Dissertationes Forestales 28

The ozone transfer between atmosphere and vegetation. A study on Scots pine in the field

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Academic dissertation

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ABSTRACT

This thesis analysed the controlling processes of the transfer of ozone at the air-plant interface. Improvement in the knowledge of these processes benefits the prediction of both atmospheric removal of O_3 and its impact on vegetation.

This study was based on the measurement and analysis of multi-year field measurements of O_3 flux to Scots pine (*Pinus sylvestris* L.) foliage with a shoot-scale gas-exchange enclosure system. In addition, the analyses made use of simultaneous CO_2 and H_2O exchange, canopy-scale O_3, CO_2 and H_2O exchange, foliage surface wetness, and environmental variables. All data was gathered at the SMEAR measuring station (southern Finland).

Enclosure gas-exchange techniques can be applied to the measure of O_3 gas-exchange in the field with consideration of artefacts arising from O_3 reactivity and low concentration. The O_3 wall-loss was corrected with information from simultaneous and continuous measurements, and was included the mass balance formulation of O_3 concentration inside the chamber.

The flux of ozone to the Scots pine foliage was generated in equal proportions by stomatal and non-stomatal controlled processes. The temporal pattern of total flux was an overlap of the patterns of biological activity and presence of wetness in the environment. The stomatal uptake was estimated from models of stomatal behaviours and the non-stomatal portion of the flux was analysed further. The observed moisture enhancement was related to the presence of liquid films on the foliage surface. Theoretical simulation of the diffusive transfer at one-stoma-scale showed that O_3 scavenging reactions located before or near the stomatal aperture can prevent or diminish the diffusion of ozone towards the intercellular air space of the mesophyll.

The discussion covers the methodological developments of this study, the relevance of the different controlling factors of ozone flux, the partition amongst its component, and the possible mechanisms of non-stomatal uptake.

Keywords: gas-exchange, deposition, shoot-scale chambers, stomatal and non-stomatal, uptake, SMEAR.

ACKNOWLEDGEMENTS

More than ten years ago I was looking for a job and a chance to spend time in Finland when, lucky me, I became involved with the Finnish research group known as APFE. Back in December 1995, I visited for the first time their few-months old SMEARII station (remember climbing that snow-frozen tower –I think I passed the test). I spent the following summer as a young trainee and by the end of 1996 I got entangled in the PhD studies that were going to frame my personal contest for the next decade.

I am thankful for the financing grants received from CIMO during the first year and from the APFE group during the first 5 years. I am even more thankful for the salary received thereafter from various projects within APFE. The final two-year sprint of this thesis was possible with the support from iLEAPS. The Chancellor office is warmly thanked for the ever positive reply to my travelling applications.

Professor Markku Kulmala is credited for providing the first contact with the group. Professor Pertti Hari is acknowledged for admitting me as a post-graduate student at the Department of Forest Ecology under his supervision. Amidst different scales of mutual personal incomprehension, I do have learnt from Pepe's methodological teachings –wish he benefited from our interaction too. Professor Timo Vesala and Docent Jaana Bäck have supervised the process and the thesis and managed to infuse motivation when needed. To all of you my thanks for the patience.

I have been lucky to get acquainted with the international scientific community that develops the applied research of ozone flux and effects to vegetation. They have provided a valuable virtual context for this thesis. I want to thank in particular Juha-Pekka Tuovinen for his careful and indispensable comments and Docent Sirkku Manninen for her unofficial support. I am very grateful to Professors Neil Cape and Satu Huttunen, who improved this thesis with their careful look as pre-examiners.

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I dedicate this thesis to my parents Josep i Angelina, my sister Teresa and her family, who have been quietly wondering what on Earth is this I am doing in Finland that has taken all that time away from them.

Timo has struggled by my side with this thesis and is surely happier than me now that it is over. Cheers to that, mate.

Karjaa, 11 September 2006, Núria Altimir Escale.

LIST OF ORIGINAL ARTICLES

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I. Altimir, N., Vesala, T., Keronen, P., Kulmala, M., Hari, P. 2002. Methodology for direct field measurements of ozone flux to foliage with shoot chambers. Atmospheric Environment, 36:1, 19-29.

II. Altimir, N., Tuovinen, J-P., Vesala, T., Kulmala, M., and Hari, P. 2004. Measurements of ozone removal by Scots pine shoots: calibration of a stomatal uptake model including the non-stomatal component. Atmospheric Environment, 38:15, 2387-2398, Special Issue on New methods of risk assessment for ozone impacts on vegetation.

III. Altimir, N., Kolari, P., Tuovinen, J.-P., Vesala, T., Bäck, J., Suni, T., Kulmala, M., Hari, P. 2006. Foliage surface ozone deposition: a role for surface moisture?, Biogeosciences, 3, 1-20.

IV. Altimir, N., Vesala, T., Aalto, T, Bäck, J., Hari, P. Competition between ozone sinks at the air-leaf interface. Manuscript.

Author's contributions. Nuria Altimir is responsible for the summary part of this thesis and the principal author of its composing articles. Nuria Altimir led the planning and writing of all articles. She was responsible for all data generation and analysis, except in Paper I Petri Keronen calculated detection limits, in Paper III Pasi Kolari calculated the seasonal parameters for the optimal model and the canopy scaling. Nuria Altimir undertook the production of shoot-scale gas-exchange and leaf surface wetness data, other data being provided by other researchers at SMEAR II measuring station.

TABLE OF CONTENTS

SUMMARY OF THE PAPERS

Paper I. This paper presents the chamber-based methodology to measure shoot-scale ozone flux. It includes a detailed presentation and analysis of the measuring system, chamber dynamics and potential bias and noise sources, with special attention to singularities arising from O_3 reactivity and dynamic behaviour of the system components. Data from 5 shoots including 19-month data during 1996 to 1998 was used to analyse and report on the system performance. We present a correction to the chamber wall-loss via simultaneous measurements of blank chambers from which to derive a wall-loss parameter that can be incorporated in the calculation of shoot gas-exchange.

The rest of the papers II-IV in this study use the methodology presented in Paper I.

Paper II. The 5-shoot data set of ozone flux calculated in Paper I is further analysed in this paper. The measurements were compared to the predictions of the scheme currently used to estimate regional-scale ozone deposition in Europe. We critically assessed the formulation and proposed improvements. First, on account of the large data set we used statistical fitting to estimate the parameter values. Second, we showed a fixed-date expression of spring recovery to be incompatible with inter-annual variability and proposed the use of a model for the stage of development. And last, a constant value of non-stomatal deposition proved to be incorrect. We found that this was rather variable and could be expressed as a function of ambient relative humidity, whose empirical formulation we derived from nocturnal measurements. Importantly, this study reports that almost 50% of the ozone removal can not be accounted by estimation of stomatal uptake.

Paper III. This work tests the hypothesis of a surface-moisture modulated sink of ozone to explain the apparent relative humidity dependence of the non-stomatal ozone flux found in Paper II. We used data from 2002-2003 and combined extensive and in-depth analysis at several scales. The work identifies a similar dependence at canopy scale as found previously at the shoot scale. Further, we are able to report that water vapour films form on the foliage surface and the way they relate to the ambient relative humidity. We can establish a correlation between this surface moisture and the shoot-scale non-stomatal ozone deposition via the theoretical expression of adsorption.

Paper IV. This work examines the partition of ozone fluxes at the leaf interface and the potential competition of external and internal reaction sites. We used a theoretical model to simulate the O_3 transfer into a small unit of leaf and estimate the flux partition amongst the several physically, chemically and physiologically differing surfaces. The theoretical scenarios were compared with experimental values obtained from shoot-scale measurements of O_3 flux. Results show that in most instances there is a significant proportion of the total flux to the foliage that is generated by the outer surfaces. The proportion is larger for smaller stomatal apertures and for comparably high absorption rates of the outer versus inner surfaces. Provided the absorption rate of the inner surfaces remains very high, the gradient towards the stomatal pore is not altered, and the existence of removal at the outer surfaces does not usually preclude the stomatal uptake. At background concentration, access of O_3 molecules to the stomatal cavity could be prevented by a high removal located in the way of stomatal uptake.

1. BACKGROUND

1.1. Motivation

Ozone (O_3) is a reactive gas present in the troposphere in the range of parts per billion (ppb), i.e. molecules of O_3 in 10^9 molecules of air. Its strong oxidative capacity makes it a key element in tropospheric chemistry and a threat to the integrity of materials, including living organisms. Knowledge and control of O_3 levels are an issue in relation to indoor air quality, building material endurance, respiratory human disorders, and plant performance. Ozone is also a greenhouse gas and its abundance is relevant to global warming.

The interaction of the lower troposphere with vegetated landscapes results in O_3 being removed from the atmosphere by reactions that lead to the oxidation of plant-related components. Details on the rate and pattern of removal on different landscapes as well as the ultimate mechanisms by which this occurs are not fully resolved. Improvement in the knowledge of these processes will benefit the prediction of both atmospheric removal and its impact on vegetation.

1.2. Tropospheric chemistry and abundance

The tropospheric O_3 concentration is locally variable because it is formed and destroyed at rates that depend on the conditions and the relative abundance of its precursors versus scavengers, which are also variable. Some stratospheric O_3 addition occurs, but in situ photochemistry largely controls the abundance of O_3 (Lelieveld and Dentener 2000). Through chemical reactions O_3 is simultaneously produced and destroyed within a well-known photochemical cycle and a variety of interactions with atmospheric and biospheric components (Fig. 1). Ozone formation is controlled by light, and the relative abundance of nitrogen oxides (NO_x) and volatile organic compounds (VOC). Deposition is the removal of O_3 from the atmosphere as a result of the interaction with the Earth’s surface, including the biosphere. There is a myriad of possible reactions that are part of the deposition process. The O_3 tropospheric chemistry has been reviewed on several occasions (Finlayson-Pitts and Pitts 1997, Stockwell et al. 1997, Prinn 2003, Monks 2005).

A wide range of O_3 concentration, from tenths to hundreds of ppb, exist simultaneously in the troposphere at any given time. The highest concentrations are usually associated with emission of precursors (NO_x, VOC) from urban areas and are often found downwind of these locations. More remote areas can show elevated concentrations during spring and summer. At any certain location O_3 displays a daily and annual concentration pattern that reflects the presence and changes in the precursors and scavengers (Monks 2000, Ribas and Peñuelas 2004). In Finland, the O_3 background concentration annual pattern in remote locations presents a spring maximum that is typical of low NO_x environments (Rummukainen et al. 1994, Laurila 1999). An example of the O_3 levels at the study site of this work is shown in Fig. 2. The daily pattern shows an early morning minimum and early afternoon maximum. The amplitude varies seasonally, thus it is smaller during winter when the production capacity is lower

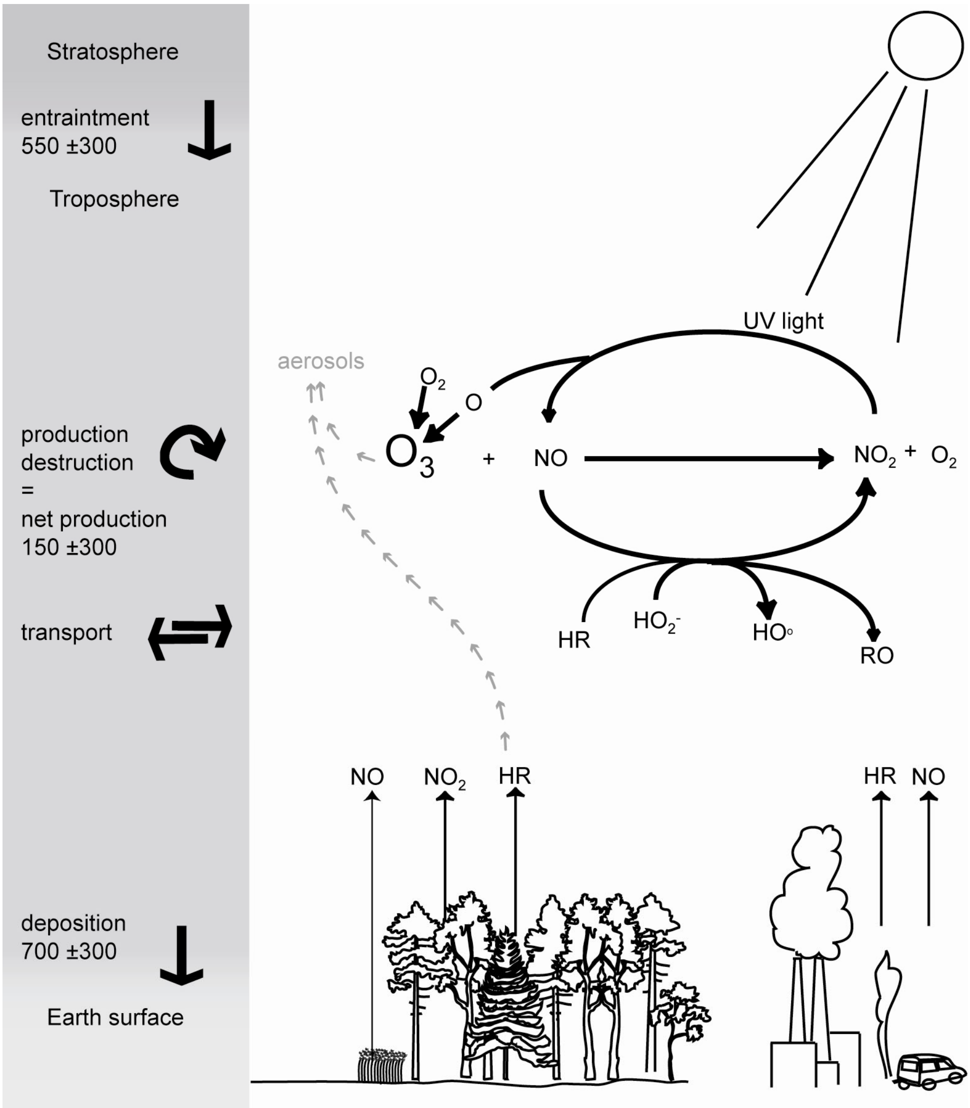


Figure 1. An overview of ozone in the troposphere: chemistry, budget and related processes. *In situ* production ensues from photochemical formation in the presence of NOx and/or hydrocarbons (HR) of natural or anthropogenic origin. The chemical coupling between O_3, NO_x and HO_x cycles includes NO_2 photolysis to NO, and the reaction of NO with O_3 into NO_2. In the presence of HRs, there happens an additional scavenging of NO and the combined effect yields a net production of O_3. In turn, O_3 itself can react directly with HRs, particularly those of biological origin. This reaction contributes to the aerosol formation. In the absence of light, the gas phase chemistry is dominated by reactions scavenging O_3 such as the oxidation of NO to NO_2 and further to NO_3 and continuous oxidation of HRs. The nitrogen oxides emitted from the soil may result in a significant consumption of O_3. Budget values (left column) are in Tg yr^{-1}±, as summary of several model results and their associated uncertainty (reported in Lelieveld and Dentener, 2000).

