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Stomatal-scale modelling of the competition between ozone sinks at the air–leaf interface

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ABSTRACT

This paper analyses the existence and relative strength of ozone (O_3) sinks at the leaf level, in particular the implications for the partition of the O_3 flux amongst the several physically, chemically and physiologically differing inner and outer surfaces of a leaf. We used a single-stomatal scale theoretical model to simulate the O_3 transfer into leaves and estimate the flux partition. The theoretical scenarios were compared with experimental values from shoot-scale measurements of O_3 flux onto Scots pine. The conditions where external sinks would prevent the stomatal diffusion involved the existence of very strong sinks at the external surfaces but yielded unrealistically high flux values. Only the possibility of a strong sink localized in the antechamber and/or pore could be of significance. Results also showed that in most instances a significant proportion of the total flux was generated by the external surfaces. For scenarios that consider strong scavenging in the mesophyll and weaker removal in the exterior, the proportion was about 60–80% for small stomatal apertures (∼0.5 μm), and 10–40% for larger apertures (∼1.5 μm). In these cases, however big the proportion of total flux is due to the external surfaces, the existence of sinks at the external surfaces does not prevent the diffusion through open and unoccluded stomata.

1. Introduction

Tropospheric ozone (O_3) is deposited at the Earth surface by various routes, including removal at the plant surfaces (Seinfield and Pandis, 1998a). The scavenging reactions can happen both at the exterior of the foliage or at its inner surfaces. Scavenging mechanisms and rates differ greatly between external and internal locations of leafs. In which of such locations the scavenging occurs has different implications for the plant, since the O_3 reacting in the interior of the leaf is most likely to impair plant performance (Sandermann, 1996). For gaseous pollutants with demonstrated phytotoxic capacity such as O_3 there is need to recommend air quality standards for the prevention of damage to vegetation (WGE, 2004). This procedure involves estimation of the amount of O_3 that meets the vegetation, including knowledge of the controlling factors of the O_3 flux to the leafs (EMEP, 2006; Wieser and Tausz, 2006).

The initial events that lead to toxicity happen upon reaction of O_3 with the apoplast of foliage cells and the subsequent generation of highly oxidative compounds in the mesophyll cells (Kangasjärvi et al., 2005). In principle thus, only the O_3 molecules that reach the apoplast are considered phytotoxic. Since the delivery of O_3 to these potential reaction sites happens through the stomatal pores, this process is largely controlled by the degree of stomatal aperture and stomatal behaviour (e.g. Heath, 1996). Although the toxicologically relevant portion of the flux is related mostly to the stomatal uptake, it does not represent the whole flux generated at the foliage, but there are other controlling processes as well. This suggestion stems from studies of O_3 removal based on flux measurement, for example, at the shoot scale as measured with gas-exchange systems (Rondón et al., 1993; Altimir et al., 2002, 2004) and at stand scale with meteorological techniques (Coe et al., 1995; Zeller and Nikolov, 2000; Fowler et al., 2001; Kurpius and Goldstein, 2003; Cieslik 2004; Gerosa et al., 2004). Results show a daily and seasonal pattern of O_3 deposition that reflects uptake through stomata and, overlaid to it, a significant and variable portion of deposition which has been called non-stomatal (e.g. Altimir et al., 2006) because it cannot be accounted for by the stomatal behaviour.

Thus, some O_3 molecules travel through the stomatal aperture and might react with the surface of guard-cells, hypodermis, or mesophyll. In addition, a portion of the O_3 molecules that react with the foliage might not go through the stomata aperture and in consequence might not react with the apoplast but likely with the

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outer foliage surface (epicuticle, thricomas and glandulae) and its components (structural, deposited, or exuded compounds) or perhaps with some plant-emitted compounds (Percy et al., 1994). With all the possible reaction sites inside and outside leafs and O_3 being very reactive, the question arises whether it is at all possible that O_3 can be significantly depleted before it reaches the stomata aperture, in other words, whether it is conceivable that reactions on the outside of the leaf could preclude or diminish the stomatal uptake. Similarly, it is not clear, if passing through the stomatal pore, at what depth total depletion actually occurs. Because of the high O_3 reactivity, it is customarily assumed that O_3 concentration at the substomatal air space is effectively 0, implying that O_3 is totally depleted by the reactions with the apoplast which forms a strong sink for ozone. However, the available experimental evidence about mesophyll concentrations of O_3 does not state really where the ozone goes just that it does not reach the mesophyll cells (Laisk et al., 1989). Experimental evidence with isolated plant epicuticle (Fruekilde et al., 1998), and cuticles (Kerstiens and Lendzian, 1989; Kerstiens et al., 1992), as well as the abovementioned flux measurements reveal a strong potential sink on surfaces other than the mesophyll apoplast.

The lack of information on the compounds involved makes a process-based description of the O_3 transfer and scavenging to leafs elusive. Instead, this study approaches the phenomena with a theoretical analysis that allows to asses the potential relevance of O_3 sinks at the outer surfaces of leafs. Finite-element models are a suitable approach to study the effect of structure on transport phenomena. The geometry of the object of study can be specified with a model grid of discrete regions or finite elements. To each of them, pertinent equilibrium equations are applied and the whole set of equations is solved for the unknown values. Thus finite element models provide information on behaviour of, for example, fluids within complex geometries that cannot be treated in closed-form analytical solutions. Previous works have applied the finite-element approach to the simulation of diffusion in a single stomatal system, for example, Aalto and Juurola (2002) examined the diffusion of CO_2. The fate of O_3 had been examined by similar methods previously in Claiborn et al. (1993) and Plöchl et al. (2000). Other physicochemical models based on one-dimension are, for example, that of Chameides (1989). All have invariably ignored any possible scavenging of O_3 at the outer surfaces. Notably, the importance of the location of sinks along the pathways was noticed in Plöchl et al. (2000). They discussed that O_3 does not reach the mesophyll possible at low concentrations and/or when there are enough reaction sites for ozone to be totally scavenged even before reaching the closest cells.

This paper uses a single stomata-scale finite element model to analyse the existence of O_3 removal at different locations in the leaf outer and inner surfaces and in particular the potential competition amongst them. We look at the implications for the partition of reaction sites at the foliage surface. The structural and field information for this study is based on Scots pine (*Pinus sylvestris* L.) shoots and needles.

