, glutathione and carotenoids and enzymes such as superoxide dismutases, catalases and several peroxidases. The complexity of the apoplastic and symplastic antioxidative capacity with different turnover rates and transport of antioxidants makes it difficult to determine the total antioxidative power.

Additional key words: Trees, effective ozone flux, oxidative defence, critical levels for ozone

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Introduction

Tropospheric ozone is considered to be the most important air pollutant in Europe and many parts of the world (Heath 1994a). Levels of ambient ozone have increased considerably over the past 60 years (Anfossi et al. 1991), and have more than doubled in the past one hundred years (Hough and Derwent 1990). They are predicted to continue to rise at an even faster rate (annual rate of 0.5–2.5%) in the future (Ashmore and Bell 1991; Marenco et al. 1994; Stockwell et al. 1997).

Ozone has been suggested to cause the largest amount of damage to vegetation as compared to any other gaseous pollutant (Fuhrer et al. 1997) and its relative importance may still increase because of the decline in the occurrence of other air pollutants.

Ozone effects have been studied on several tree species. Pine and spruce are the most studied coniferous species whereas poplar, beech and birch are on top among the deciduous species (Bortier et al. 2000a). For tree species, the choice of the exposure system is a difficult point in those studies. Growth chambers and open-top chambers (OTCs) are only useful to fumigate seedlings and young trees respectively. In addition the trees can only be treated during a relatively short period of their life cycle. Open field fumigations appear to be an accurate exposure system but it remains difficult to fumigate mature trees (Bortier et al. 2000a). Protecting chemicals such as ethylene diurea (EDU), are probably good tools for studying ozone effects but until now not much, if any, experiments have been carried out with mature trees (Bortier et al. 2001b).

Plants exposed during a sufficient period of time to an elevated concentration of ozone in ambient air can be the subject of physiological disturbances, visible injury, growth effects, early senescence, and yield losses and mortality. Simple exposure indices (average concentration over a time period) often failed to predict ozone damage (Fuhrer 1996). One of the reasons, recognised already long time ago, was the very strong diurnal pattern of ozone concentrations in ambient air. For that reason, exposure was described as 7 or 8 h averages comprising the mid-day peak concentrations. However, this is not fully describing the chronic exposure of plants to moderate ozone concentrations. Biologists have recommended a long term cumulative parameter that would relate more closely to vegetation response (Heck and Cowling 1997). The AOT40 approach (Accumulated ozone exposure Over a Threshold of 40 nl l^{-1}) i.e. the sum of the hourly ozone concentrations above a cut off of 40 nl l^{-1} during daylight hours when global radiation exceeds 50 W m^{-2}, appeared to be much more satisfying than the very simple 7 or 8 h averages. For forests, the provisional critical level (CL) for ozone in Europe is an AOT40 of 10 μl l^{-1} h over 6 months during daylight hours (UN-ECE 1996). However the simplest AOT40 index, the so-called Level I, is aiming at a general risk assessment for Europe of the potential ozone effects, but not with the aim to estimate actual growth loss. This latter aim is the so called Level II approach, where modifying factors that influence the ozone impact, such as soil moisture, temperature and light, are incorporated in the index (Fuhrer et al. 1997). These modifying factors do influence stomatal opening and hence ozone uptake.

The European CL for ozone is primarily based on measurements at the top of the canopy in chambers (OTCs, growth chambers, and others). The correct application to ambient conditions is questionable because ozone concentrations in the proximity of the canopy are differing from the ambient air concentration at reference height (Grünhage et al. 2000).

Recent studies are facing to model the ozone concentration at the top of the canopy. Indeed taking into account stomatal conductivity, models have been developed to calculate the ozone flux or ozone uptake by the plants (Emberson et al. 1998). The ozone flux (FO_3, expressed in nmol m^{-2} s^{-1}) is the rate at which ozone enters the leaf and it is a more biologically relevant parameter in relating plant response to ozone exposure. However, the totally accumulated ozone flux (CFO_3, expressed in mmol m^{-2}), does not take into account the species-specific differences in ozone sensitivity.