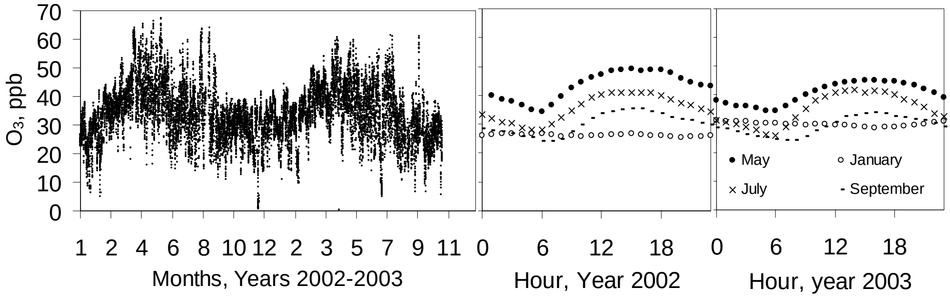


Figure 2. Example of the seasonal and daily pattern of O_3 concentration at a remote location in Southern Finland. Data is from SMEAR station in Hyytiälä for the years 2002 and 2003.

Globally, the average tropospheric O_3 concentration is increasing. The background ozone concentration (the level not attributed to direct anthropogenic influence) has increased since industrialization. The pre-industrial background ozone concentration of 10-15 ppb has at least doubled (e.g. Voltz and Kley 1988). Owing to the local variability of O_3 abundance the long-term trends at individual measuring stations is quite varied (Oltmans et al. 2006). However overall measurements e.g. from the Northern Hemisphere show an increase of 0.5-2% per year, which has raised modern background ozone levels to 20-45 ppb. Although the maximum levels have decreased in the last decade likely due to abatement techniques applied to emissions of precursors (NO_x,VOC), the minimum continues to rise (Vingarzan 2004), a situation that is encountered also in Finland (Laurila et al. 2004, Solberg et al. 2005). In general, the rise is mainly attributed to increased NOx and to less extent methane emissions (Fusco and Logan 2003). These two O_3 precursors and also CO are projected to increase in the future and yield background O_3 levels over 50 ppb (IPCC 2001, Vingarzan 2004). Asia, where local O_3 levels are rising (Huixiang et al. 2005) will likely become a relevant source of tropospheric O_3 precursors and sustain the global increase in the background O_3. Local and regional abatement strategies might be insufficient in view of the global-scale increase in tropospheric ozone precursors (Collins et al. 2000). O_3 concentration and behaviour is sensitive to anthropogenic emissions which means emission control could really affect the future scenario (Stevenson et al. 2006)

The amount of tropospheric ozone relates to processes ranging from local air quality to global warming. O_3 is a relevant gas in tropospheric chemistry. Importantly, it is precursor of the hydroxyl radical (OH), which controls the atmospheric life-time of many gases and thus governs the oxidative properties and self-cleansing mechanisms of the troposphere (Finlayson-Pitts and Pitts 1997, Prinn 2003). Also, ozonolysis of organic volatiles yields low vapour pressure products that contribute to aerosol formation (Bonn and Moortgat 2003, Kulmala et al. 2003). O_3 is a greenhouse gas itself with 25% of the CO_2 forcing (IPCC 2001, Shindell et al. 2006). The many interlinking effects on the biosphere make it an important factor to be considered within the global change, including its effects on plants, species interactions and ecosystem function (Ashmore 2005).

1.3. Effects on vegetation

O_3 is phytotoxic. When stomata are open O_3 can gain access to the interior of leaves. There, the reaction at the cell surface produces a burst of oxidative species. That initial reaction triggers a cascade of events that can manifest in a range of effects from localised visible injury to decreased growth. O_3 represent a stressor to the plant and as such, interferes with plant fitness and performance.

This capacity has been demonstrated over the last decades by many works that linked the presence of O_3 with the appearance of deleterious effects in the plants (reviewed in Unsworth and Ormrod 1982, Darrall 1989, Lefhon 1991, Saxe 1991, Krupa et al. 1995, Fox and Mickler 1997, Sandermann et al. 1997). Air pollution smog was attributed to cause plant damage during the 40's and 50's, particularly in the Los Angeles area (e.g. Haggen-Smit et al. 1952). By mid-1960 it seemed clear that high occurring levels of ambient O_3 affected pine in eastern North America and Ponderosa pine in southern California (reviewed in Fox and Mickler 1997). Investigations were also early to realise that damage was dependent on the uptake and physiology of the plant (e.g. Macdowall et al. 1964) and in particular on the stomatal conductance (Reich 1987).

The evidence of the phytotoxic properties of O_3 is supported by several approaches. Through field observations, the type and extent of damage can be assessed and the correlation with ambient air quality can be established. National-based monitoring programmes guided by the ICP-Forest provide background data in Europe. O_3 specific effects in the field have proved particularly elusive, but diagnosis tools are being continuously developed based on microscopical structures (Günthardt-Goerg et al. 2000, Kivimäenpää 2003), visual injury (Vollenweider et al. 2003, Vollenweider and Günthardt-Goerg 2005), morphological changes and tissue chemistry (Grulke et al. 2003), and biochemical markers (Calzada et al. 2001). The symptoms displayed in the most sensitive species are actually used as bioindicators of the presence of O_3 (Ribas et al. 1998, 2003).

Analysis of the causal relation between O_3 exposure and modifications in plant performance has been approached via experimentation in laboratory, fumigation chambers and open-air fumigation systems (Saxe 1991, Sandermann et al. 1997, Karnosky et al. 2005). Succeeding generations of experiments are ever tending towards more relevant conditions in terms of exposure regimes and environments, as well as plant age or species mixtures. The consequences of O_3 exposure included essentially reduction in photosynthesis and changes in stomatal behaviour. High short exposures produced acute effects as visible chlorotic and necrotic lesions, low and longer exposures produce chronic effects via altered metabolisms, and prolonged low exposures lead to accelerated senescence. Decreased productivity and growth was found to be mediated by reduced carbon assimilation, increased costs of tolerance or repair mechanisms, altered phloem transport (Grantz 2003), changes in carbon allocation (Andersen 2003). Since the sensitivity to O_3 phytotoxicity is species-specific, the consequences in ecosystem competition are also examined. A concern related to the study of tree species is the complication of scaling the impacts researched in young individuals to mature trees (Kolb and Matyssek 2001, Samuelson and Kelly 2001). Furthermore, the response of individual trees is expressed in changes in the ecosystem structure and function, a level that is experimentally approached by large-scale fumigations (Karnosky et al. 2005).

Ozone could also possibly affect the plant through modification of the protective epicuticular surfaces. An altered epicuticular surface is less efficient in all its protective functions. As epicuticular wax structure is lost, needle wettability, permeability, and cuticular water loss increase, with potential consequences for the plant (e.g. Schreuder et al. 2001). For sensitive, annual or deciduous species these effects might be comparatively smaller than the toxicity caused by internal uptake. However, the foliage of evergreen species endures exposure to the ambient conditions for several growing seasons. The rate of erosion of epicuticular waxes of coniferous species relates to the level of air pollution (Turunen and Huttunen 1990, Huttunen 1994) and it can be accelerated by O_3 (Barnes et al. 1988, Bytnerowicz and Turunen 1994). Conifer epi- and cuticular properties and structures are affected e.g. by acid rain (Turunen et al. 1995, 1997), trichloroacetic acid (Sutinen et al. 1995), or nitric acid vapour (Bytnerowicz et al. 1998). Pollutant cause e.g.: disintegration of wax structures such as fusion of wax tubes and stomatal occlusion, increased wettability and ion leaching or modified chemical composition; which can be a consequence of direct reaction with epicuticular components *in situ* or a result of altered wax biosynthesis.

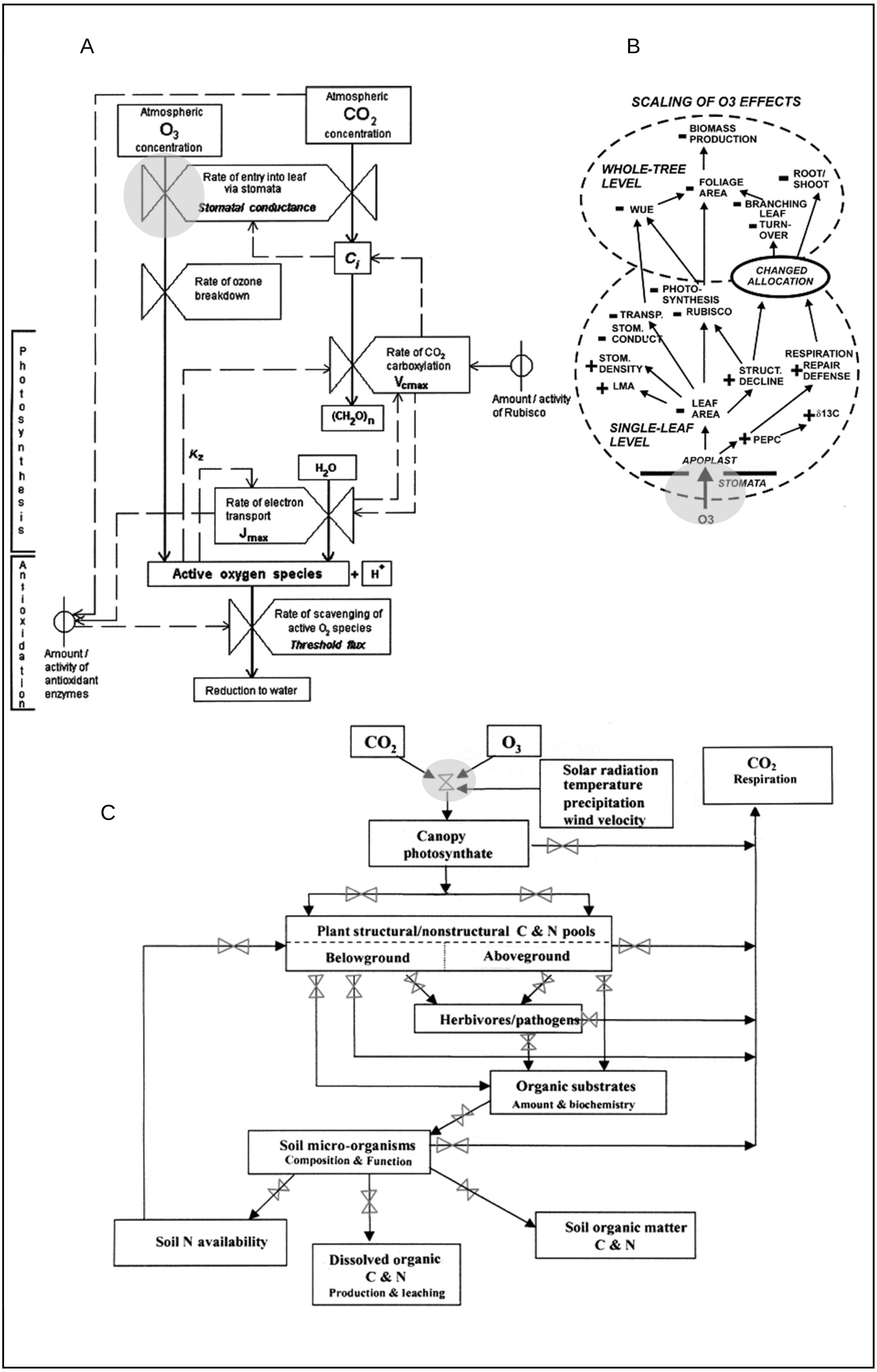
The mode of action of ozone toxicity is synthesised in conceptual models such as the three examples in Fig. 3, which cover the process at different scales. Together, they provide an overview of the chain of potential effects and disturbances and the spread of them throughout the functional scales, from the initial entrance of O_3 through the stomata to the change in ecosystem functioning. Research on the mode of action of O_3 toxicity is to elucidate the relevant controlling processes and influencing factors. The transfer of O_3 from the atmosphere to the interior of the leaves is the first necessary event for related toxicity, damage, or long-term effects to exist. The understanding of this transfer process is needed to quantify the uptake rates. Together with the understanding of the physiological responses, the toxicologically relevant O_3 flux should be also quantified. Research on the mode of action, particularly at a detailed scale, also informs on the location and nature of the initial interaction between O_3 and the biological material. This is in turn valuable information to the prediction of the uptake rates (Section 2).