2. The air–leaf interface

The outer and inner surfaces of foliage are different not only in their respective position but also in their composition. The outer surface is covered by the epicuticular waxes that provide impermeability to the loss of water vapour and protection to the continuous exposure to the exterior environment. Details on the structure, physiology, function and environmental effect on the cuticle have been reviewed in several occasions (Percy et al., 1994; Shepherd and Griffiths, 2006). Epicuticular waxes are on top of the cuticle including the walls of the stomatal antechamber in conifers. In Scots pine, about 40% of the waxes consists of secondary alcohol nonacosan-10-ol, about 15% are estolides and additional 15% alkylesters followed in abundance by diterpenes, diols, fatty acids, alkanes, di-esters and some minor amount of primary alcohols and hydroxy fatty acids. The pine waxes are arranged as interlacing tubes of around 0.06–1 μm long that are especially conspicuous and abundant in the vicinity of the stomatal aperture, along the stomatal pore rows. The waxes themselves are not very reactive towards O_3 (Jetter et al., 1996) but all the components trapped or associated to them likely offer more possibilities for reactions (Fruekilde et al., 1998) including the surface microflora (Schreiber and Schönherr, 1993). On the epicuticular waxes there can be a mixture of particulates, salts, ions, and condensable vapours that either originate from the ambient air or are emitted or leached from the interior of the plant. Water vapour gathers on all these components as surface moisture in amounts that range from films to dew droplets (Burkhardt and Eiden, 1994; Brewer and Smith, 1997; Burkhardt et al., 1999). Liquid water on the foliage is also provided by interception of rain droplets. The epicuticle and cuticle proper are effective barriers to O_3 and the main route of passage to the interior of the foliage is through open stomatal pores. The experiments that show the low cuticle permeances of O_3 (Kerstiens and Lendzian, 1989; Lendzian and Kerstiens, 1991) suggest that O_3 does not permeate through the cuticle because it reacts with it, thus the epi- and cuticle are a location of reaction sites for O_3 at the leaf–air interface. Surface characteristics such as the number of trichomes has been suggested to offer protection against ozone perhaps by sorbing O_3 from the boundary layer (Elkiey et al., 1979; Elkiey and Ormrod, 1980).

The inner surfaces of the foliage are covered with apoplastic liquid film which forms a continuum surrounding the mesophyll and guard cell walls. Mesophyll air spaces are thus lined with the apoplastic liquid. The apoplastic liquid contains, for example, the antioxidant ascorbic acid (AA) in concentration of several mM (Conklin and Barth, 2004). In addition to both reduced and oxidized ascorbate, the apoplast space also contains superoxide dismutase and peroxidases. AA is the main antioxidant

in plants (Chen and Gallie, 2004) and has been considered the main apoplastic O_3 scavenger and main defence against O_3 damage. Although AA has a prominent role in this protective scavenging, it is neither complete nor is the only factor (Ranieri et al., 1999; Turcsányi et al., 2000; Van Hove et al., 2001; Moldau and Bichele, 2002). Other apoplastic components are also able to scavenge O_3 or its reaction products (Castillo and Greppin, 1988; Langebartels et al., 1991). Also, cell wall components are sensitive to oxidation, for example, phenolic compounds (Wiese and Pell, 2003). The overall capacity of the apoplast to scavenge O_3 is thus even higher than the direct reaction with AA. For example, based on experiments with high O_3 pulses, Moldau and Bichele (2002) estimated an overall loss coefficient in the order of 10^5 to 10^6 s^{-1}, higher than the direct reaction of O_3 with AA ($\sim 10^4$ s^{-1}).

The guard-cells are effectively in contact both with the exterior and interior sides of the foliage. Their outermost side is part of the antechamber and might present a waxy cover whereas the inner surface of the cell is covered by the cell wall. The cell wall is thickened at the walls of the stomatal pore no doubt as protection to the almost constant contact with the outer environment.

3. Methods

We used a theoretical model to simulate the O_3 transfer into foliage with a different combination of inner and outer foliage surface characteristics and estimated the flux partition amongst the different foliage surfaces. The theoretical scenarios were framed within the range of values compatible with experimental data of shoot-scale O_3 flux.

3.1. Model

We calculated the mass transfer of O_3 at the foliage surface with a finite element three-dimensional cylindrically symmetrical model of one stoma that solves the Laplace equation of diffusion in the stomatal geometry (Vesala et al., 1995; Aalto, 1998). The model bounds the gas-phase environment of a stomatal pore, the viscous layer above it, and the intercellular airspace immediately below (Fig. 1). The calculation method is based in finding the value of concentration at each point of an imaginary infinitesimal grid spread within the described volume. The inputs to the model are the geometrical measures and the conditions existing at the boundaries. Strictly, the boundaries in the model refer to the edges of a gas phase volume. Mostly, the model boundaries represent the limit with a physical surface, like the outer (epicuticular) surface of the needle, and the surface of the stomatal and mesophyll cells. The model boundaries can also be used to indicate virtual surfaces. This is the case with the boundaries that mark the limits of the diffusive layer on top of the foliage.

To find the solution of the Laplace equation, the conditions at the boundaries need to be specified. We can specify either concentration at that boundary (C, mmol m^{-3}) or a flux through the boundary (J, mmol m^{-2} s^{-1}). Since the flux is proportional to the existing concentration ($J = CW$), what we specify is the constant of proportionality between the flux and concentration (W, μm s^{-1}). W, which we refer generally in this paper as absorption rate, represents the strength by which a certain boundary removes O_3 from the gas phase and accounts for the combination of all transfer and removal mechanisms. W could be though of as describing the passage of ozone to the other side of the boundary where—in case of O_3—we assume 0 concentration. The general interpretation of boundary settings are as follows: $C = 0$ at a certain boundary means that O_3 reaches the surface but is reacting so quickly that in practise the concentration in that position is zero. It is thus the equivalent to a very strong sink on the surface; $C > 0$, to give a known concentration, we only use it to set the ambient concentration at the top of the viscous layer; $W = 0$, no transfer through that boundary, it means the surface is impermeable, or in case of O_3 inert; $W > 0$, transfer across the boundary at a given rate.

3.2. Basic model settings

Conditions are isothermic at 20 °C. The corresponding diffusion coefficient of O_3 in air (D_{O_3} [°C, atm]) was calculated according to Massman (1998) as D_{O_3} [20,1] = 0.1444 cm^2 s^{-1}. The geometry was chosen to represent the slightly sunken position of stomatal cells of a conifer, with open stomatal pore, Fig. 1. The dimensions agree with previous reports (Vesala et al., 1995). In order to simulate homogeneous conditions between the neighbouring stomata, no lateral flow was allowed. The boundary layer was set to simulate the ventilated conditions in the measuring shoot chamber and was thus relatively shallow (50 μm) (Jones, 1992). The ambient ozone concentration was chosen to represent Finnish rural background, $[O_3]$ = 30 ppb (Laurila and Lättilä, 2004).

We used several combinations of conditions at the boundary to represent the following basic scenarios: (1) all surfaces are equal sinks, all have the same removal rate; (2) all surfaces are very strong sinks, all have C = 0; (3) strong sink at the inner surfaces, outer surface inert; (4) strong sink at the inner surfaces, outer surfaces also remove and (5) only removal at the outer surfaces, closed stomata. Intermediate cases between scenario 2 and 3 were also considered. Table 1 shows the details for these scenarios. Note that scenario 4 was run with the stomatal pore treated both as inner and outer surface.