The antioxidative capacity of the plants plays a major role in their reaction to ozone and needs to be included in models describing ozone effects on vegetation. Musselman and Massman (1999) define this defence response "D(t)" as a function of the photosynthetic activity because this process provides the plant with photosynthates needed for defensive mechanisms. The term effective ozone flux "EF" is expressed as a function of absorbed ozone dose or uptake into the leaf at a given point in time "F(t)"and the defensive response "D(t)" at that time: EF= F(t) – D(t). Plöchl et al. (2000) on the other hand, developed a mathematical model, which enables the semi-quantification of ozone detoxification, based upon direct reaction of the pollutant with ascorbate located in the aqueous matrix associated with the cell wall (i.e. the apoplast).

Ozone effects on growth and physiology of trees

Comparison of species sensitivity based on different experiments is extremely difficult, due to differences in exposure system, ozone exposure, growth conditions, provenance and genotypes of the trees etc. Another aspect is which parameter is considered: visible injury, growth or physiological performance, biochemical traits etc.

In most cases visible injury and growth are the major criteria considered for the comparison of sensitivity and when critical levels of ozone are discussed. Biomass production is an integrating parameter that will only be affected as a result of damage or disruption to metabolic and physiological processes (Broadmeadow 1998). Changes in growth can, for example, be detected by destructive harvests at the end of the experiments. However, since trees are very long-living species, it is not easy to gather sufficient reliable and comparable data on final biomass production. Instead, diameter increment can be used as a non-destructive method for analysing growth (Bortier et al. 2000c). This approach also offers the advantage that it can be expressed as a relative increase if the initial trees are variable at the start of the experiment. Another candidate for this purpose could be a more physiological parameter. Ozone flux into the plants during daytime can cause biochemical changes, which result in decreased photosynthesis (Heath 1994b; Musselman and Massman 1999). Since CO_2-assimilation is the main source of biomass production, it seems appropriate to consider the plant's photosynthetic capacity as an indicator of the risk for growth reduction. Bortier et al. (2000b) exposed two species, a fast growing poplar and a more slowly growing beech, to exactly the same experimental conditions for one growing season in OTCs. Three treatments were applied: charcoal-filtered (CF), non-filtered (NF) and non-filtered air plus 30 nl l^{-1} ozone (NF+). The AOT40s calculated from April to September were 4055 nl l^{-1} h for the NF and 8880 nl l^{-1} h for the NF+ treatments. The relative ozone sensitivity of these trees was compared with reference both to the relative growth rate of stem diameter and of height growth. Over the growing season non-destructive measurements of light saturated photosynthesis (A_{sat}), stomatal conductance (g_s), chlorophyll content and chlorophyll fluorescence parameters were carried out. With regard to the growth parameters such as stem diameter and height, beech showed a less clear response to the applied ozone treatments than poplar, confirming the general idea that fast-growing species and genotypes (clones) are more sensitive than slower-growing ones. The data of the photosynthetic performance of both species correlated well with these observations: for poplar, ozone strongly reduced A_{sat} (CO_2 assimilation at saturating light intensity), g_s, chlorophyll content and slightly lowered F_v/F_m (potential quantum yield of photosystem II). Compared to poplar, the influence of ozone on the photosynthetic capacity of beech was small however: A_{sat} and g_s in the NF+ treatment were almost at all times slightly reduced compared to the control treatment, while chlorophyll content remained unaffected and F_v/F_m was only reduced in September (Bortier et al. 2000b). From this experiment we may conclude that the impact of ozone on certain photosynthetic parameters such as A_{sat} can be regarded as an indication of the relative sensitivity of a certain species.