1.3.1. Initial reactions

The mode of action has been examined with more detailed experiments to clarify the processes involved in the mechanism of the O_3 toxicity. These are usually targeted investigation about the chemistry between O_3 and biological components, or the physiological and molecular basis of the mechanisms and provide details on the initial reactions of O_3 with the plant surface.

O_3 can react and break down many biological molecules, as expected from its chemical nature. This can be easily demonstrated, particularly under *in vitro* experimental conditions where delivery is maximized with high O_3 concentrations and the components of interest

Figure 3 (facing page). Examples of conceptual models of the mode of action of O_3 toxicity in plants. Shaded areas highlight the process of O_3 uptake A) model of photosynthetic response to O_3 exposure for aspen (reprinted from Martin et al. (2001) ©2001, with permission from Elsevier); B) scaling scheme of O_3 effects on plant internal processes in young birch plants (reprinted from Kolb and Matyssek (2001) ©2001, with permission from Elsevier); C) flow of carbon from photosynthesis through forest ecosystem and switches where the authors hypothesise that responses to elevated O_3 and CO_2 can alter the carbon flow through above and below ground food webs (reprinted from Karnosky et al. (2005), with permission from Blackwell Publishing).


A
B
C
Atmospheric O_3 concentration
Atmospheric CO_2 concentration
Rate of entry into leaf via stomata
Stomatal conductance
C_i
Rate of ozone breakdown
Rate of CO_2 carboxylation
V_{cmax}
Amount / activity of Rubisco
$(CH_2O)_n$
K_z
H_2O
Rate of electron transport
J_{max}
Active oxygen species
+ H^+
Rate of scavenging of active O_2 species
Threshold flux
Reduction to water
Amount / activity of antioxidant enzymes
Photosynthesis
Antioxidation
SCALING OF O3 EFFECTS
WHOLE-TREE LEVEL
- BIOMASS PRODUCTION
- FOLIAGE AREA
- ROOT/SHOOT
- WUE
- BRANCHING
LEAF - TURN-OVER
CHANGED ALLOCATION
- PHOTO-SYNTHESIS
- TRANSP.
- RUBISCO
STOM. - CONDUCT
+ STOM. DENSITY
+ LMA
+ STRUCT. DECLINE
RESPIRATION + REPAIR DEFENSE
- LEAF AREA
+ δ13C
+ PEPC
SINGLE-LEAF LEVEL
APOPLAST
STOMATA
O3
CO_2
O_3
Solar radiation temperature precipitation wind velocity
CO_2 Respiration
Canopy photosynthate
Plant structural/nonstructural C & N pools
Belowground
Aboveground
Herbivores/pathogens
Organic substrates
Amount & biochemistry
Soil micro-organisms
Composition & Function
Soil N availability
Dissolved organic C & N
Production & leaching
Soil organic matter C & N

are isolated. It was initially suggested that the mechanism of toxicity was initiated by the direct oxidation of membrane components, protein ozonolysis and lipid peroxidation, which altered membrane permeability (Taylor et al. 1982, Heath 1994). However, in a compartmentalised cell and at realistic concentrations, O_3 molecules might rarely have a chance to permeate unreacted till the membrane. Rather, O_3 is scavenged upon reaction with compounds in the apoplast or gas phase, as the following mechanisms suggest: Upon contact with the apoplast, O_3 readily reacts resulting in production of reactive oxygen species (ROS) such as H_2O_2, OH, O_2^-. This triggers oxidative signalling, a cascade of events that the plant uses to respond to environmental and metabolic fluctuations. Plants use ROS as second messengers in signal transduction cascades in processes associated with plant growth and development; it is one of the major ways in which plants transmit information about changes in the environment. Ozone damage ensues not for the direct action of O_3 but for its effect on the signalling mechanisms. If the O_3 concentration is high enough, an acute response will follow with the same chain of events as the defence response of plant to pathogens. The initial lesion (in the guard cells) spreads as oxidative bursts to the neighbouring cells. The whole sequence of events confers the hypersensitive response (HR) and trigger programmed cell death (PCD), a genetically programmed cell suicide events that eventually lead to the visible lesion. The molecular details of HR and PCD are quite well-known, including the activation of ethylene, salicylic acid (SA) and jasmonic acid (JA) signalling pathways and the consequent induction of defence-gene expression (Kangasjärvi et al. 1994, Vahala 2003, Kangasjärvi et al. 2005). This type of acute response will happen if ROS accumulates in high enough concentration to start the signal transduction and this depends in great part on the relative amount of antioxidants present in the apoplast. This amount varies, which is one of the reasons why the sensitivity to O_3 varies. Ascorbic acid (AA) is an antioxidant present in high concentrations in the apoplast, whose levels are known to correlate with ozone tolerance. In the apoplast, AA scavenges O_3 and the ROS generated by O_3 so that the acute sensitive responses started by the oxidative signalling can be avoided. Concentration too low to induce HR and PCD might however induce premature aging by interfering in the promotion or timing of senescence with induction of related hormones such as SA and ethylene. Accelerated cell senescence is promoted via changes in the redox status of the chloroplast.

Although ascorbate has a prominent role in this protective scavenging (Conklin and Barth 2004), it is neither complete (Luwe et al. 1993, Ranieri et al. 1999, Turcsányi et al. 2000) nor is it the only factor. Other components are also able to scavenge ozone or the reaction products. For example, cell wall components like phenolic compounds are sensitive to oxidation upon O_3 uptake (Wiese and Pell 2003).

The location and incidence of all these reactions and responses can have different consequences for the plant. The stomatal guard cells are first in the pathway of O_3 towards the inner mesophyll. The ROS from O_3 breakdown in the apoplast of guard cells induce stomatal closure and/or inhibition of stomatal opening, eventually decreasing stomatal conductance. H_2O_2 is actually a signalling intermediate towards stomatal closure and, being AA the major H_2O_2 scavenger, the AA redox state controls this guard cell signalling (from Chen and Gallie 2004).

The relevance of the reactions of O_3 with hydrocarbons, particularly biogenic volatile organic compounds (BVOC), in the intercellular gas-phase has been a long discussed but a less known process. It was initially proposed that the observed enhancement of ethylene elicited by O_3 would be contributing to the damage (Mehlhorn and Wellburn 1987). The direct reaction of O_3 and hydrocarbons in the plant could lead to formation of hydroxyhydroperoxides, peroxide and OH radicals, which elicit the oxidative damage (Salter and Hewitt 1992). Some estimations on the relevance of this process concluded that the reaction of O_3 with hydrocarbons in the plant could be responsible for only a very small fraction of the total scavenging and thus was a negligible process (Chamaeides 1989, Taylor et al. 1994). Studies at low concentrations in spruce found no relation (Lindskog and Potter 1995). But it is likely the calculation behind these studies were an underestimation based on preliminary knowledge of the amount, nature, and location of the hydrocarbons. By contrast, recent works demonstrate that some of these hydrocarbons could be acting rather as antioxidants and offer protection through O_3 quenching (Loreto and Velikova 2001, Loreto et al. 2001, 2004, Velikova et al. 2005). Emission of BVOCs would be induced by the presence of O_3 upon reaching a certain flux threshold (Beauchamp et al. 2005). Whether the protective action happens on the membrane, cell wall or intercellular space is not clear yet.

As to the outer surfaces, O_3 can affect the wax composition and structure via changes in the wax biosynthesis (Percy et al. 1994). But it has been questioned whether it could induce morphological changes via direct reactions with the wax components due to their saturated nature as well as lack of evidence (Cape 1994). Jetter et al. (1996) showed that O_3 delivered in high doses did not alter the chemical composition or structure of isolated and recrystallised pure secondary alkanol tubules (the main component of coniferous epicuticula). Thus, the direct reaction of O_3 molecules with this major wax component is unlikely to happen at ambient conditions. However, in natural conditions, the chemical nature of the epicuticular surface is varied, determined by a more complex wax composition and by all other compounds that reside on the surface. Fruekilde et al. (1998) fumigated an extraction of unpurified epicuticula and found products of ozonolysis, thus providing a demonstration of O_3 reactions with plant surface components. *In situ* effects of O_3 to the epicuticular most likely happen through interaction not with the main wax components but with other compounds associated to them (for details see Sec 2.2). The permeability of cuticles to O_3 is very low indeed. Experiments with isolated cuticles show permeances in the range of 0.001mm s^{-1}, many orders of magnitude smaller than the permeances through open stomata (Kerstiens and Lendzian 1989, Lendzian and Kerstiens 1991). One of the corollaries to these observations is that 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. Ozone does not permeate through the cuticle because it reacts with it, thus another important corollary is that the epicuticle and cuticle are a location of reaction sites for O_3 at the leaf-air interface.

1.4. Prevention of damage, risk assessment

For gaseous pollutants with demonstrated phytotoxic capacity such as O_3 there is a social and political need to recommend air quality standards for the prevention of damage to crops, forest and natural vegetation. A common ecotoxicological approach is the study of the relation between presence of toxicant and effect on an organism, with the aim to identify the levels that are likely to produce an undesired effect.

The simplest indicator of damage risk is exposure, that is, the magnitude of the ambient concentrations occurring near the object of protection -in this case vegetation. A basic exposure index would be e.g. the mean of the hourly concentration over a period, or a cumulative sum (SUM0). In order to account for the stronger phytotoxic potential of higher concentrations the ambient concentration can be modified with weighting functions such as sigmoidally weighted exposures (W126) or accumulated exposure over a threshold (AOTx) (for a review see e.g. Lee et al. 1988). However, these indices are not satisfactory because they do not reflect the temporal variation and interaction of the many factors that modify the plant response in the short or long run (Coyle et al. 2003). For example, water availability is decisive for forest physiological activity in dry sites but is not accounted for in an index such as AOT40 (Pannek et al. 2002). In fact, higher ambient concentrations occur when uptake is reduced. Also, the ambient concentration is in practice measured at some distance from the vegetation canopy where the concentration is different (Grünhage et al. 1999, Sofiev and Tuovinen 2001). Furthermore, AOT40 is inadequate to take into account the contribution of lower concentrations, which are common e.g. in northern Europe and are the range of concentrations that will change in the future (Ashmore et al. 2002)

It is considered more appropriate to establish cause-effect relationships based on the amount of O_3 going into the foliage, instead of the amount of O_3 present in the air. A flux-based index would relate directly to the actual amount of ozone reaching the sensitive sites, what has been called the Pollutant Absorbed Dose (e.g. Fowler and Cape 1982, Grünhage and Haenel 1997, Grünhage et al. 1998). In a recent review, Musselman et al. (2006) considered that since detoxification and biological response is not accounted for by the current flux-based indices, the likely overestimation of the effects renders the flux-indices less useful than the exposure-based metrics. Indeed, a more appropriate approach would be to evaluate this absorbed dose against the internal state of the plant as defined by the level of defence mechanisms and thus establish the Effective Pollutant Dose (e.g. Massman et al. 2000, Massman 2004). To such effect, details of the antioxidant capacity in field conditions are being elucidated (Wieser et al. 2002, Haberer et al. 2006); and defence and repair are included in integrative process models (Ollinger and Reich 1997, Martin et al. 2001).

In Europe, the scientific recommendations for planning air quality standards and abatement techniques come from a working group under the auspices of the UN Economic Commission for Europe. The basic approach is determining the critical level (Kärenlampi and Skärby 1996). The last scientific revision of the critical levels recommended the use of flux-based indices within the possibilities of the available knowledge (Karlsson et al. 2003). Several works using experimental data on O_3 effects have shown that damage relates rather to the accumulated absorbed dose than to the exposure-based indices (e.g. Pleijel et al. 2000, Karlsson et al. 2004).

The concentration of ozone close to the leaf, indeed inside the leaf, is a closer measure of toxicity but it needs to be referred to the outer concentration because that is what can be measured. The ozone concentration in the ambient air, laminar layer, and inside the foliage relate to each other non-linearly and through a variety of interactions. The knowledge of the nature and magnitude of these interactions is the first step to relate the measurable ambient levels with the toxicologically relevant O_3 concentration.