3.3. Experimental data

The total flux calculated by the model was compared to experimental values of shoot-scale O_3 flux. Data was obtained from field measurements of Scots pine foliage from the long-term measuring station SMEARII, in Hyytiälä, Southern Finland. (Hari and Kulmala, 2005.). The measuring set-up was a multiline gas-exchange open flow system, which providing simultaneous O_3, CO_2 and water vapour fluxes. Single Scots pine shoots were

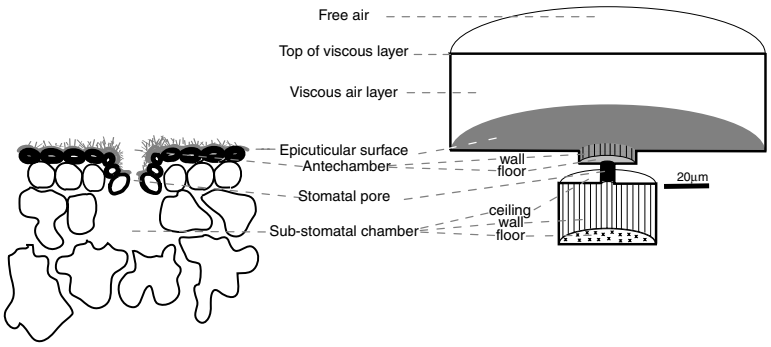

Fig. 1. Scheme of the elements associated to one stomatal aperture. Left-hand panel: detail of a cross-cut of Scots pine needle showing one stoma and the adjacent cell types and air spaces, the content of the cells has been omitted. Right-hand panel: cross-cut through the middle of the model cylinders showing the corresponding geometry of the model boundaries. Shadings and patterns distinguish different surfaces.

Table 1. Details of the boundary settings for the basic scenarios and other intermediate cases

	Scenario							
			Intermediate cases					
Location	1	2	I	II	III	3	4	5
Epicuticular surface	W	$C = 0$	INERT	INERT	INERT	INERT	W	W
Antechamber walls	W	$C = 0$	$C = 0$	INERT	INERT	INERT	W	W
Antechamber floor	W	$C = 0$	$C = 0$	$C = 0$	INERT	INERT	W	W
Pore	W	$C = 0$	$C = 0$	$C = 0$	$C = 0$	INERT	W or $C = 0$	INERT
Sub-sto. chamber ceiling	W	$C = 0$	$C = 0$	$C = 0$	$C = 0$	$C = 0$	$C = 0$	INERT
Sub-sto. chamber walls	W	$C = 0$	$C = 0$	$C = 0$	$C = 0$	$C = 0$	$C = 0$	INERT
Sub-sto. chamber floor	W	$C = 0$	$C = 0$	$C = 0$	$C = 0$	$C = 0$	$C = 0$	INERT

Table 2. Experimental values of shoot-scale O_3 sink strength (mm s^{-1}) during selected condition, temperature = 20 ± 1 °C and $[O_3] = 30 \pm 2.5$ ppb. Data from Scots pine during the growing season

	Measured total	Estimated stomatal	Estimated non-stomatal
All data	0.2–1.4	0–1	0–0.6
Nocturnal	0.2–0.5	0	0.2–0.5
Dry diurnal	0.2–0.4	0.1–0.2	0.1–0.3
Humid diurnal	0.6–1.4	0.4–1	0–0.4

enclosed in transparent and ventilated 1 L trap-type chambers at upper canopy level. The chambers remained open most of the time. To measure shoot exchange, the chamber closed and the gas flux was calculated from the concentration change inside the chamber during closure, which lasted less than a minute. Equivalent measurements from an empty chamber were used for blank correction (Altimir et al., 2002). For more details, we refer the reader to previous descriptions (e.g. Altimir et al., 2002, 2004, 2006; Hari et al., 1999)

This study used measurements from 2002 and 2003. From these, we selected data from the growing season (June–August) that matched the chosen temperature and O_3 concentration within 20 ± 1 °C and 30 ± 2.5 ppb. Table 2 lists the range of measured values for all the data and for particular conditions within these, such as nocturnal measurements. Diurnal measurements are separated between dry and humid conditions. This distinction follows the documented effect of moisture enhancing the O_3 flux to foliage (Altimir et al., 2006). The O_3 flux values obtained from these field measurements correspond to the total flux. This means that it includes the O_3 removal by all foliage components, from external epicuticular surfaces to internal mesophyll space. Such values correspond to the modelled values of the flux at the top of the viscous layer according to the model used in this paper. Measured fluxes were obtained at the shoot-scale whereas modelled values were calculated for single stomata. For these flux values to be comparable, shoot and stomata-scale spatial scaling was needed. We achieved this via knowledge of the stomatal density (170 stomata mm^{-2}). The stomatal density also determined the dimension of the outermost cylinder, which was set to represent the average distance to the neighbouring stomata.

Table 2 also details the corresponding ranges for the estimated stomatal and non-stomatal portions of the O_3, uptake. The stomatal uptake corresponds to the portion of the uptake that can be accounted for by the stomatal behaviour. The rest of the uptake is considered then non-stomatal removal. The calculations, methodology, and results have been previously reported in Altimir et al. (2006). In short, the stomatal conductance was calculated with a stomata-photosynthesis model based on the optimality of stomatal behaviour (as initially proposed by Cowan and Farquhar (1977) and further developed in, e.g. Hari et al.,

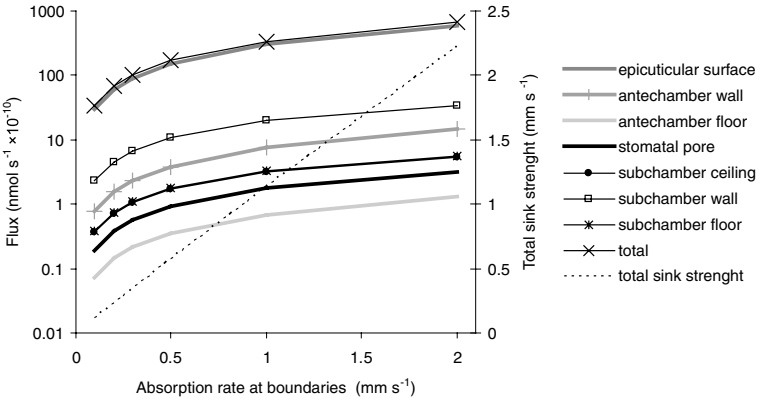

Fig. 2. The predominance of the O_3 removal at the epicuticular surface in case all surfaces have equivalent absorption rates. Flux received at the different boundaries according to scenario 1, where all boundaries have the same absorption rate, W, as specified by the x-axis. For all values of the absorption rate, the flux to the epicuticular surface is almost identical to the total flux. Total refers to the values at the top of the viscous layer. Stomatal radius = 2.5 μm.

1986; Mäkela et al., 1996, 2004). The stomatal conductance to water vapour thus obtained was transformed into stomatal conductance to O_3 via scaling by the diffusion coefficient. In these previous studies the concentration of O_3 at the surfaces was considered negligible, as it has been customary in the analysis of O_3 fluxes. Therefore, the flux became proportional to O_3 concentration with the proportionality factor being the stomatal conductance (m s^{-1}). In this paper, the stomatal conductance is considered analogous to the absorption rate, W. The stomatal and non-stomatal values estimated from the total measured flux give the range of expected values for particular scenarios.

4. Results

Whatever conditions, if all surfaces would remove O_3 with the same strength, most of it would be scavenged by the outermost surface (Fig. 2). Fit with experimental data was achieved with absorption rates between 0.1 and 2 mm s^{-1}, for which at least 90% of the flux was due to the outer surfaces. At very high absorption rates (all surface $C = 0$, scenario 2) the proportion increases to practically 100% (Fig. 3). However, a sink so strong that would deplete homogeneously the concentration to 0 at the epicuticular surface would produce a flux at least 2 orders of magnitude larger than the measured ranges, implying the outer surfaces cannot be so strong a sink.