In another experiment however where beech seedlings were exposed to episodes of ozone in environmentally controlled growth chambers during one growing season, Bortier et al. (2001a) did not observe a correlation between the ozone mediated photosynthetic reduction and growth. Diameter increment and biomass were not significantly affected by ozone treatments, whereas the light saturated CO_2 assimilation rate was significantly reduced. One must bare in mind, however, that the light intensity in the growth chambers was well below the saturation point and that most likely the difference in photosynthetic performance between control and ozone treated plants was negligible at this point, resulting in only minor biomass changes. In the OTC experiment, under natural light conditions, ozone peaks coincide with very high light intensities at which the photosynthetic performance is at its maximum. Since it was proved that ozone reduces light saturated CO_2 assimilation, it is more likely to detect corresponding biomass differences if plants are growing under higher light intensities.

The question remains what determines the differences in response between poplar and beech, or more generally, what explains the differential sensitivity of species? One possible explanation raised earlier (Reich 1987; Runeckles 1992) is that species with lower stomatal conductances, in this case beech, will have lower ozone uptake rates. On the other hand, ozone exposure generally results in a decline in stomatal aperture, and plants that display a more rapid stomatal closure in response to O_3 are often reported to be the most "resistant" in population-level studies (Winner et al. 1991). However, stomatal closure is not a universal response to O_3 exposure, and Reiling and Davison's (1995) detailed investigations on *Plantago major* suggest that patterns of stomatal response may be considerably more complex than is often credited. Indeed, O_3 induced declines in stomatal aperture may be of limited protective value, since stomatal closure is commonly a downstream consequence of damage to the photosynthetic apparatus (Lyons et al. 2000). In a study on clones of *Populus maximowiczii* × *Populus trichocarpa*, Koch et al. (1998) found that differences in both physiological properties, like stomatal conductance, as well as gene expression patterns correlated with differences in ozone sensitivity. The different physiological and molecular responses to ozone suggested that ozone tolerance involved the activation of salicylic-acid- and jasmonic-acid-mediated signalling pathways, which may be important in triggering defence responses against oxidative stress.

Although physiological assessments offer more detailed information at different organisation levels, it is very difficult to relate them to final effects on growth and vitality of forest trees. They do however offer a non-destructive means to identify the risk for ozone mediated growth reductions. On the other hand one can also question if growth should be the only criterion for the assessment of critical levels. What about disease susceptibility, ecosystem vulnerability etc.? Therefore it is necessary to combine both approaches and integrate physiological parameters into growth models to obtain the necessary tools for more reliable predictions. The problem remains: how to validate the models?

Ozone deposition on forest stands

Ozone deposition on forest stands is different as compared to crops. Forest trees have in general a large leaf area index (LAI), but the top of their canopy is much higher than it is for crops. In addition, wind velocity is higher and the ozone concentration might be higher at the top of the canopy. Ozone measured at a reference height does not reflect the real ozone concentration at the top of the canopy, i.e. the upper surface boundary of the quasi-laminar layer if the micrometeorological big-leaf approach is applied. The application of those measured concentrations at reference height needs to be transformed to the effective phytotoxic concentrations at the top of the canopy. This could be done on the basis of micrometeorological models (Grünhage and Haenel 1997; Grünhage et al. 1997, 2000).

On the basis of the models developed, Grünhage et al. (2001), recalculated exposure-response relationships for European conifer and deciduous tree species based on literature data. They used a simple relationship based on the ozone concentration at the top of the canopy and the exposure time. However, the concentration on top of the canopy is also influenced by the ozone flux to the canopy, when there is ozone deposition and uptake.