2. THE PROCESS: O_3 FLUX AT THE AIR-FOLIAGE INTERFACE

2.1. Flux as detection of removal

Resolving the mechanisms of ozone removal on the foliage is answering the question of where and with what the O_3 reacts. A true experimental test of the location of ozone removal is very difficult without "seeing" where the ozone goes. In principle, it could be possible to detect the reaction products of ozone, or some physiological responses such as peroxidase activity with microscopic and analytical techniques. In practice, this is a complex approach. Other chemical species are made visible with isotopic markers, but O_3 has no easily measurable isotope.

An alternative is to detect the consequences of the removal by the measurement of the flux the removal tends to generate. This is the approach used in this study.

Flux measurement techniques are convenient methods used in plant physiology and micrometeorology e.g. in H_2O and CO_2 or heat flux estimations. The processes expressed by the measured flux, that is, the processes that contribute to generate the flux we measure, will depend on the set-up in use. Ideally, we would like that the measured value would be a direct proxy of the process of interest on the object of interest. In practice, this is not always achievable and the available measured value reflects additional processes and/or objects that also need to be understood in order to interpret the flux.

2.1.1. Related concepts and terminology

Flux is the expression of transport rate per unit area and it is a net expression of all the individual movements. In atmospheric sciences, the flux of a trace gas or particles that results in its removal from the atmosphere into a surface is called deposition –in the absence of precipitation, dry deposition (Seinfield and Pandis 1998). O_3, being reactive, is expected to react and disappear as such upon contact with a surface. Thus, O_3 deposition is often referred simply as ozone removal, implying removal from everywhere (Paper I, Cieslik and Labatut 1997).

The final step of deposition is sometimes called uptake. This word has taken different meaning in different disciplines. In atmospheric sciences any surface can be said to uptake chemical species and the mechanisms for that can be various. Studies focused on vegetation, however, use the word uptake mostly referring to the transfer into the interior of the foliage. This happens through the stomatal pores, thus it is more precise to call it stomatal uptake. External plant surfaces also "uptake" O_3 in the general sense but uptake into the leaf interior does not happen through these surfaces. Because passage to the interior through the cuticle is not possible the cuticle uptake is often neglected in calculations of flux (e.g. Baldocchi et al. 1987).

To emphasize the idea of irreversibility, the surface is pictured as a sink, opposed to the idea of source. The capability of any given surface to remove ozone is called sink strength. Sink strength is the net expression of the combined occurrence of all the removal reactions present on that surface. The strength of removal or sink strength relates to the combined effect of all chemical reactions that together produce the net removal of O_3.

Another related concept and widely used expression is that of conductance and its inverse, resistance. This is a deep-rooted terminology inside both atmospheric sciences and plant physiology and has its origins in the electrical analogue (Gaastra 1959). Conductance is strictly the constant of proportionality between the flux and the concentration gradient. It is a bulk parameter that may aggregate physical, chemical and biological processes affecting the flux. The concept of conductance is akin to the deposition velocity used in atmospheric sciences (section 2.3).

A common division between the various mechanisms of removal at the foliage is the stomatal versus the non-stomatal. The principal purpose in the wording of stomatal and non-stomatal is to reflect the partitioning of the ozone flux between the portion that passes through the stomatal pore (stomatal) and that which does not (non-stomatal). This division is implicitly connected with the interest to know whether the ultimate sites of O_3 removal are in the mesophyll (stomatal) or in some other place (non-stomatal).

This overlapping and complementing terminology (Fig. 4) are different ways of referring to the group of physical and chemical processes that configure the interactions at the air-foliage interface.

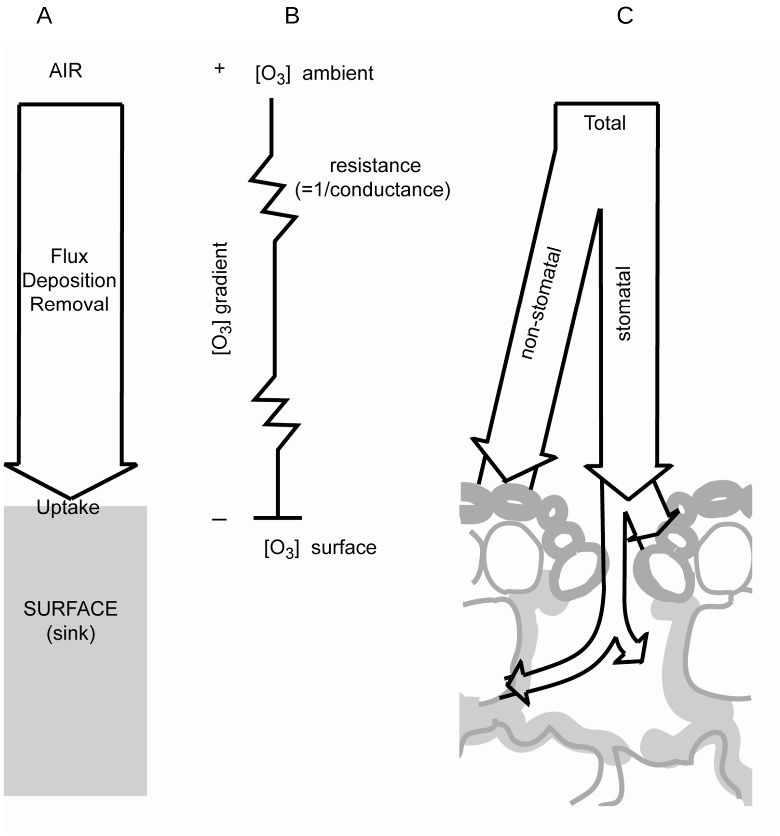


Figure 4. Outline of the terminology and concepts commonly used to refer to the O_3 flux at the air-foliage interface. A) the net direction of the flux is towards the surface, equivalent expressions showed; B) the most common conceptual model is that of the resistance analogue; C) the total flux partitions into stomatal and non-stomatal components, represented in a scheme of a crosscut through one stomatal pore of a Scots pine needle.

Table 1. Overview of the scales approached in this study. Shadowed areas indicate the scales treated in the separate papers.

		I	II	III	IV
km					
m	Region				
cm	Forest			▨	
mm	Shoot	▨	▨	▨	▨
µm	Leaf			▨	▨
nm	Cytological structure				▨
	(Bio)molecules				

2.2. The air-foliage interface

This study is concerned with the exchange of ozone happening at the interface where air and plant foliage meet. The processes and structures that intervene in the flux of ozone at this interface encompass a wide range of scales. This study dealt from the stand scale to the level of the single-stoma depending on the question under scrutiny (Fig. 5, Table 1). Note, however, this study does not aim to develop a thorough scaling between the levels but rather focuses on identifying and analysing relevant processes. This section provides a condensed description of the components and processes at the the air-foliage interface, based on the literature detailed throughout the sections and papers.

To proceed with the flux analysis it is useful to think in terms of imaginary volumes and identify the processes that add or remove from it the compound of interest. Two kinds of basic processes are actually taking place: chemical reactions and transport. O_3 is a reactive molecule that readily oxidises a variety of compounds, whether in the gas-phase or in heterogeneous reactions. On any given surface, when the quality of that surface changes over time, the removal of ozone will be expected to change according to the history of that surface. On the surface of foliage, in addition, major changes in the quality of the surface are related to the plant activity. Transport phenomena act by controlling the access of O_3 to the potential reaction partners/sites. Turbulent transport is important in canopy-scale mixing (Fig. 5a), whereas molecular diffusion controls the transport at smaller scales, e.g. very close to surfaces (Fig. 5d). The micrometeorological conditions govern over other processes through their effect on the canopy ventilation and dissipation of the concentration differences.

Plant activity influences the flux of O_3 through its effect on those two basic processes, reactions and transport. The exchange of O_3 at the air foliage interface contains a clear signature of the biological nature of the foliage. The behaviour of the stomatal pores, the primary surface of gas-exchange for the plants, has a major impact on the behaviour of the O_3 flux to foliage and canopies overall. Stomata take a small proportion of the whole leaf surface, typically in the order of few percents. Open stomata provide access to the expanse interior surface of the leaf and, in the other direction, facilitate release of compounds from the interior outwards.

When open, the pores make accessible the internal surfaces. This inner surface is the apoplast surrounding the stomatal and mesophyll cells, a complex composed by the cellular

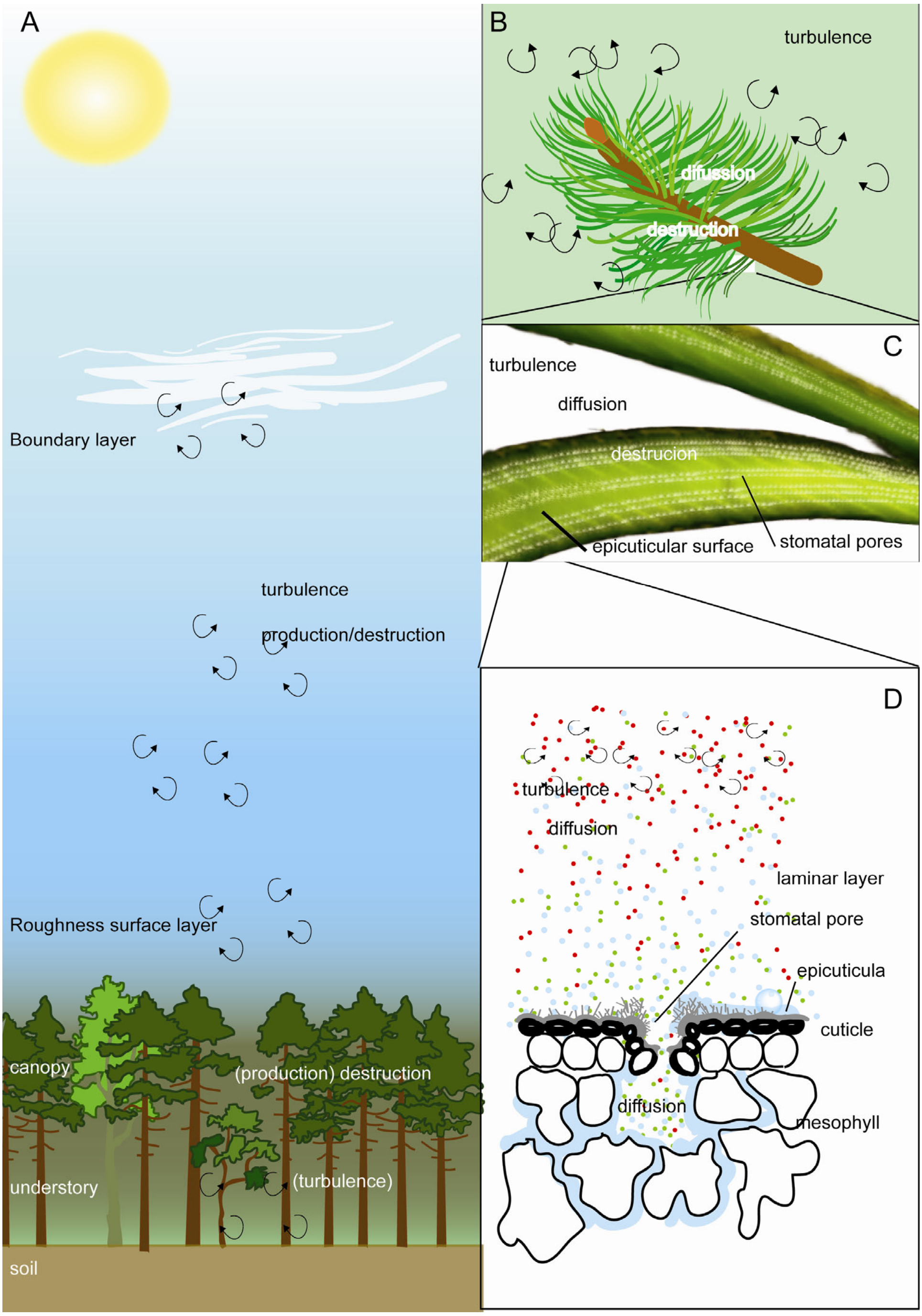


Figure 5. The air-foliage interface at different scales. A) canopy and atmospheric boundary layer; B) single shoot; C) foliage surface; D) cross-cut through a pine needled showing details of the epicuticular surface, stomatal pore and laminar boundary layer. Dots denote gaseous species where red is O_3, blue is water vapour, and green are BVOCs.

walls and a lining of aqueous solution.

The outer foliage surface is covered with epicuticular waxes and a complex multitude of compounds that are trapped or associated amongst its structures.. In addition of the components of the epicuticular waxes, on the leaf surface we can find a mixture of particulates, salts/ions (including Na^+, K^+, NH_4^+, Ca^{2+}, Cl^-, NO_3^-, SO_4^{2-}), ad/absorbed inorganic gases, and condensable vapours (water, BVOC) that either came from the ambient air (gas and aerosol deposition) or were secreted or leached from the interior of the plant. Bacteria and fungi can be found living on this epicuticular complex. O_3 also reacts with this outer surface. The signature of these reactions in the measured flux is not expected to be as predictable as the stomatal signature.

Outer and inner surfaces are surrounded by air whose composition is very much dominated by the gases the plant emits, the viscous boundary layer and the intercellular air space (Fig. 5d). Thus, it is an environment of high relative humidity, potentially high concentration of biogenic organic volatile compounds (BVOC), and a microclimate different from the air surrounding the plant. O_3 reacts with some of these compounds.