Setting any surface with $C = 0$ had a very dramatic effect. Ozone disappeared at the encounter with any such surface, which effectively precluded any flux at deeper locations (Fig. 3). Assuming the outer surface would be inert, a strong sink on the antechamber or even only the pore walls would reduce the proportion reaching the mesophyll to less than 1% (Figs. 3 I, II, III). The same is seen in the partition amongst the mesophyll surfaces: Considering all a very strong sink makes the closest surface immediately near the stomatal aperture to receive most of the O_3 (Fig. 3).

In the cases were all the boundaries would have the same removal rate, the partition of the flux is in practice not affected by the stomatal aperture. However, in the case where the inner and outer surfaces have different rates, the stomatal aperture becomes relevant. We used the data set of estimated stomatal conductance for ozone to find the values of the stomatal pore radius. For this we run scenario 3 at different aperture radii and compared the resulting total flux with the range of experimental values. We found the radius to be in the range of 0.5–2 μm, with 0.5 μm happening on diurnal dry conditions and 1.5 μm being in the mid range for less limiting conditions. Having set the functional stomatal aperture, we further use the experimental values to find the range of absorption rates on the outer surfaces. To simulate diurnal situations we assigned a pore aperture suitable to the different conditions. During dry conditions we use

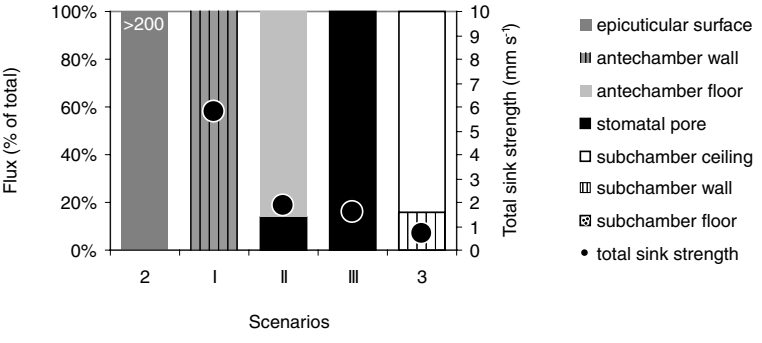

Fig. 3. The effect of $C = 0$. Partition of the total flux when concentration 0 exists at all or some of the surfaces. The bars denote different scenarios. Total refers to the values at the top of the viscous layer. The percentage of flux to the subchamber floor is in all cases extremely small (10^{-14} to 10^{-3}%). The value of the total sink strength for scenario 2 is very large and out of the figure scale (>200 mm s^{-1}).

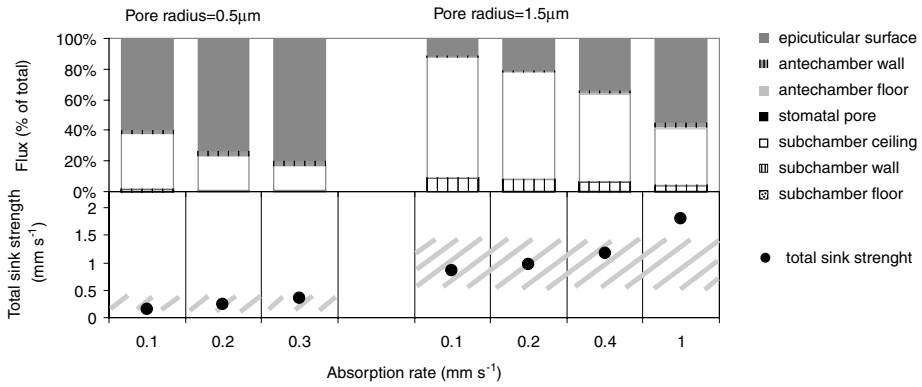

Fig. 4. Scenario 4, the inner surfaces are strong sinks and the outer surfaces including the stomatal pore walls have a certain absorption rate, as specified by the *x*-axis. Two groups of data are represented: on the left the diurnal dry conditions with a small pore aperture and on the right diurnal humid conditions with a wider pore. Upper: partition of the flux to the different surfaces. Lower: dots show the sink strength at the top of the boundary layer and horizontal diagonal-stripped areas show the range from the experimental data.

0.5 μm radii whereas during humid conditions we use a range of larger stomatal apertures. Figure 4 gives the summary of this comparison, which shows that the smaller the stomatal aperture the more predominant the absorption to the external surfaces.

Nocturnal closed stomata were simulated so that the only possible sinks are at the outer surfaces (scenario 5). The experimental values measured at night should then be equivalent to the absorption rate at the outer surfaces and from the agreement found this seems to be the case. A fit with the experimental values measured at night was achieved when the outer surfaces exhibit absorption rates in the order of 0.2–0.4 mm s^{-1}.

In the case where the inner surfaces are assumed a strong sink and the outer surfaces are given some absorption rate, the characteristics of the guard-cell surfaces are crucial. Figure 5 shows how much the partition of the flux changes depending on the absorption rate specified at the walls of the stomatal pore. Note a high absorption rate at the pore walls affects the delivery of ozone mostly to the inner surfaces. In the extreme case where the stomatal pore would be a very strong sink most of the ozone molecules would essentially react there. The opposite situation would be to assign to the stomatal pore an absorption rate equal to the outer surfaces (Fig. 4). In this case, for the same stomatal aperture, the higher the outer surface sink, the lower the proportion of stomatal flux. And for the same outer surface sink, the wider the pore the higher the proportion of stomatal flux. Depending on the combination the proportion of flux at the outer surface can be as low as 10% or as high as 75%. At sink strengths between 0.1 and 1 mm s^{-1}, the stomatal flux is not precluded but it represents a small proportion of the total flux, particularly when the stomatal pore is narrow. Thus for the selected diurnal dry data even weak sinks at the outer surfaces reduce the stomatal flux by 40%. Figure 6 shows in more detail the differences in the flux partition at different stomatal apertures.

We checked how different scenarios affect the passage of ozone through the stomata by comparing the generated profile of simulated concentration along the central axis of the system (Fig. 7). The basic scenario where the internal concentration is 0 and the outer surfaces have a certain removal rate yield (scenario 4) essentially the same concentration profile as if the outer surface is considered inert (scenario 3). In this case, the existence and magnitude of sink at the outer surfaces has a negligible effect. On the other hand, if any of the outer surfaces is a very strong sink (scenarios 2, I, II and III) the passage is essentially prevented because the concentration at the entrance of the stomatal pore is reduced to 0. In the case both inner and outer surfaces present the same removal rate (scenario 1), the higher this rate

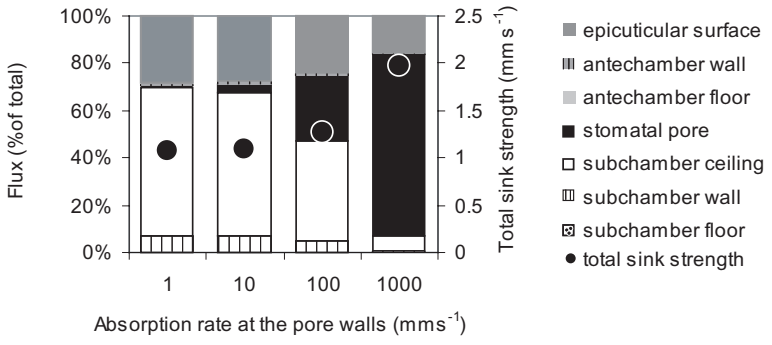

Fig. 5. Effect of the absorption rate of the pore walls on the partition of the mass flow at the different surfaces. This represents a scenario 4 with pore radii 1.5 μm, inner surfaces $C = 0$ and outer surfaces $W = 0.3$. Shades and patterns refer to the boundaries as in Fig 1. The percentage of flux to the subchamber floor is in all cases extremely small ($\sim 10^{-4\%}$).