Stomatal ozone uptake

Plant injury is most closely related to the fraction of ozone entering the plant through the stomata, i.e. the ozone flux (Fredericksen et al. 1996; Sandermann et al. 1997). Stomatal opening is to a large extent determining the ozone flux. Plants exhibiting a higher rate of stomatal uptake undergo in many cases larger effects of ozone damage (Reich 1987). Ozone itself is influencing stomatal aperture, because the stomates are injured so that they close prematurely and slow CO_2 movement into the leaf. Some toxic products might migrate into the chloroplast where they react and/or ionic balances are altered to induce metabolic shifts (Heath 1994a). Stomatal closure can also be the result of disturbances in the photosynthetic apparatus (Farage et al. 1991). In both cases the protective effect might be too late, as it is a result of early ozone damage on the cellular level.

In order to approach an exposure measure, with a direct relationship to effects, the ozone exposure should be described as an uptake dose, based on ozone flux estimates. However, it is not easy to measure the stomatal conductance of a mature tree leaf at any time of the day. Based on ozone flux estimates and following simulation experiments, models can be developed to describe the ozone exposure as an uptake dose. A stomatal conductance simulation model was developed by Emberson et al. (1998), based on earlier models by Jarvis (1976) and Körner et al. (1995). The model approach is presented by Emberson et al. (2000a, b) for Norway spruce and several European crops respectively. It calculates the stomatal conductance (g_s) as a function of leaf phenology and four environmental variables: photosynthetic flux density, temperature, vapour pressure deficit and soil moisture deficit (SMD). Models have already been developed for wheat (Pleijel et al. 2000), potato (Pleijel et al. 2002) and Norway spruce saplings (Karlsson et al. 2000).

Processes involved in ozone uptake

Once entered the stomata, ozone may react in the substomatal cavity or intracellular spaces with unsaturated hydrocarbons in the substomatal cavity and apoplast (ozonolysis) to give rise to H_2O_2 formation. Hydrogen peroxide can also be formed in ambient air and can enter the plants via the stomata. Ozone itself may also dissolve in the extracellular fluid (apoplast) and transformations of dissolved ozone in the apoplastic fluid may generate many toxic oxygen species, including free radicals, which are able to injure cell membranes. It is not likely that ozone molecules are entering the symplast of the cell through the plasmalemma. Ozone is reacting with all kinds of molecules with double bounds and will react also with the plasma membrane. However, the molecule needs to be dissolved first in the apoplast and it is not so very soluble in water compared to other pollutants such as SO_2 and NO_2. Nevertheless, dissolved ozone is transformed in a whole series of well soluble reactive oxygen species such as: hydroxyl radicals ($OH^{\cdot}$), singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Kanofsky and Sima 1991). Singlet oxygen and hydroxyl radicals are the most toxic components and will almost immediately oxidise organic molecules. Singlet oxygen is extremely reactive (residence time 2 μs in aqueous environment) and preferentially oxidises methionine, histidine and tryptophan (Krinsky 1979).

Ozonolysis takes also place in the gas phase in the sub-stomatal cavity and the intracellular spaces. Indeed plants emit gaseous substances with double

bounds such as ethylene (Tingey et al. 1976; Mehlhorn and Wellburn 1987; Mehlhorn et al. 1991; Langebartels et al. 1991) and other alkenes (terpenes, isoprene, etc.) (Elstner et al. 1985; Salter and Hewitt 1992). Ozone is opening the double bound to form a primary ozonide that is transformed in an aldehyde and a Criegee zwitterion, this is in the water phase transformed in a hydroxy hydroperoxide and decomposing in an aldehyde and hydrogen peroxide. Ozonolysis is forming H_2O_2, perfectly soluble in water (Hewitt et al. 1990; Hewitt and Terry 1992). The existence of ozone-alkene reactions was demonstrated *in vivo* through the detection of hydroxy hydroperoxides in isoprene emitting plants (Hewitt et al. 1990). The significance of such reactions *in vivo* is discussed in Lyons et al. (2000). Hydrogen peroxide is less toxic to the plant cell than most of the free radicals formed, but it can be transported through the membranes to the cytoplasm and the chloroplasts. Among other substances i.e. salicilic acid, it could be one of the second messengers during oxidative stress signalling (Mudd 1998).