Potentially, O_3 can be removed at the air-foliage interface in several ways: via gas-phase and heterogeneous reactions both near and at the outer and inner surfaces of the foliage. Some of these reactions have a biological signature and some not. Some reaction partners or conditions are regulated by the plant and some not.

However, the biological and non-biological signature of the removal are not totally independent. Methodologically, since temperature, light, and VPD (vapour pressure deficit) or RH (relative humidity) affect both components it becomes in practice difficult to separate the effects on the basis of correlation, a difficulty already commented by Mikkelsen et al. (2004). Phenomenologically, the interrelations of all the components at the leaf-air interface are tight and e.g. the evaporation of surface water vapour and the emission of BVOC affect the so-called non-stomatal deposition and can be themselves dependent of the stomatal conductance.

2.3. Existing approaches to the prediction of O_3 flux

There are several theoretical ways to express atmospheric and near-surface movements. However, an overwhelming proportion of the numerical schemes to calculate deposition and uptake of O_3 to vegetation are based on bulk parameters and the formulation of deposition velocity and the electrical resistance analogue model (Gaastra 1959, Seinfield and Pandis 1998). The transport is defined by an array of resistances; each one represents a particular process happening in a particular location along the pathway. Thus, the formalization of the model requires a conceptual compartmentalization of the system and a description of the resistance magnitude. Many versions of the resistance structure are in use, from those that lump a whole landscape in one resistance to those that make a layered representation of the system and consider a variety of deposition pathways. As to the description of the resistance magnitude, either it is described by a function or assigned an experimental value, depending on the available information on the process. The atmospheric resistances related to aerodynamic and boundary layer transport are commonly described by functions based on similarity theory (Monteith and Unsworth 1990, Kaimal and Finnigan 1994). But the surface resistance, which describes the transport at the air-surface interface, is particularly elusive to characterise. For a vegetated landscape, the bulk surface resistance represents actually an aggregation of biological and non-biological signatures from a possibly complex structure. There exist potentially many removal sites and thus many parallel routes of deposition: O_3 can be removed on the upper or lower canopy, the forest floor, the soil; removal at the foliage can be on the outer or inner surfaces. Each of these parallel routes might be controlled by different factors and might thus need its own description.

The analysis of deposition has been linked to the advancement of micrometeorological techniques and theory. Evaluations of the related methods have been reviewed in several occasions and contexts (Businger 1986, Dabberdt et al. 1993, Grünhage et al. 2000, Weseley and Hicks 2000, Erisman et al. 2004).

Many models in use that include prediction of O_3 deposition are part of large-scale applications that dispense with the fine-scale temporal and spatial variations of the controlling processes. Surface conductance is parameterised on the basis of tabulated values for different surface and vegetation types, time of the year, or other conditions. Although it is impractical to include all fine-grain details in a regional-scale model, major known controlling factors should be taken into account. And one of these factors is the particular ability of the surface to uptake the depositing gas (Wesely et al. 1982). An example of this is in Wesely (1989), which has become the classical parameterisation of surface resistance. Wesely divided the surface conductance in several basic pathways and described the resistances considering factors such as solubility and reactivity of the deposited gas, temperature, stomatal behaviour, effects of dew, rain, and snow. This is still the basis for most of the current models that include deposition (e.g. Wang et al. 1998; CHIMERE in Vautard et al. 2005; ALADIN in Lagzi et al. 2004; or EMEP in Simpson et al. 2003).

The development of the surface conductance parameterisation has advanced at different speeds for its different components. The biological signature that is affected by the stomatal aperture was readily recognizable and implemented. Research focused on CO_2 and water vapour exchange made available rather complete multilayered models including stomatal behaviour linked to photosynthesis models, radiative transfer, leaf energy balance, turbulent diffusion, sometimes with additional gas-phase chemistry. Assuming that the main route to deposition was the stomatal diffusion, these models could be adapted to calculate the deposition of O_3 and other gases. But whereas the stomatal pathway seemed to be well-characterised, the other deposition routes were not included (Baldocchi et al. 1998), described with a constant value (Baldocchi et al. 1987, Walton et al. 1997, Padro et al. 1998, Zeller and Nikolov 2000) or described according to Wesely (1989) (Meyers et al. 1998).

Measurements of O_3 flux to several vegetation types and under varied conditions showed that the stomatal uptake could not account for all the measured flux. The unexplained “non-stomatal” portion of the flux was found to be far from negligible. The mechanisms behind this “non-stomatal” flux are still under discussion. It seems to have been quite unmanageable to attribute a process and a descriptive function to the observed values and variation. Contradicting results and multiple apparent influences have rendered the parameterisation of the non-stomatal ozone sink elusive. The lack of understanding of the mechanism driving this “non-stomatal” O_3 deposition to foliage hinders its quantification and leads to its parameterisation, so far, with constant values. Non-stomatal deposition, particularly that involving external plant surfaces, is a major unknown in our present understanding of biosphere-atmosphere gas-exchange (PORG 1998, Wesely and Hicks 2000, Erisman et al. 2004).

Recent works propose parameterisations where the non-stomatal deposition could be derived from meteorological measurements and follow the dynamics of the environmental factors. Zhang et al. (2002) presented a parameterisation for the non-stomatal sink of ozone determined by ambient relative humidity, canopy leaf area index and friction velocity. The work was not mechanistically explicit about the processes and the parameterisation included empirically determined parameters.

To advance in the understanding of pollutant uptake by leaves, it would be useful to have a closer look at the sites and characteristics of the initial reactions in the outer and inner leaf surfaces (Cape 1998). A call to have a close-up look at the processes is still in place. Some works have developed detailed descriptions of the physico-chemical nature of O_3 deposition and removal on the outer surfaces of plants. For example, the O_3 deposition on dew droplets was approached through transport theory by Chameides (1987) and Fuentes (1992). The conceptual models developed require the value of several parameters to be known, e.g. rate of accumulation of dew water on foliage and total amount, average radius of dew drops, or overall rate constant for the undergoing chemical reactions. More recently, Wu et al. (2003) presented a cuticle resistance model that approaches the physico-chemical nature of the cuticles as polymer membranes and describes the absorption of a gas into the cuticle through cuticle/air partition coefficients. It differentiates the process on dry and wet surfaces, proposes a description of the thickness of the water film on the leaf, and the inclusion of pH to take into account the effect of the solution composition. Also, detailed descriptions exist of the likely relevant physical and biological processes involved in the uptake of O_3 into the leaf interior. The resistance analogy is often the framework of reference (Chameides 1989, Runeckles 1991, Kesselmeier et al. 1997). Transport theory applied to 2 or 3 dimensional models has also been used (Claiborn et al. 1993, Plöchl et al. 2000). Whichever the model, the challenge is to recognize and incorporate the relevant physical and chemical processes, including transport in gas and liquid phases, and through surfaces, as well as the location of reaction sites.

This type of close-look approaches, combined with pertinent measurements can hopefully provide tools to the description of O_3 deposition to external surfaces. But the application of these conceptual models to e.g. regional deposition schemes requires information that is not readily available. The qualitative details of the processes might be known but the lack of quantification and characterization of the parameters involved limits the application. There is not enough knowledge for instance –taking the above mentioned example- on what is the overall rate constant for the undergoing chemical reactions in dew droplets.

2.3.1. Surface flux decomposition: basic assumptions

The vast majority of the estimation of O_3 flux to and into the foliage relies in two generally adopted assumptions. First, that the behaviour of O_3 is equivalent to that of other scalars, typically water vapour. Second, that the concentration inside the leaf is zero.

To estimate the stomatal uptake of O_3 the common routine is to transform the water vapour stomatal conductance to O_3 stomatal conductance by scaling between different gas diffusivities. This surrogate method assumes a linear relationship between the conductances for O_3 and water vapour and implies the two gases follow the same diffusive pathway and have the same chemical behaviour. The surrogation is inadequate if these implications do not hold. It has been discussed that possible differences in the diffusive path of O_3 vs. water vapour would bias the surrogation between these gases (Taylor et al. 1982). Also, the reactive nature of the O_3 molecules is different from the comparatively inert water vapour and thus the consequences of the reactivity would be unlikely predicted via surrogation.

Therefore, an estimation of O_3 flux to foliage based solely on surrogation from water vapour stomatal conductance would constrain the information on O_3 flux to the knowledge on water vapour flux and consider the diffusive stomatal uptake as the only accountable sink of O_3 to vegetation. As we know, comparisons with measurements of O_3 flux show that such estimated stomatal uptake does not account for all the measured flux. Therefore, it is generally incorrect to use only surrogation to predict total O_3 flux to vegetation. In any case, surrogation remains the only used method to estimate the contribution of stomatal uptake to the total O_3 flux and deviations from ideal surrogation are reflected in the term that is commonly analysed as non-stomatal contribution.

On account of O_3 reactivity, it is customarily assumed that O_3 concentration at the mesophyll air space is effectively 0, implying O_3 is totally depleted by the reactions with the apoplast. This is almost exclusively based in the results of Laisk et al. (1989). The available experimental evidence about mesophyll concentration of O_3 does not state really where the O_3 goes just that it does not seem to reach very deep into the mesophyll. The concentration of O_3 in the substomatal space could conceivably be different than 0 if the amount of O_3 entering through stomata would overwhelm the available reaction partners, such as in the experiment with high concentration pulses in Moldau and Bichele (2002). What is the situation in nature and ambient conditions is under discussion and likely a subject of upcoming investigations. If the assumption of zero mesophyll concentration would not hold, the present estimations of stomatal ozone flux would be an overestimation.

This study does not analyse these basic assumptions commonly used in the decomposition and analysis of O_3 flux. Thus, by their use it is assumed that the O_3 concentration is depleted to zero in the mesophyll space at about the same location where water vapour reaches saturation.

3. AIMS AND QUESTIONS

The aim of this study was to analyse the controlling processes of the transfer of ozone at the air-plant interface. The study is based on field measurements of ozone flux. The conclusions are to be applicable to the evaluation and improvement of current dose-response relationships for ozone toxicity to vegetation, and of dry deposition calculations of tropospheric ozone to plant canopies.

In particular, this study and its composing papers focus on the following working questions:

- How to obtain direct non-surrogated measurements of ozone flux in the field by the enclosure gas-exchange technique? Paper I
- What are the main controlling factors of ozone flux to foliage? And more specifically, what is the relative contribution of the stomatal versus the non-stomatal sinks to the total flux generated by foliage? Paper II, III, IV
- Is it possible that the reactions of ozone in the air and on the surface prevent its diffusion through the stomata? Paper IV
- What conditions produce maximum uptake? Paper II, III, IV

4. METHODOLOGY

Various measuring and modelling methodologies are utilized in the separate papers (Table 2). This study is largely based on the measurement and analysis of field data of O_3 flux to Scots pine (*Pinus sylvestris* L.) foliage measured at the shoot-scale with a gas-exchange enclosure system. Together with O_3, also shoot-scale CO_2 and water vapour exchange were measured and relevant to Paper I, II and III. Other foremost measurements for Paper III were canopy scale exchange of O_3, CO_2 and water vapour, and the wetness of foliage surface. In addition, a suite of complementary measurements of environmental variables was used in Papers I-III. Also, this work made use of models and algorithms in a variety of ways and intentions. They were mostly algorithms developed outside this study.

Table 2. List of measurements and models used in the separate papers of this study.

measurement	**Instrument/reference**	**I**	**II**	**III**	**IV**
Shoot scale O_3 flux	Chamber, UV absorption analyser (API 400, USA)	X	X	X	X
Shoot scale CO_2 and water vapour flux	Chamber, infrared gas analyser (URAS 4, Hartmann & Braun, Germany)	X	X	X	
Photosynthetic Active Radiation	Flat quantum sensor	X	X	X	
Temperature	Cu-Ko thermocouple	X	X	X	
Pressure	Barometer (DPI260, Druck, UK).	X	X	X	
Soil Water Content	Time domain reflectometers and site specific water retention curves		X		
Relative Humidity	From dew point temperature, chilled mirror sensor (M4 Dew point monitor, General Eastern, USA).		X	X	
Rain	ARG-100 tipping bucket counter (Vector Instruments, UK)		X	X	
Droplet detection	DRD 11-A raindetector (Vaisala, Finland)				
Leaf surface wetness	Clip sensors Burkhardt & Eiden 1994			X	
Canopy scale fluxes	Eddy Covariance Aubinet et al. 2000 UV absorption analyser (TEI 49, Thermo Environmental, USA) for O_3			X	
model	**reference**	**I**	**II**	**III**	**IV**
Linear and non-linear fit	standard	X	X		
Stomatal behaviour, multiplicative	Emberson 2000		X		
Seasonal development	Mäkelä et al. 2004		X		
Stomatal behaviour, optimal	Hari and Mäkelä 2003			X	
EC flux decomposition	e.g. Monteith and Unsworth 1990			X	
Canopy model	Kolari et al. 2006			X	
Adsorption isotherm	Adamson 1960			X	
3D single stomata	Vesala et al. 1995				X