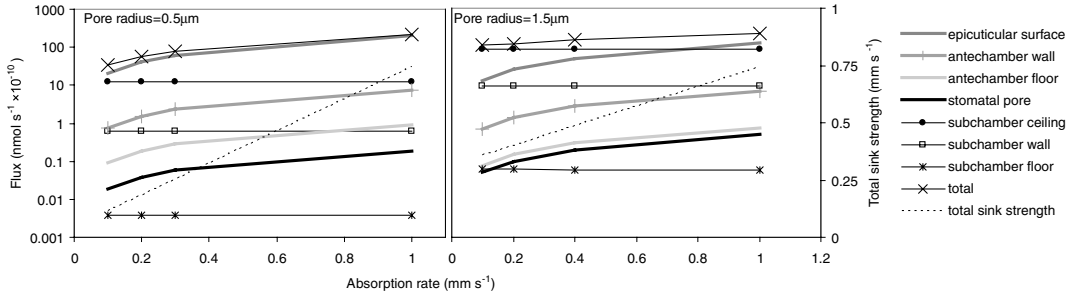

Fig. 6. Details of the effect of stomatal aperture in the partition of flux to different boundaries scenario 4, the inner surfaces are strong sink and the outer surfaces including the stomatal pore walls have an absorption rate, W, as specifies by x-axis

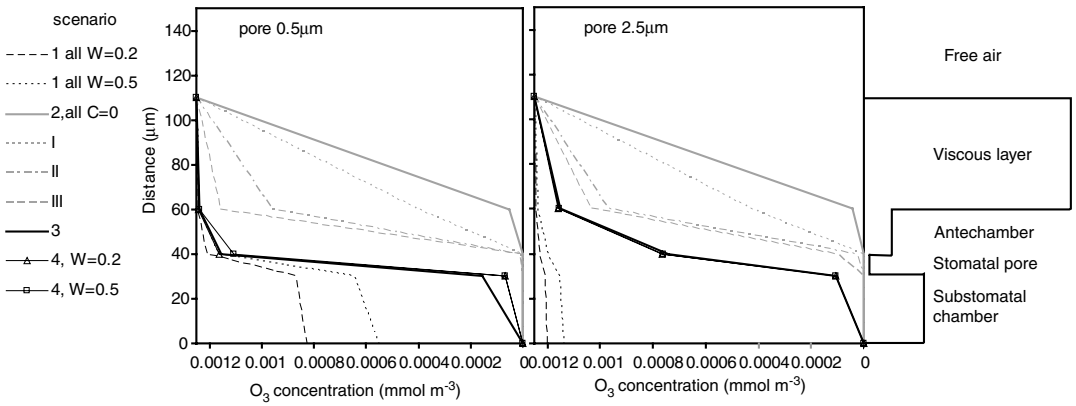

Fig. 7. Profiles of the modelled O_3 concentration along the geometrical centre of the cylinders for different scenarios and pore radius. The scenarios can be grouped in three cases: (1) discontinuous black lines: O_3 reaches the substomatal chamber with a concentration that depends on the absorption rate on and the stomatal pore size; (2) grey lines: O_3 would not reach the stomatal chamber because is depleted to 0 in the surfaces above it; and (3) solid line and symbols: O_3 reaches the stomatal chamber and there is depleted to 0.

the larger the concentration gradient. It would seem then that unless the sink on the outer surfaces would be of the same strength as on the mesophyll it has no effect in the profiles.

5. Discussion

This work reproduced several situations where the proportion of O_3 removal at the outer leaf surfaces could be of equal or larger magnitude than the proportion of O_3 at the inner leaf surfaces. This happened for example if all surfaces were to have the same characteristics, an expectable result on account of the relatively small area of stomata aperture versus outer surface. Everything else being equal, the difference between surfaces is their area and their location along the diffusive path. The epicuticular surface is both the largest one and the first encountered by the diffusing O_3 molecules and would thus receive most of the ozone.

Another situation where the proportion of removal outside the leaf is larger than inside is the case of small stomatal aperture. Small stomatal apertures are reduced by high vapour pressure deficit as to minimize the water vapour loss-like in the diurnal dry cases considered in Fig. 4. Also, stomatal aperture is reduced by lack of light, such as during night. Some plant species sustain a certain degree of stomatal opening at night and the effect of the potential nocturnal O_3 uptake is believed to be of some toxicological relevance (Musselman and Minnick, 2000). It would seem that the extent to which nocturnal uptake could contribute to the load of oxidative stress in plants would depend on the relative strength of ozone sinks at the outer surface of the foliage.

However big the proportion of total flux is due to the external surfaces, the fact is that in many of the simulated cases the existence of this external removal does not prevent the diffusion through an open and unoccluded stomata. There are, however, some possibilities for the outer sinks to represent a real competition to the diffusive flow through the stomatal pore. One of them is that the external removal would be stronger than the internal. The second is that, regardless of the strength of the inner sink, the outer sink would be so strong as to consume most of the O_3. However, such a strong sink at the whole of the epicuticular surface is in contradiction with the levels of measured ozone flux (cf. Fig. 3, Table 2). There exists the possibility that the very strong sink could be localized in the antechamber and/or pore. In this case, these areas would still remove most of the ozone but would generate a flux in the range of the measured values.

This possibility would be related with the architecture of the epicuticular surface around the stomatal pore. Conifer stomata present abundant waxes around the pore, which conditions to some degree the gaseous passage and the potential accumulation of compounds in the pore area. The stomatal pores of some conifers are clearly occluded by wax plugs that can substantially reduce the water vapour conductance (Brodribb and Hill, 1997). During the life-time of needles, disintegration of wax structures such as fusion of wax tubes and stomatal occlusion occurs. Any occlusion of the stomatal pore could contribute to reduce the ozone passage. The model structure used in this paper did not structurally account for such occlusions, but a strong sink located near the stomatal aperture could be taken to simulate this situation.

Yet one more possibility would be that the walls of the stomatal pore, which is the surface of the guard cells of the stomatal apparatus, would have the same apoplastic composition as the mesophyll cell and the same capacity to regenerate the oxidized compounds.