The free radicals formed are able to react with the fatty acids of the cell membrane to cause leakage and destruction of the cell. Radicals can start a chain reaction forming alkylradicals and subsequently alkyl peroxyl radicals. The end products of the reactions are malondialdehyde (MDA) and another radical that can continue the chain reaction. The MDA is an indicator of fatty acid peroxidation (Mehlhorn et al. 1991; Farage et al. 1991).

In addition the reaction of dissolved ozone and H_2O_2 is forming superoxide ($O_2^{\cdot-}$), hydroxyl radicals and oxygen. The radicals formed are able to start the chain reaction (Wellburn 1994).

Defence systems

After an exposure to ozone, as for other external stressors, reactive oxygen species (ROS) are primarily formed within the apoplastic fluid of tree leaves. It is therefore believed that the antioxidative capacity within the apoplast of exposed leaves is of great importance in determining ozone resistance. It is pointed out in this communication, that the apoplastic antioxidative capacity should be approached with caution, before introducing this parameter in models describing true ozone flux. What is the best parameter to estimate apoplastic antioxidative capacity?

Many apoplastic antioxidants in relation to ozone have been reported earlier, the most mentioned being ascorbate (Mächler et al. 1995; Vanacker et al. 1998; Imai et al. 1999; Moldau 1999; Turcsányi et al. 2000; Lyons et al. 2000; Plöchl et al. 2000). Indeed, *in vitro* experiments do indicate high reaction constants between ascorbate and ozone, reactive oxygen species and other radicals (Sturgeon et al. 1998), but its relative antioxidative capacity *in vivo* is still unknown. Moreover, the decay of O_3 through direct reaction with cell wall ascorbate is not sufficient to explain the different degrees of ozone sensitivity in two poplar clones, i.e. the ozone sensitive *P. deltoides* × *P. maximowiczii* cv Eridano and the more resistant *Populus* × *euramericana* cv I-214 (Ranieri et al. 1999).

Lignin biosynthesis and oxidative defence

The capacity to scavenge ROS has been assigned to a great variety of molecules other than ascorbate. Examples of low-molecular antioxidants are phenolics (such as ferulic acid, caffeic acid, catechol, syringic acid and *p*-coumaric acid) (Polle 1998; Arnao et al. 1999), polyamines (Bors et al. 1989; Langebartels et al. 1991), diketogulonate (Deutsch 1998) and glutathione (Dixon et al. 1998). The involvement of phenolic compounds in the sensitivity of poplar to ozone was shown by Biagioni et al. (1997). After a single pulse exposure of a resistant poplar clone there was a marked increase of phenolic compounds. In the sensitive clone, only the behaviour of caffeic acid was different; an increase was only found a week after the treatment.

Phenolics are present in the apoplastic fluid and play an important role in lignin biosynthesis. Lignin is a complex macromolecule that originates from the oxidative polymerisation of cinnamyl alcohols as principal monomeric units. The biosynthesis starts with phenylalanine, enzymatically transferred to cinnamate, and further to coumarate, caffeate, ferrulate, hydroxyferrulate and sinapate, which form, again enzymatically hydroxycinnamoyl and further hydroxycinnamaldehydes. The phenolic acids formed have antioxidative properties and they exchange electrons with apoplastic ascorbate (Polle 1998). Cinnamyl alcohol dehydrogenase (CAD) is considered to be a key enzyme in the lignification pathway because it catalyses the final step in the synthesis of the monolignols, thereby converting the cinnamaldehydes to the corresponding alcohol (Baucher et al. 1995, 1996). These lignin monomers (or monolignols) are p-coumaryl, coniferyl and sinapyl alcohols. Woody angiosperm lignin contains mainly coniferyl and sinapyl alcohols. The final step in the synthesis of lignin is the polymerization of cinnamyl alcohols. Two distinct classes of enzymes, peroxidases and laccases, have been proposed to perform the polymerization of monolignols into lignin (Baucher et al. 1996, 1998; Baudet 1998; Mäder and Füssl 1982; Lagrimini et al. 1987). Transgenic plants with 10-fold higher peroxidase activity in the leaves were characterised by wilting of the leaves and browning of wounded tissues (Lagrimini et al. 1990; Lagrimini 1991). In needles of Norway spruce the apoplastic peroxidases and the lignification process