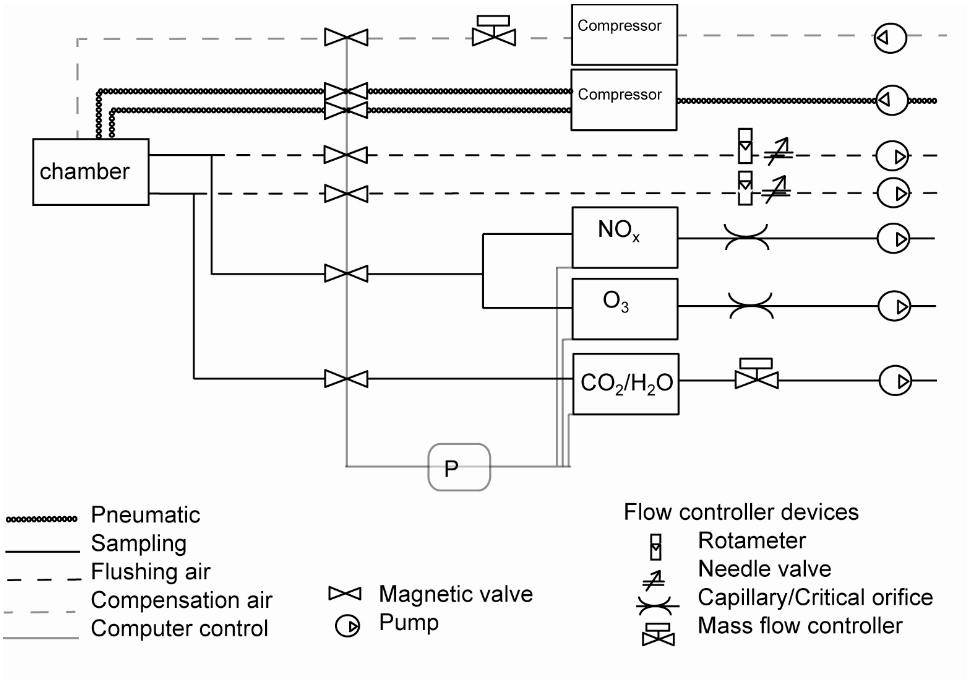


Figure 6. Basic components of the gas-exchange set up, for clarity only one of the 12 existing chambers and sampling lines is shown. A pneumatic system moves the lids that open and close the chambers. During measurement the air is sampled towards the gas analysers; otherwise the air is flushed. The air is re-routed via magnetic valves whose behaviour is controlled automatically from a computer. The gas analysers readings are also automatically stored in the computer.

4.1. Measurements

4.1.1. Site and station

All the data were collected at the SMEAR II station, which is located in a Scots pine stand in Hyytiälä, southern Finland. The most notable characteristics of the measurements are their field conditions, their temporal resolution, and the simultaneity of collection of many variables.

SMEAR II is a highly automated measuring station that has been simultaneously gathering information on a wide array of variables for the study of mass and energy transfer between soil, plants, and the atmosphere since 1995 (Haataja and Vesala 1997, Vesala et al. 1998, Hari and Kulmala 2005). The station design is particularly suited to study the time development and time behaviour of forest-atmosphere interaction. The combination of high time resolution over long time provides data suited to the study of temporal patterns,

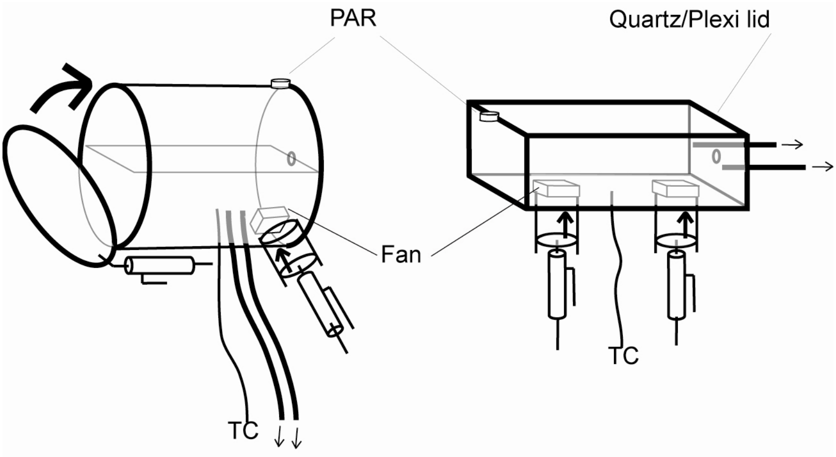


Figure 7. Details of the two chamber designs used in this study: (left) 3-L cylinder (Paper I, II) and (right) 1-L box with exchangeable lid (Paper II-IV). The position of the PAR sensors, Fans, and Thermocouples (TC), sample tubing and pneumatic systems are shown. Bold arrows show the direction of the moving parts, operated by pneumatic cylinders; thin arrows show the direction of air flow inside the tubing. The shoot is clasped at the side small hole of the chamber.

from daily to annual. In particular, the shoot gas-exchange set-up used in this study is focused clearly on the systematic sampling in time rather than in space. We therefore gathered long time series for any one shoot but not from many shoots simultaneously, the amount being limited by technical constraints.

The long trend and simultaneous measurements yields information that is otherwise not captured. These are field measurements and thus data are obtained under many conditions and combination of conditions, as they realistically happen. Such field records can be considered a documentation of the natural events. From this point of view, field measurements are irreplaceable tools to test theoretical models, particularly those that aim to reflect the complexity of natural field conditions. In addition, extensive field data detect events the existence or frequency of which would otherwise go unrecorded. The simultaneity of many variables being measured at the same place allows readiness and depth of analysis also for questions that were not though of in the original design.

4.1.2. Shoot-scale measurements

The gas-exchange of single pine shoots was measured with a multi-line automatic open flow system (Fig. 6). The shoots were place inside transparent and ventilated trap-type chambers that remain open most of the time (Fig. 7). To measure shoot exchange, the chamber closes and the gas flux is calculated from the concentration change inside the chamber during closure:

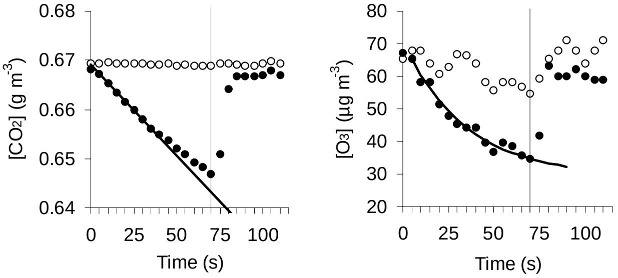


Figure 8. Details of the concentration change of CO_2 and O_3 during one chamber closure. O_3 data is noisier. Empty symbol: blank reference chamber; full symbol: chamber with a shoot. (After Paper I).

$$\frac{V \cdot dC}{dt} = q \cdot (C_a - C(t)) - A \cdot F \tag{1}$$

where the left-hand term is the time derivative of the mass of the studied gas inside the chamber, and the right-hand terms are the gas mass flux produced by the sampling towards the gas analysers and the shoot, respectively. V (m^3) is the internal volume of the enclosure, q ($m^3 s^{-1}$) is the air flow rate through the chamber generated by the gas sampling, C_a (g m^{-3}) is ambient gas concentration, A (m^2) is the shoot all-sided needle area. F is the net flux of CO_2 or water vapour (g m^{-2} s^{-1}), which is obtained by a linear fit to the initial third of the chamber closure.

The calculation of O_3 fluxes is slightly complicated by comparison to CO_2, which is reflected by reference to the concentration change during one chamber closure (Fig. 8). The reactive nature of O_3 results in significant losses onto the chamber material and the lower ambient concentration reduces the signal to noise ratio challenging the measuring system. To take into account these features, we used simultaneous measures of the O_3 flux produced by an empty equivalent chamber. Due to the noise in the concentration measurements, all the measurements during the length of the chamber closure (~1 min) were used in the fit to the mass balance expression, not only the initial third as for CO_2. The O_3 flux during the closure was thus assumed to be concentration dependent. The mass balance equation for O_3 was:

$$\frac{V \cdot dC(t)}{dt} = q \cdot (C_a - C(t)) - V \cdot K \cdot C(t) - A \cdot g_{T,O_3} \cdot C(t) \tag{2}$$

where the second and last term on the left-hand side are the O_3 mass flux produced by the chamber walls and the shoot respectively. K (s^{-1}) is rate constant of O_3 loss to the chamber walls, and $g_{T,O3}$ (m s^{-1}) is total shoot conductance. K was fitted on measurements from the empty chamber (omitting $Ag_{T,O3}C(t)$) and its value was used when fitting $g_{T,O3}$ to measurements with a shoot. The fitted parameters, K and $g_{T,O3}$, are thus not a flux but a proportionality constant to be multiplied by ambient concentration in order to obtain an expression of flux. The “T” in $g_{T,O3}$ stands for “total” referring to the aggregation of several components.

Paper I discusses in detail the dynamics of the system and the explanation for the proposed outlined methodology. The results in Paper II-IV are obtained with the methodology developed in this first paper.

4.1.3. Canopy-scale flux measurements

The gas-exchange of the whole canopy was measured using the micrometeorological technique of Eddy Covariance (EC). The EC method measures the vertical turbulent transport of matter and energy though the imaginary plane at the measurement height. Technically, it is based on high frequency measurements of vertical wind speed and the scalars of interest (e.g. gas concentration), and the calculation of the covariance between them. The details on set-ups and the processing of the data have been developed by other workers and presented elsewhere (Buzorious et al. 1998, Rannik 1998, Keronen et al., 2003).

4.1.4. Leaf surface wetness

The surface wetness of foliage was measured with small clip-type sensors (Burkhardt and Eiden, 1994). A sensor consisted of two electrodes that aligned on both sides of the foliage length-wise so that the only plane where moisture could build up was the foliage surface. The presence of moisture is related to the electrical resistance between the sensor's electrodes. Several of these were clasped onto the surface of 2-3 pine needle-pairs both inside and outside the gas-exchange chambers.

4.2. Models and algorithms

4.2.1. Methodological

The methodology to measure O_3 gas-exchange models the mass-balance inside the measuring chamber. This was developed in Paper I and has been treated in a previous section.

Parameter fitting was done by minimization of squared residuals by standard methods as specified in each paper.

4.2.2. Stomatal behaviour and structure

The model analysed in Paper II follows the scheme outlined in Emberson et al. (2000), which describes the stomatal behaviour with an empirical multiplicative algorithm. A nominal maximum value of stomatal conductance is modified with generic functions that describe the isolated effect of different factors (light, temperature, VPD, SWP, age, etc). The values of the maximum conductance and the parameters for the modifying functions are derived from the relation displayed by the measured values. This description assumes that the various factors do not interact. This approach is largely empirical and thus has limited prediction power outside the measured conditions. However, it was the algorithm of choice to implement in the O_3 deposition module within the EMEP (European Monitoring and Evaluation Programme) chemical transport model.

The stomata-photosynthesis model used in Paper III is based on the optimality of stomatal behaviour initially proposed by Cowan and Farquhar (1977). The basic idea suggests that natural selection should favour plants whose control of gas-exchange tends to maximize the C uptake per unit of water transpired. Thus, we can think in terms of the water use efficiency or the transpirational cost of photosynthesis. The model was developed from a set of assumptions that defined an optimisation problem for stomatal behaviour so it could be solved by the Lagrangian method (Hari et al. 1986, Mäkelä et al. 1996). The model is described in Paper III and in more detail in Hari and Mäkelä 2003. This model has been largely developed with the data provided by the several SMEAR stations and its performance has been extensively reported (Hari et al. 1986, Berninger and Hari 1993; Mäkelä et al. 1996, Hari et al. 1999, 2000, Hari and Mäkelä 2003, Mäkelä et al. 2004). This model is also the base for the upper canopy scaling used in Paper III.

The stomatal diffusion model used in Paper III is identical to the one presented in Vesala et al. (1995) and it was used here without modifications other than the input parameters.

5. RESULTS AND DISCUSSION

5.1. Methodological development

5.1.1. A unique and advantageous system

To our knowledge, this is the most extensive series of direct measurements of ozone flux at the shoot (leaf, branch) scale in the field. By direct we mean that the O_3 analyser is plugged into the gas-exchange system, not only that the ambient O_3 is monitored. Paper I reviewed chamber-based O_3 flux measurements and found only two other field systems (Havraneck and Wieser 1994, Mikkelsen and Ro-Poulusen 1995). The measuring systems described were of a different design (flow-through instead of transient) and used for comparatively shorter periods than the SMEARII chamber systems. Wieser and Emberson (2003) report single growing season measurements but from different locations. Other multiyear series of O_3 flux exist, but they are stand-level fluxes measured by micrometeorological techniques. Amongst them we find 1995-1998 measurements on moorland vegetation at Auchencorth Moss (Fowler et al. 2001), 1996-2000 series in a Danish Norway spruce forest (Mikkelsen et al. 2004), and on grassland in Edinburgh during 2001-2004 (Coyle 2005).

The availability of such an extensive and frequent data set allows the selection of groups of conditions for analysis. This advantage was used in Paper II-IV. In Paper II we could avoid fitting parameters to the multiplicative model of stomatal behaviour via the system of the boundary line technique. Instead, we could choose data sets for which the value of the other parameters was minimised and use standard statistical fitting. We could also analyse separately nocturnal/diurnal or dry/wet conditions. This differentiation was also important in the analysis for Paper III and was in fact one of the major advantages of the analyses (Burkhard 2005). Finally in Paper IV, we could put together a reasonable data set to match the restricted isothermic and steady-state conditions of the theoretical model in use.