The expression that O_3 is removed upon contact with any surface is often used in deposition literature to describe the ultimate deposition fate of the O_3 molecules for which most surfaces behave as perfect sinks. Ozone is not expected to permeate through the epicuticular waxes, the cell wall, neither is expected to be found in significant amounts in solution. However, if all O_3 molecules impinging on a surface would automatically and irreversibly react the concentration at all surfaces would be near-0. As we have seen, this scenario would preclude any significant passage through the stomatal pore. The fact that most surfaces behave as perfect sinks for ozone refers rather to the fact that ozone is neither accumulating at the surface nor re-emitted. O_3 is a strong oxidizer (standard reduction potential of 2.07 V, a value larger than for most materials), the oxidation of most species by O_3 is thermodynamically favourable. But yet, the removal rate of ozone depends on the amount of reactants or reaction sites and whether the kinetics is favourable to a fast reaction.

There are in the literature several suggestions for reaction sites and partners that might remove O_3 at the air foliage interface. Rondón et al. (1993) and Coyle (2005) speculated on the possibility of photochemical reactions mediated by the foliage surface. Another mechanism would be that destruction of ozone at the surfaces happens by thermal decomposition (Fowler et al., 2001). In view of the presence of liquid film in the leaf surfaces and the fact that their presence relates to increased deposition levels (Altimir, 2006), the role of heterogeneous reactions seems to be important. The low solubility of O_3 has been invoked to expect limited reactions with dissolved compounds, but there is actually a lack of understanding on the heterogeneous reactions for oxidants. Coyle (2005) showed that the heterogeneous chemistry arising from certain combinations of ambient SO_2 and NH_3 can account for the level of estimated non-stomatal removal. The removal of other substances with low Henry law coefficient has also been found to increase likely through heterogeneous reactions (Turnipseed et al., 2006). Another suggestion is the reaction between ozone and emitted BVOCs, particularly the most reactive ones, in accordance with canopy-scale field observations (Mikkelsen et al., 2000; Stroud et al., 2005) and leaf level laboratory experiments (Velikova et al., 2005). BVOC dissolved in the epicuticular waxes could play a similar role. Concentrations of BVOC are highest close to the foliage, and some are very reactive, for example, β-caryoplyllene. There is increasing evidence to the suggestion that the scavenging by BVOC can act also as a defence mechanisms against ozone (e.g. Loreto and Velikova, 2001). This protective quenching of O_3 implies an effective depletion of the concentration before they reach the sensitive reaction sites in the apoplast. This could happen at the gas-phase or at the surface, depending on the volatability and emission pathway of the compound.

It is interesting to note that most of the mechanisms currently considered to have a role as sinks of O_3 in the external plant surfaces are likely to be simultaneous with small stomatal apertures. For example, higher temperature promotes thermal decomposition and also stronger vapour pressure deficits, which in turn would promotes stomatal closure, and also emission of BVOC. Stomatal aperture is reduced during night, which coincides with the formation of epicuticular water films (particularly in the moisture of calm nights) that might mediate heterogeneous reactions. All these situations would thus seem to fit into the scenario 4 with small stomatal apertures and large proportion of the total flux going to the external surfaces (Fig. 4). In the cases the partition to the external surfaces dominates, the total deposition will be dominated by these phenomena. In these situations, the amount of ozone scavenged outside the leaf is larger than the scavenged inside the leaf. Also, the dynamics of the total ozone deposition are likely to be dominated by the dynamics of these processes mediated at the external leaf surfaces instead of the biological regulation of the stomatal pore aperture.

Some potentially relevant chemical reactions are listed in Table 3 along with the first or pseudo-first order reaction rate with O_3 and a comparison with the absorption rate simulated in this study. Unimolecular decomposition in solution seems to be quite slow, but can become faster depending on the compound in dissolution. For example, reactions with S(IV) in solution seem to be sufficiently fast. This would be likely the case in free water solutions such as dew and macroscopic droplets. The rates of removal attributed to the direct reaction with ascorbate are by far fast enough. The scavenging of O_3 by emitted organic compounds (BVOC) could also be very effective. The chemistry happening at the leaf interfaces is likely to be complex due to the simultaneous and varying presence of a suite of compounds. The O_3-scavenging effectiveness of any reaction also depends on the relative speed with which the reaction partner is depleted and renewed. For example, the speed in the reduction of AA can limit the effectiveness of ascorbate (Luwe et al., 1993; Moldau and Bichele, 2002); or for example, heterogeneous reactions might need a certain sustained pH to take place (Seinfield and Pandis,

Table 3. Potential reactions of O_3 at the air–leaf interface. (ni = no information)

Reference	Reaction	Value	Units	mm s^{-1}
Sehested et al. (1991)	Unimolecular decomposition in solution	10^{-4}	s^{-1}	0.0000055
Hsu et al. (2002)	Unimolecular decomposition in solution	2.4×10^{-4}	s^{-1}	0.0000132
Westerhoff et al. (1999)	Unimolecular decomposition solution with organics	8.8×10^{-3}	s^{-1}	0.000484
Seinfield and Pandis (1998b)	Reaction with S(IV) in solution	10–100	s^{-1}	5.5
Claiborn et al. (1993)	Mesophyll ascorbic acid	10^4	s^{-1}	550
Moldau and Bichele (2002)	Reactive absorption attributed to liquid phase	10^4–10^6	s^{-1}	>550
Atkinson (1997)	β-caryophyllene ozonolysis	11600×10^{18}	$cm^3\ mol^{-1}\ s^{-1}$	ni
This paper	Boundary layer	5.5	s^{-1}	0.3
This paper	Pore	60	s^{-1}	0.3

1998b). At every moment, thus, the sink strength depends on the history of the surface, affected by environmental exposure and plant response.

6. Conclusion

We have assessed the potential relevance of O_3 sinks at the outer surfaces of leafs with a theoretical approach using a single-stomata scale finite-element model to simulate the O_3 transfer into leaves. Experimental values of shoot-scale field measurements of O_3 flux onto Scots pine were used as a reference.

We identified situations when the diffusion of ozone through stomatal aperture and towards the intercellular air space of the mesophyll can be notably diminished. This would include very low presence of reaction sites in the mesophyll, or extremely strong sink located on the outer surfaces. The particular case in which a strong sink would be localized in the stomatal pore could be the most realistic situation. The relevance, or existence, of these possibilities in natural conditions would need to be assessed further.

For a common scenario that considers a strong scavenging in the mesophyll and a weaker removal in the external surfaces we found that the existence of sinks at the external surfaces does not prevent the diffusion through open and un-occluded stomata. Although, stomatal flux is not prevented, the strength of the sinks at the external surfaces effects the partition of the total ozone flux to leafs, and this effect is larger the smaller the stomatal pore.

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References

Aalto, T. 1998. Gas exchange of Scots pine shoots: stomatal modelling and field measurements. *Report Series in Aerosol Sciences* 40. Helsinki, Finland, Finnish Association for Aerosol Research.

Aalto, T. and Juurola, E. 2002. A three-dimensional model of CO_2 transport in airspaces and mesophyll cells of a silver birch leaf. *Plant, Cell Environ.* **25**, 1399–1409.

Altimir, N. 2006. *The Ozone Transfer between Atmosphere and Vegetation. A Study on Scots Pine in the Field*. PhD Thesis, Department of Forest Ecology, University of Helsinki, Finland.