have been studied (Polle et al. 1994), and the relation between ozone exposure and apoplastic guaiacol peroxidase and lipid peroxidation examined (Kronfuss et al. 1996).

The question remains: how efficient are phenolic acids, compared to ascorbate, in scavenging ROS? Generally, this question remains unanswered, but a few naturally occurring components seem to have greater instantaneous antioxidative power than ascorbate (e.g. diketogulonate, ferulic acid, *p*-coumaric acid, gallic acid, resveratrol and quercetin) (Deutsch 1998; Arnao et al. 1999). Because electron transfer can take place between ascorbate and other antioxidants (e.g. phenolic acids) (Takahama and Oniki 1992), possible synergistic effects should also be considered.

Total antioxidative capacity

To examine the relative importance of ascorbate in apoplastic antioxidation, one has to analyse the total antioxidative capacity (TAC) of the apoplast. Numerous tests for determination of TAC in chlorophyll-containing tissues are already available (Arnao et al. 1999). By expressing this antioxidative capacity in terms of ascorbate-equivalents, and comparing these results with actual ascorbate content, one can estimate the relative importance of ascorbate in the total detoxification power of the apoplast (the same accounts for other antioxidants, of course).

The levels of most important antioxidants in the apoplastic fluid are at least an order of magnitude lower than in the symplast. Furthermore, there are no means of regenerating oxidised antioxidants in the apoplast (Luwe and Heber 1995). So, considering only the instantaneous levels, it seems that apoplastic antioxidants are not well suited for detoxifying external oxidising agents. However, apoplastic antioxidants (e.g. ascorbate, glutathione) are continuously being exchanged with the cytoplasm through a number of plasma membrane transporters (Jamaï et al. 1996; Horemans et al. 2000). As a consequence, the apoplastic antioxidant pool is metabolically linked to its cytoplasmic counterpart, where regeneration can take place. Finally, the re-reduced antioxidants are transported back from the cytoplasm into the apoplastic fluid.

Transport of antioxidant is best documented for ascorbate. When an ascorbate molecule is oxidised by an ozone molecule, it forms H_2O and a monodehydroascorbate radical (MDA). Two MDA molecules can spontaneously disproportionate to one molecule of reduced ascorbate (ASC) and one molecule of fully oxidised ascorbate (dehydroascorbate or DHA) (Bielski et al. 1981). The apoplastic DHA is then exchanged for cytoplasmic ASC, either physically (Horemans et al. 1998, 2000), or by transferring electrons through a cyt b561 complex (Asard et al. 1998). There are indications that apoplastic ASC is, at some basic level, constantly being oxidised by ASC dependent oxidases and peroxidases (Takahama and Oniki 1992; Smirnoff 1996). This means that without a cytoplasmic exchange, the ratio of reduced to oxidised ASC (= redox status) in the apoplast would quickly drop. Nevertheless, the redox status of apoplastic ASC is reported to differ from zero in non-stressed situations (Castillo and Greppin 1988; Takahama and Oniki 1992; Takahama 1994; Luwe and Heber 1995), which proves that the cytoplasmic exchange must be fairly active *in vivo*.