Another obvious advantage of a long series is the capture of dynamics in the measured processes, from daily to annual resolution. In fact this is one of the design principles of the SMEAR station. Thus this study was able to report on seasonal trends and changes in controlling factors of ozone fluxes (II, III), and we could detect the dynamic behaviour of

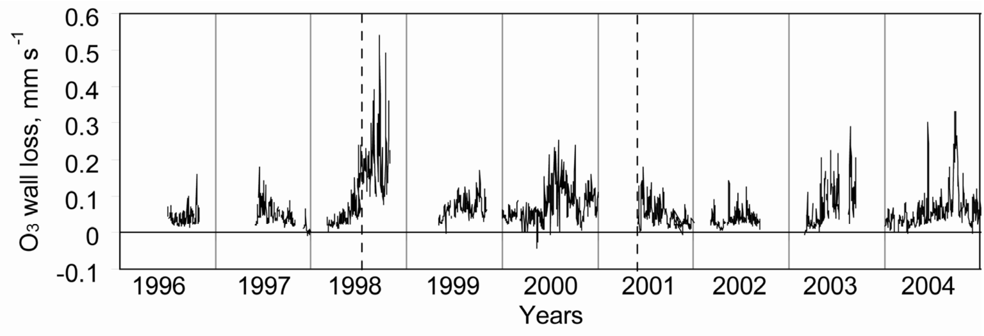


Figure 9. Level of O_3 wall-loss in the gas-exchange chambers during 1996-2004. There is variation between year and between chamber types. The three zones marked with the broken line correspond to the following chamber types and materials of the inner walls: methacrylate cylinder, methacrylate box, and box coated with Teflon. Values are daily averages for one chamber at a time.

the chamber-wall losses (I) and the non-stomatal portion of the flux (II, III).

5.1.2. Wall-loss

The methodology presented in Paper I is basically an admission of the dynamic nature of the chamber wall-loss (Fig. 9) and a proposal to take it into account (Eq. 2). This is in contrast to other blank chamber approaches that treat the wall-loss as a static (semi)constant and base the corrections on sparse before-and-after blank measurements (discussed in Paper I). Some past and recent reported gas-exchange chamber designs claim to archive negligible ozone absorption, typically by minimising the material uptake by use of glass or Teflon (van Hove et al. 1988, Fuentes and Gillespie 1992, Grulke and Paoletti 2005, Tholl et al. 2006). This is not only a merit of the material choice but mostly of the controlled and stable laboratory-like conditions where they were used. There is no perfect material; Teflon coating is known to release volatiles when fresh and the wall of an empty chamber painted in aluminium produced aerosols upon flushing with O_3 (Joutsensaari et al. 2005). However, under controlled conditions, many of these effects disappear with time; if the conditions do not change, the material becomes conditioned or saturated. By contrast, material saturation or a situation of equilibrium is not expected to happen in the field chambers used here, for two reasons: the ambient concentrations are low and the conditions around the system are constantly changing. Thus the necessary admission of the dynamic nature of the chamber wall-loss and the need to take it into account.

Apart from the short minute when the chamber closes for measurement, the rest of the time the chamber remains open and ambient air flushes through its interior. It is assumed that at the moment of closure, the conditions that could govern the wall-loss of any chamber are equivalent e.g. in terms of wetness, aerosol loading, and BVOC or dust content etc. This was more easily achievable if all chambers were let to have the same exposure history, that is, they were installed, cleaned, or had their internal material changed at the same time and of course only same versions of chamber design were used for comparison. The treatment of the blank chamber has been discussed by Raivonen et al. (2003) in the context of NO_x flux measurements. They found difference between individual chambers and thus a source of uncertainty when one of the chambers is used as blank reference to the rest. They proposed a regular comparison between chambers for an intracalibration of the blank levels. A careful treatment of the blank chamber is critical the smaller the ratio between the signals of the shoot and the blank chamber. We found the difference between chambers in case of O_3 deposition to walls to be less systematic and less variable that in case of NO_x emission. As reported in Paper II, the standard deviation between the wall-loss (K in s^{-1}, Eq. 1, Paper I) of several chambers was about 25% of the average. Incidentally, the chamber difference reported in Paper I (Fig. 4) are due not to the intrinsic different between chambers but to the fact that the data-sets for different chambers contained different time periods.

During closure, the proximity of the shoot could influence differently the conditions of the wall versus those of the blank chamber, e.g. by the effect of the condensable vapours emitted by the foliage, resulting in increased humidity caused by transpiration or adsorption of emitted BVOC on the chamber wall. We know that at high ambient RH the chamber wall material adsorbs moisture that is released during chamber closure and consequently disturbs the measurement of transpiration. A VPD dependent correction is applied to minimise the problem (Kolari et al. 2004). However, we do not consider the increase in RH during closure to be sufficient to have a disturbing effect. Recently, Ruuskanen et al. (2005) reported on monoterpene emission measurements using the SMEAR field chambers. They showed the blank chamber to display the capability of slowly emit, at a temperature dependent ratio, monoterpenes that were absorbed in the Teflon chamber walls. Kulmala et al. (1999) present an earlier examination of the influence of the gas-phase chemistry during chamber closure, including monoterpenes. They concluded that some chemical reactions needed to be taken into account in case of NO or terpenes but none was significantly affecting the measure of O_3 deposition during the chamber closure.

For the sake of simplicity, this work has not attempted a detailed description of the chemico-physical processes behind the chamber material behaviour. All influences are gathered in one parameter (K in s^{-1}, Eq. 1, Paper I) which is experimentally determined. We opted for a correction method that reflected the dynamics of the phenomenon and are aware of its uncertainty level. The good agreement between shoot and canopy fluxes suggests that there is not a major problem with the chamber artefacts and their correction.

5.1.3. What this data is and is not

Chamber-based or shoot-scale data can be easily criticised; problems of representativity or modification of the natural environment by the enclosure are commonly invoked to support the supposedly limited nature of these measurements. All measurements have drawbacks; the usefulness of certain measurements depends on their adequacy to provide answers to a particular question.

The set-up of the gas-exchange measuring system is very useful to the analysis of patterns and their dependency on environmental factors but a bit less so to the determination of (representative) absolute values. For example, it can be discussed how much the values obtained in this study can be generalized to all Scots pine shoots or forests. It should be noted that the main results of the study are mostly reported in relative values.

The measured foliage is in slightly different condition than it would be outside the chamber, a situation commonly encountered with enclosure techniques. The air inside the chamber is usually warmer than the air outside (both as measured by thermocouples), and there is some additional warming during the chamber closure. The average temperature

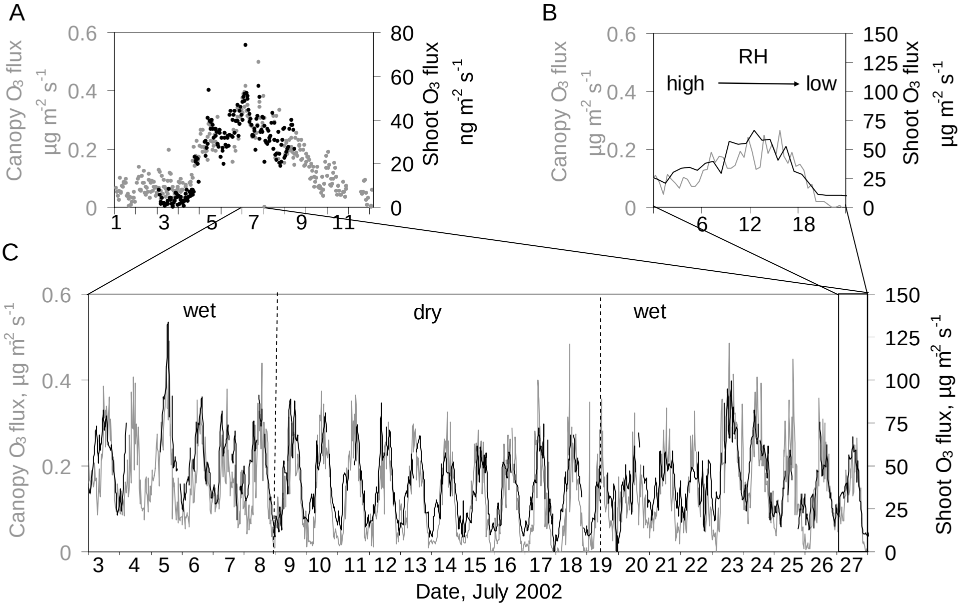


Figure 10. The general pattern of O_3 flux at SMEAR station is an overlap of the patterns of biological activity and moisture. Example time series for A) one year; B) one day; and C) several days. Data in grey is for the canopy scale expressed over m^2 of ground area and in black for the shoot scale expressed over m^2 of total all-sided needle area.

difference for the different chambers ranges from 0.7 to 1.2°C, with absolute maximum differences around 5°C.

Due to the field nature of the measurements, there was a limit to the analysis of the individual effects of the different environmental factors. This is due to the correlation existing between the many environmental factors, which renders the individual effects inseparable. The targeted study of a single factor is studied better under controlled laboratory conditions. This limitation was discussed in particular in Paper III.

5.2 Analysis of the ozone flux

5.2.1. The controlling factors of ozone flux to foliage

The measured O_3 flux has a biological signature that is evident through this study in the shape of seasonal and diurnal patterns that mimic those of the biological activity. That the O_3 flux is affected by the biological activity is particularly clear in spring: the spring recovery is accompanied by active gas-exchange and detectable net CO_2 fluxes that are matched in magnitude and pattern by O_3 deposition. During the growing season, the basic level of O_3 deposition follows the biological activity; the amplitude of the daily patterns matches that of the biologically controlled gas-exchange. The decline in activity during autumn is reflected in a decrease of deposition till the winter levels are reached. During winter there is a background level of deposition that does not display any regular daily pattern. A clear daily pattern appears with the spring recovery of vegetation.

The expression “biological activity” pretends to relate to the general intensity of the plant metabolism. As an indicator of the activity, we have used the measure of CO_2 gas-exchange (Paper I, III) or the daily C uptake (Paper II). The mechanisms by which the biological activity affects the flux of O_3 are possibly multiple. CO_2 gas-exchange relates to the stomatal aperture, the rate of photosynthesis, BVOC emissions, or transpiration, and any of these can have a role in the mechanisms by which biological activity affects the O_3 flux.

Other signatures apart from the biological are also evident. What was most obvious at our site was the enhancement of deposition during moist and wet conditions. We observed this enhancement at both shoot scale (Paper II, III) and canopy scale (Paper III). Thus the pattern of O_3 flux at our site resulted from an overlap between the pattern of biological activity and the presence of moisture. This overlap was evident at many temporal scales (Fig. 10).

The analysis showed that, once the effect of stomatal conductance had been taken into account, the enhancement during wet conditions remained and it related to the ambient RH. An example of the relation of the shoot-scale ozone flux with environmental conditions is depicted in Fig. 11. This figure is an extension of Fig. 8 in Paper III and exemplifies two different points: the apparent difference between total, stomatal and non-stomatal expressions of ozone removal, and the differences between data for dry and wet conditions. The daily patterns of fluxes, conductances and environmental factors for the same data is shown in Fig. 12. In all cases, the transformation of fluxes to conductances seems to reduce the scatter in the data but the relations with the environmental factors are similar–except of course for O_3 concentration. According to the definition in use, the flux reflects the combined effect of the O_3 concentration and the surface sink and the conductance is a reflection of the surface sink only, without the influence of the O_3 abundance. O_3 concentration peaks in early afternoon, whereas stomatal conductance peaks before midday. This results in a symmetrical daily pattern of total and stomatal flux. The difference between dry and wet data is also apparent particularly in the non-stomatal flux and conductance: this seems to correspond with the RH during wet conditions and with temperature during dry (Paper III). Finally, the daily patterns show the coincidence or disagreement of maximum and minimum between the environmental factors and the fluxes and deposition. For example, high RH, low irradiance, and low ozone concentration usually coincide with high non-stomatal conductance, which lead to interpret an enhancement mechanism mediated by RH, instead of temperature (Paper III). However, at the canopy scale humid nights are usually a result of stable conditions, in which the air around the canopy does not circulate very much, the aerodynamic resistance to transport is large. An enhanced deposition during night presents actually a conflict between lack of ventilation and existence of sinks. Stable conditions develop a shallow atmospheric boundary layer that results in accumulation of emitted compounds, or depletion of deposited, inside the canopy while these conditions remain. Whereas some studies concluded that the high aerodynamic resistance rather precludes O_3 removal (Pleijel et al. 1996), others proposed that terpene concentration increases during calm nights and enhances ozone scavenging (Johansson and Janson 1993, Mikkelsen et al. 2000). This happens simultaneously to the accumulation of wetness in the ambient and in surfaces so we might wonder on the relative importance of these potential mechanisms. Resolving this needs analysis beyond the gathering of field measurements. This exemplifies the data limitations commented in section 5.1.3. However,