Altimir, N., Vesala, T., Keronen, P., Kulmala, M. and Hari, P. 2002. Methodology for direct field measurements of ozone flux to foliage with shoot chambers. *Atmos. Environ.* **36**, 19–29.

Altimir, N., Tuovinen, J.-P., Vesala, T., Kulmala, M. and Hari, P. 2004. Measurements of ozone removal to Scots pine shoots: calibration of a stomatal uptake model including the non-stomatal component. *Atmos. Environ.* **38**, 2387–2398.

Altimir, N., Kolari, P., Tuovinen, J.-P., Vesala, T., Bäck, J. and co-authors. 2006. Foliage surface ozone deposition: a role for surface moisture? *Biogeosciences* **3**, 209–228.

Atkinson, R. 1997. Gas-phase tropospheric chemistry of volatile organic compounds: 1. alkanes and alkenes. *J. Phys. Chem. Ref. Data*, **26**, 215–290.

Brewer, C. A. and Smith, W. K. 1997. Patterns of leaf surface wetness for montane and subalpine plants. *Plant, Cell Environ.* **20**, 1–11.

Brodribb, T. and Hill, S. 1997. Imbricacy and stomatal wax plugs reduce maximum leaf conductance in southern hemisphere conifers. *Aust. J. Bot.* **45**, 657–668.

Burkhardt, J. and Eiden, R. 1994. Thin water films on coniferous needles. With an appendix "A new device for the study of water vapour condensation and gaseous deposition to plant surfaces and particle samples" by J. Burkhardt and J. Gerchau. *Atmos. Environ.* **28**, 2001–2017.

Burkhardt, J., Kaiser, H., Goldbach, H. and Kappen, L. 1999. Measurements of electrical leaf surface conductance reveal recondensation of transpired water vapour on leaf surfaces. *Plant, Cell Environ.* **22**, 189–196.

Castillo, F. J. and Greppin, H. 1988. Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. leaves after ozone exposure. *Exp. Environ. Bot.* **28**, 231–238.

Chameides, W. L. 1989. The chemistry of ozone deposition to plant leaves: role of ascorbic acid. *Environ. Sci. Technol.* **23**, 595–600.

Chen, Z. and Gallie, D. R. 2004. The ascorbic acid redox state controls guard cell signalling and stomatal movements. *Plant Cell* **16**, 1143–1162.

Cieslik, S. 2004. Ozone uptake at various surface types: a comparison between dose and exposure. *Atmos. Environ.* **38**, 2409–2420.

Claiborn, C. S., Carbonell, R. G. and Aneja, V. P. 1993. Transport and fate of reactive trace gases in red spruce needles .2. Interpretations of flux experiments using gas-transport theory. *Environ. Sci. Technol.* **27**, 2593–2605.

Coe, H., Gallagher, M. W., Choularton, T. W. and Dore, C. 1995. Canopy scale measurements of stomatal and cuticular O_3 uptake by Sitka spruce. *Atmos. Environ.* **29**, 1413–1423.

Conklin, P. L. and Barth, C. 2004. Ascorbic acid, a familiar small molecule interwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant, Cell Environ.* **27**, 969–970.

Cowan, I. and Farquhar, G. 1977. Stomatal function in relation to leaf metabolism and environment. 31. In: *Society of Experimental Biology Symposia* **31**, 471–505.

Coyle, M. 2005. *The Gaseous Exchange of Ozone at Terrestrial Surfaces: Non-Stomatal Deposition to Grassland*. PhD Thesis, School of Geosciences, Faculty of Science and Engineering, The University of Edinburgh.

EMEP 2006. Transboundary acidication, eutrophication and ground level ozone in Europe from 1990 to 2004 in support for the review of the Gothenburg Protocol. EMEP Status Report. Norwegian Meteorological Institute.

Elkiey, T. and Ormrod, D. P. 1980. Sorption of ozone and sulfur dioxide by petunia leaves. *J. Environ. Qual.* **9**, 93–95.

Elkiey, T., Ormrod, D. P. and Pelletier, R. L. 1979. Stomatal and leaf surface features as related to the ozone sensitivity of petunia cultivars. *J. Am. Hort. Soc.* **104**, 510–514.

Fowler, D., Flechard, C., Cape, J. N., Storeton-West, R. L. and Coyle, M. 2001. Measurements of ozone deposition to vegetation quantifying the flux, the stomatal and non-stomatal components. *Water Air Soil Pollut.* **130**, 63–74.

Fruekilde, P., Hjorth, J., Jense, N. R., Kotzias, D. and Larse, B. 1998. Ozonolysis at vegetation surfaces: a source of acetone, 4-oxopentanal, 6-methyl-5-hepten-2-one, and geranyl acetone in the troposphere. *Atmos. Environ.* **32**, 1893–1902.

Gerosa, G., Marzuoli, R., Cieslik, S. and Ballarin-Denti, A. 2004. Stomatal ozone fluxes over a barley field in Italy. "Effective exposure" as a possible link between exposure- and flux-based approaches. *Atmos. Environ.* **38**, 2421–2432.

Hari, P. and Kulmala, M. 2005. Station for measuring ecosystem–atmosphere relations (SMEAR II). *Boreal Environ. Res.* **10**, 315–322.

Hari, P., Mäkela, A., Korpilahti, E. and Holtan, H. L. 1986. Optimal control of gas exchange. *Tree Physiol.* **2**, 169–175.

Hari, P., Keronen, P., Bäck, J., Altimir, N., Linkosalo, T. and co-authors. 1999. An improvement of the method for calibrating measurements of photosynthetic CO_2 flux. *Plant, Cell Environ.* **22**, 1297–1301.

Heath, R. L. 1996. The modification of photosynthetic capacity induced by ozone exposure. In: *Photosynthesis and the Environment* (ed. Baker, N. R.). Kluwer Academic Publishers, Dordrecht, the Netherlands, 409–433.

Hsu, Y.-C., Chen, T.-Y., Chen, J.-H. and Ay, C.-W. 2002. Ozone transfer into water in a gas-inducing reactor. *Ind. Eng. Chem. Res* **41**, 120–127.

Jetter, R., Riederer, M. and Lendzian, K. J. 1996. The effects of dry O_3, SO_2 and NO_2 on reconstituted epicuticular wax tubules. *New Phytol.* **133**, 207–216.

Jones, H. G. 1992. *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*. Cambridge University Press, Cambridge.

Kangasjärvi, J., Jaspers, P. and Kollist, H. 2005. Signalling and cell death in ozone-exposed plants. *Plant Cell Environ.* **28**, 1021–1036.

Kerstiens, G. and Lendzian, K. J. 1989. Interaction between ozone and plant cuticles. I. Ozone deposition and permeability. *New Phytol.* **112**, 13–19.

Kerstiens, G., Federholzner, R. and Lendzian, K. J. 1992. Dry deposition and cuticular uptake of pollutant gases. *Agric., Ecosyst. Environ.* **42**, 239–253.

Kurpius, M. R. and Goldstein, A. H. 2003. Gas-phase chemistry dominates O_3 loss to a forest, implying a sourse of aerosols and hydroxyl radicals to the atmosphere. *Geophys. Res. Lett.* **30**, 24-1–24-4.

Laisk, A., Kull, O. and Moldau, H. 1989. Ozone concentration in leaf intercelular air space is close to zero. *Plant Physiol.* **90**, 1163–1167.