The turnover rate of apoplastic ASC not only results from transmembrane transport (or electron shuttling), but also from the size and turnover rate of the cytoplasmic ascorbate pool. So, although generally instantaneous pool sizes of ASC are analysed, transport and turnover rates could be at least as important in detoxifying ozone. Measuring turnover rates is practically feasible using radiolabelling techniques (Imai et al. 1999; Pallanca and Smirnoff 2000), although it forms a much greater challenge than measuring the absolute concentrations only. Other, more indirect methods for measuring ASC-turnover, quantify the enzymatic activity of enzymes related to ASC regeneration (e.g. MDA-reductase, DHA-reductase, GSH-reductase). These form a major attribution to the mechanism of ASC-reduction, but they are poor estimates for the absolute turnover rate. Also the estimation of ASC transport is to be considered as a novel parameter in ozone detoxification.

Until now, it was assumed here that no injury of the plasma membrane occurred and that ROS could not pass the membrane. This might not always be the case. Even at ambient ozone concentrations, H_2O_2 could permeate the plasma membrane and cause intracellular oxidative damage (Foyer et al. 1997). Apart from being a product of apoplastic ozone reactivity, H_2O_2 can also arise endogenously by activity of NADPH-oxidase-like enzymes during the oxidative burst (Low and Merida 1996; Schraudner et al. 1998). For this, one must also take into account the symplastic antioxidants, again in relation to the TAC.

So, in summary we can say that antioxidative capacity is to be considered in calculations of ozone flux. Descriptive models, based on ascorbate, have already been set up and are now verified with experimental data (Lyons et al. 2000). However, for more complete models, transport and turnover rates of ascorbate will have to be introduced in the calculations. Also the possibilities of other, maybe more efficient, antioxidants are to be considered by measuring the total antioxidative power of apoplast and symplast. Finally, the possibility will be created to quantify the 'antioxidative resistance' and introduce it into the electrical analogues of ozone-transport.

Stress effects on the cellular level

Long-term ozone exposure is causing stress of the plant cells, which results in increased production of H_2O_2 and other reactive oxygen species in the chloroplasts (Alsher et al. 1998). H_2O_2 is a toxic agent and, in addition, it is able to form extremely toxic hydroxyradicals. To some extent the enhanced formation of reactive oxygen species is also a result of increased respiration, since repair of membranes consumes carbohydrates derived from respiration. Indeed, it is primarily the leaf's maintenance respiration that is increased while the growth respiration is unaffected (Amthor and Cumming 1988). Blocking the photosynthetic electron transport caused by a number of processes, some being driven by ozone exposure, initiate the formation of singlet oxygen, H_2O_2 and other reactive oxygen species (Hippeli and Elstner 1996).

Plants can cope with those oxidative species by using antioxidants such as ascorbate, α-tocopherol, glutathione, carotenoids and enzymes such as superoxide dismutase (SOD), catalase and several peroxidases (Hippeli and Elstner 1996). In addition, all these tools are very helpful to the plant to avoid that the most toxic species are formed. Plants are also able to recover from ozone injury but as repair processes are driven by dark respiration, the length of the night might be very important to prevent the plants from chronic injury. This is probably the reason why plants are more susceptible in summer to ozone damage in Nordic countries. It was shown recently that potato appeared to suffer much more from ozone exposure in Scandinavia compared to the mid-latitude European countries (De Temmerman et al. 2002). It appeared from Artificial Neural Network analysis that day length was a very important parameter. More likely, the nights become too short in summertime to recover from ozone damage and the effect of cumulative exposure becomes very negative.

Conclusions

In order to obtain a reliable critical level for ozone, modelling of the effective ozone flux is a very promising issue to determine the risk for forests. It is a crucial intermediate step to translate data obtained in artificial exposure systems to natural conditions. In addition, models to determine stomatal ozone uptake give already very good results but need to be further developed and tested.

Much more research is needed to determine the defensive response because of its complexity, and the following issues seem to be crucial

- the antioxidative power of phenolic acids is not well known;
- the turnover rate of antioxidants is unknown;
- constitutive antioxidant levels do not determine resistance.

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