Langebartels, C., Kerner, K., Leonardi, S., Schrauder, M., Trost, M. and co-authors. 1991. Biochemical plant responses to ozone. I. Differential induction of polyamine and ethylene biosynthesis in tobacco. *Plant Physiol.* **95**, 882–889.

Laurila, T. and Lättilä, H. 1994. Surface ozone exposure measured in Finland. *Atmos. Environ.* **28**, 103–114.

Lendzian, K. J. and Kerstiens, G. 1991. Sorption and transport of gases and vapors in plant cuticles. *Rev. Environ. Contamin. Toxicol.* **121**, 65–128.

Loreto, F. and Velikova, V. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol.* **127**, 1781–1787.

Luwe, M. W. F., Takahama, U. and Heber, U. 1993. Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia-Oleracea* L.) leaves. *Plant Physiol.* **101**, 969–976.

Massman, W. J. 1998. A review of the molecular diffusivities of H_2O, CO_2, CH_4, CO, O_3, SO_2, NH_3, N_2O, NO and NO_2 in air, O_2 and N_2 near STP. *Atmos. Environ.* **32**, 1111–1127.

Mäkela, A., Berninger, F. and Hari, P. 1996. Optimal control of gas exchange during drough. *Theoretical analysis. Ann. Bot.* **77**, 461–467.

Mäkela, A., Hari, P., Berninger, F., Hänninen, H. and Nikinmaa, E. 2004. Acclimation of photosynthetic capacity in Scots pine to the annual cycle of temperature. *Tree Physiol.* **24**, 369–376.

Mikkelsen, T. N., Ro-Poulsen, H., Pilegaard, K., Hovmad, M. F., Jensen, N. O. and co-authors. 2000. Ozone uptake by an evergreen forest canopy: temporal variation and possible mechanisms. *Environ. Pollut.* **109**, 423–429.

Moldau, H. and Bichele, I. 2002. Plasmalemma protection by the apoplast as assessed from above-zero ozone concentrations in leaf intercellular air spaces. *Planta* **214**, 484–487.

Musselman, R. C. and Minnick, T. J. 2000. Nocturnal stomatal conductance and ambient air quality standards for ozone. *Atmos. Environ.* **34**, 719–733.

Percy, K., Cape, J. N., Jagels, R. and Simpson, C. J. 1994. Air pollutants and the leaf cuticle. *NATO ASI Series Series G: Ecological Sciences*. Springer-Verlag, Berlin.

Plöchl, M., Lyons, T., Ollerenshaw, J. and Barnes, J. 2000. Simulating ozone detoxification in the leaf apoplast through the direct reaction with ascorbate. *Planta* **210**, 454–467.

Ranieri, A., Castagna, A., Padu, E., Moldau, H. and co-authors. 1999. The decay of O_3 through direct reaction with cell wall ascorbate is

not sufficient to explain the different degrees of O_3-sensitivity in two poplar clones. *J. Plant Physiol.* **154**, 250–255.

Rondón, A., Johansson, C. and Granat, L. 1993. Dry deposition of nitrogen dioxide and ozone to coniferous forest. *J. Geophys. Res.* **98**, 5159–5172.

Sandermann, H. 1996. Ozone and plant health. *Ann. Rev. Phytopathol.* **34**, 347–366.

Schreiber, L. and Schönherr, J. 1993. Determination of foliar uptake of chemicals: influence of leaf surface microflora. *Plant, Cell Environ.* **16**, 743–748.

Sehested, K., Corfitzen, H., Holcman, J., Fischer, C. H. and Hart, E. J. 1991. The primary reaction in the decompositon of ozone in acidic aqueous solutions. *Environ. Sci. Technol.* **25**, 1589–1596.

Seinfield, J. H. and Pandis, S. N. 1998a. Atmospheric chemistry and physics. *From Air Pollution to Climate Change. Chapter 19: Dry Deposition*. John Wiley & Sons, New York. 958–996.

Seinfield, J. H. and Pandis, S. N. 1998b. Atmospheric chemistry and physics. *From Air Pollution to Climate Change. Chapter 6: Chemistry of the Atmospheric Aqueous Phase*. John Wiley & Sons, New York. 337–405.

Shepherd, T. and Griffiths, W. 2006. The effects of stress on plant cuticular waxes. *New Phytol.* **171**, 469–499.

Stroud, C. A., Makar, P. A., Karl, T., Guenther, A., Geron, C. and co-authors. 2005. Role of canopy-scale photochemistry in modifying biogenic-atmosphere exchange of reactive terpene species: results from the CELTIC field study. *J. Geophys. Res.* **110**, doi:10.1029/2005JD005775-

Turcsanyi, E., Lyons, T., Plochl, M. and Barnes, J. 2000. Does ascorbate in the mesophyll cell walls form the first line of defence against ozone? Testing the concept using broad bean (*Vicia faba* L.). *J Exp. Bot.* **51**, 901–910.

Turnipseed, A. A., Huey, L. G., Nemitz, E., Stickel, R., Higgs, J., Tanner, D. J. and co-authors. 2006. Eddy covariance fluxes of peroxyacetyl nitrates (PANs) and NO_y to a coniferous forest. *J. Geophys. Res.* **111**, doi:10.1029/2005JD006631-

Van Hove, L. W. A., Bossen, M. E., San Gabino, B. G. and Sgreva, C. 2001. The ability of apoplastic ascorbate to protect poplar leaves against ambient ozone concentrations: a quantitative approach. *Environ Pollut.* **114**, 371–382.

Velikova, V., Pinelli, P., Pasqualini, S., Reale, L., Ferranti, F. and co-authors. 2005. Isoprene decreases the concentration of nitric oxide in leaves exposed to elevated ozone. *New Phytol.* **166**, 419–426.

Vesala, T., Hämeri, K., Ahonen, T., Kulmala, M., Hari, P., and co-authors. 1995. Experimental and numerical analysis of stomatal absorption of sulphur dioxide and transpiration in pine needles. *Atmos. Environ.* **29**, 825–836.

Westerhoff, P., Aiken, G., Amy, G. and Debroux, J. 1999. Relationship between the structure of natural organic matter and its reactivity towards molecular ozone and hydroxyl radicals. *Water Res.* **33**, 2265–2276.

WGE. 2004. Review and assessment of air pollution effects and their recorded trends. Working Group on Effects, *Convention on Long-range Transboundary Air Pollution*. Natural Environmental Research Council, United Kigdom. xvi+99 pp66. ISBN 1-870393-77-5.

Wiese, C. B. and Pell, E. J. 2003. Oxidative modification of the cell wall in tomato plants exposed to ozone. *Plant Physiol. Biochem.* **41**, 375–382.

Wieser, G. and Tausz, M., 2006. *Proceedings on the workshop "Critical Levels of Ozone: Further Applying and Developing the Flux-based Concept" Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW)*, Vienna, Austria, ISBN 3-901347-65-9.

Zeller, K. F. and Nikolov, N. T. 2000. Quantifying simultaneous fluxes of ozone, carbon dioxide and water vapor above a subalpine forest ecosystem. *Environ. Pollut.* **107**, 1–20.