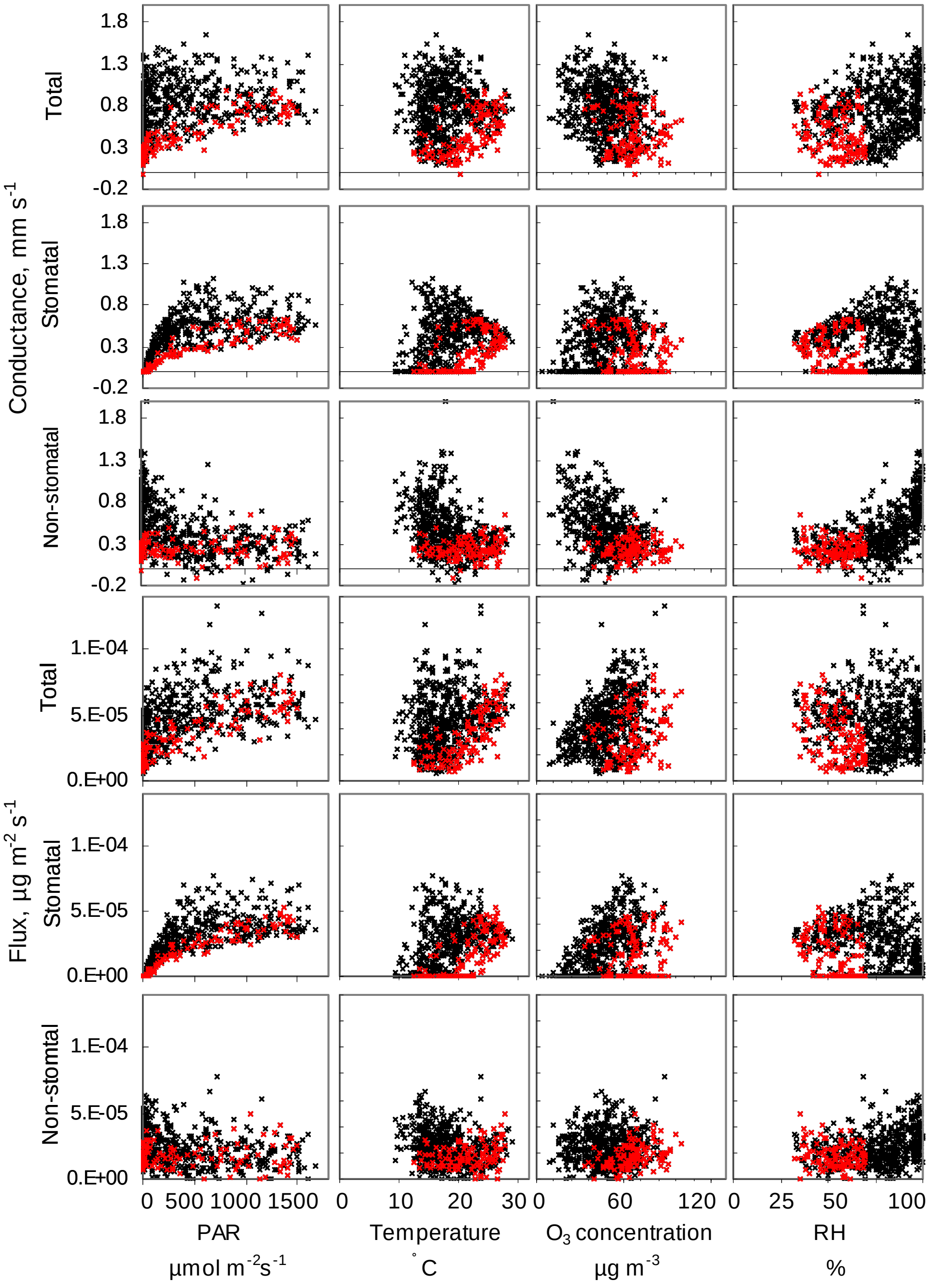


Figure 11. The relation of O_3 flux and conductances with several environmental factors Data is from one chamber during July 2002. Data points in red mark the data during dry conditions, when presence of moisture on the surfaces was assumed null.

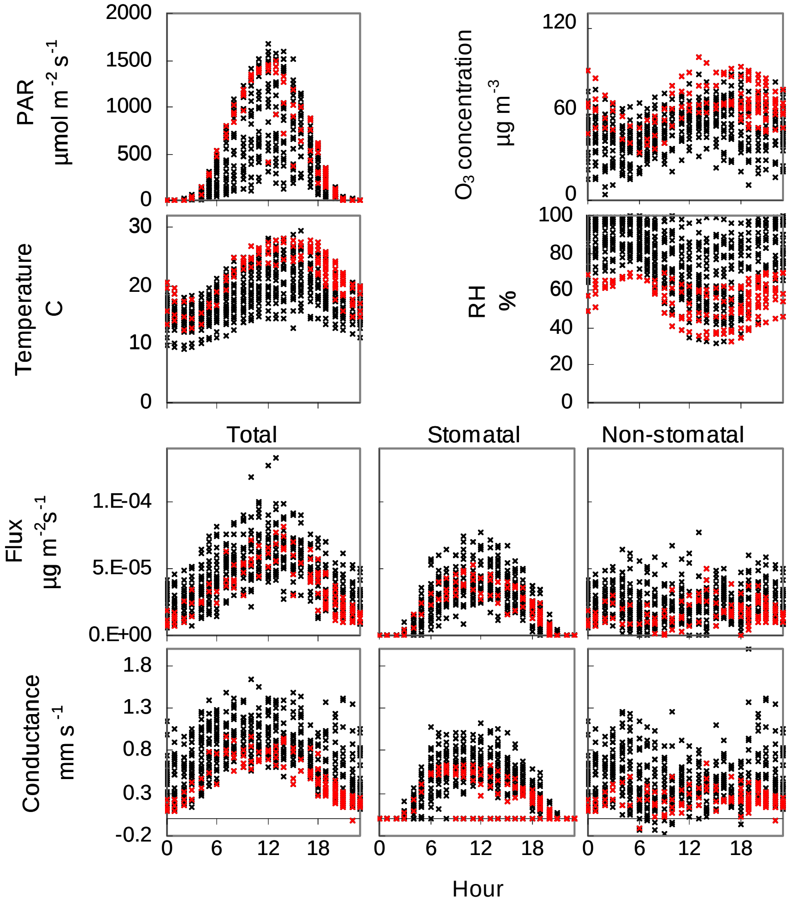


Figure 12. Daily patterns of environmental factors, and O_3 fluxes and conductances. Data as in Fig. 11.

correlations observed in the field can be the only available source of information of what happens in nature.

5.2.2. Partition of the ozone flux

An important part of the ozone deposition on foliage can not be predicted as stomatal uptake alone. Whether calculated as accumulated flux over the growing season (Paper II) or as the average percentage over the total conductance (Paper III), the results of this work show that the stomatal removal accounts for only half of the measured total flux. Thus we can not predict the total flux only from estimations of stomatal uptake; non-stomatal routes of removal might account for the unexplained portion of the flux.

It is clearly shown that ozone removal also happens during night and that these nocturnal levels are often half as big as diurnal levels. This observation, together with the

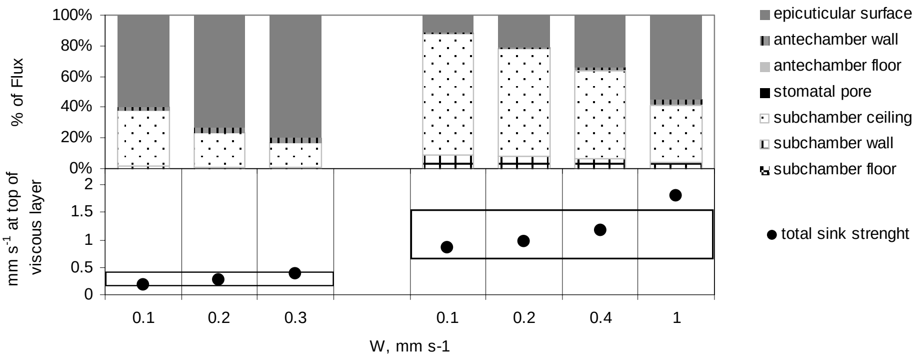


Figure 13. Partition of the flux to different surfaces according to the analysis in Paper IV. In this simulation the inner surfaces are represented as a strong sink and the outer surfaces including the stomatal pore walls have an absorption rate as specified by x-axis. Upper: partition of the flux to the different surfaces. Lower: the total sink strength at the top of the laminar boundary layer, where dots are simulated values and boxes show the range from the experimental data. Two groups of data are represented: on the left the diurnal dry conditions and on the right diurnal humid conditions. Stomatal aperture is 0.5µm in dry conditions and 1.5µm in humid conditions.

assumption of negligible nocturnal stomatal aperture, has been presented as a demonstration that ozone deposition is also mediated by mechanisms different from stomatal uptake (Paper II). The same results are obtained from analyses of diurnal measurements where the stomatal uptake has been estimated and thus extracted from the measured fluxes (Paper III). Amongst different possible candidates, these particular measurements relate the non-stomatal removal with the levels of ambient moisture (Paper II-III).

Other works report contribution of non-stomatal sinks to the total removal at the canopy scale in the order of 50% to 70% as analysed from canopy scale measurements. This has been studied for a variety of ecosystems such as forests of Sitka spruce (Coe et al. 1995), spruce-fir (Zeller & Nikolov 2000), or Ponderosa pine (Kurpius and Goldstein 2003), as well as low vegetation such as moorland (Fowler et al., 2001), barley field (Gerosa et al. 2004), and at several Mediterranean sites (Cieslik 2004). Measurements at the shoot scale have also revealed levels of deposition that exceed the prediction by stomatal uptake such as the measurements on Scots pine (Rondón et al. 1993) or laboratory measurements on poplar (van Hove et al. 1999).

Detailed theoretical examination of the partition of sinks at the air-leaf interface (Paper IV) showed that the proportion of removal at the epicuticular surfaces is larger the smaller the stomatal aperture is and the stronger the sink at the outer surfaces are (Fig. 13). This would mean that at times when the non-stomatal sink is considered to have minimum values (during dry conditions) it could actually contribute more to the total flux than when the absolute value would be larger (in humid conditions). In other words, the absolute value of the non-stomatal sink does not relate to the proportion it represents from the total sink. This result agrees with the proportion of the two types of sinks that results from the analysis of the field measurement: the average ratio remains around 50/50 for both dry and moist cases (Table 1 in Paper III).

5.3. Mechanisms/processes

The mechanism(s) that generate the O_3 removal at the foliage interface can be various and many have been proposed. There are in the literature several suggestions for reaction sites and partners that might remove O_3 at the air foliage interface (Fig. 14). The ultimate basic mechanism is a chemical reaction that transforms the O_3 molecule into something else. Which is the chemical reaction taking place, what is the abundance of potential reaction partners and mediators, and what controls the reaction rate as well as the abundance of partners and mediators are the questions to be answered to detail the removal mechanism(s). This work shows that stomatal behaviour and ambient RH can be combined to predict the level of total O_3 flux to the foliage, and has discussed possible details of the mechanisms. But the existence and relevance of these would need further testing.

Paper III answers the question whether surface moisture plays a role in O_3 flux to foliage. The presence of wetness enhanced the flux not only in the presence of liquid droplets but also during existence of moisture film on the plant surfaces. This was seen to occur both at the shoot and canopy scale. It was an advantage to analyse chamber-based flux and surface wetness measurements because those were related to the processes mediated by the foliage in a more direct way that we could have inferred from the canopy scale measurements. We could show a moisture-modulated surface sink for O_3 but we did not test some of the potential explaining processes and thus did not detail the exact mechanisms. We assumed the different strength of such sink on surface films depends on the chemical composition of the films and estimated the value for a pseudo first order chemical removal. This seemed to be larger than could be explained by simple O_3 decomposition in water (Paper III). We suspect however that the estimated values were at the limit of the reasonable uncertainty. Rather, the chemical details could be better approached by a combination of laboratory experiments and field sampling of the existing components.

Preliminary scrutiny of non-stomatal data is shown in Fig. 15. Fig. 15a compares the seasonal patterns of the calculated non-stomatal sinks and the chemical sink strength after removal of the RH enhancement (as calculated in Paper III, Eq. 5-6). A preliminary look shows different patterns between the years and quite much variability in the data.

One of the likely scavenging mechanisms is the reaction between ozone and emitted BVOCs. The emitted BVOC, particularly the most reactive ones, can produce a significant sink for ozone near and inside the vegetation canopy, according to canopy-scale observations (Mikkelsen et al. 2000, Kurpius and Goldstein 2003, Di Carlo et al. 2004, Goldstein et al. 2004; Mikkelsen et al. 2004, Stroud et al. 2005). Smaller scale studies of the early reactions of BVOCs upon emission propose ozonolysis very close to the emission sites as a potential effective scavenging mechanism of O_3 (Velikova et al. 2005). Since the emission of BVOCs has a marked seasonal pattern (Hakola et al. 2003, Tarvainen et al. 2005, Holtze et al. 2006,) we could expect to detect the scavenging in the seasonal pattern of ozone deposition. For example, a clear enhancement during spring should appear. However, such an effect could not be detected in the pattern of total flux or deposition, neither for shoot nor for canopy (Paper II-III). The spring values agreed very well with the stomatal estimations. The agreement was attributed to the dryness of the environment and

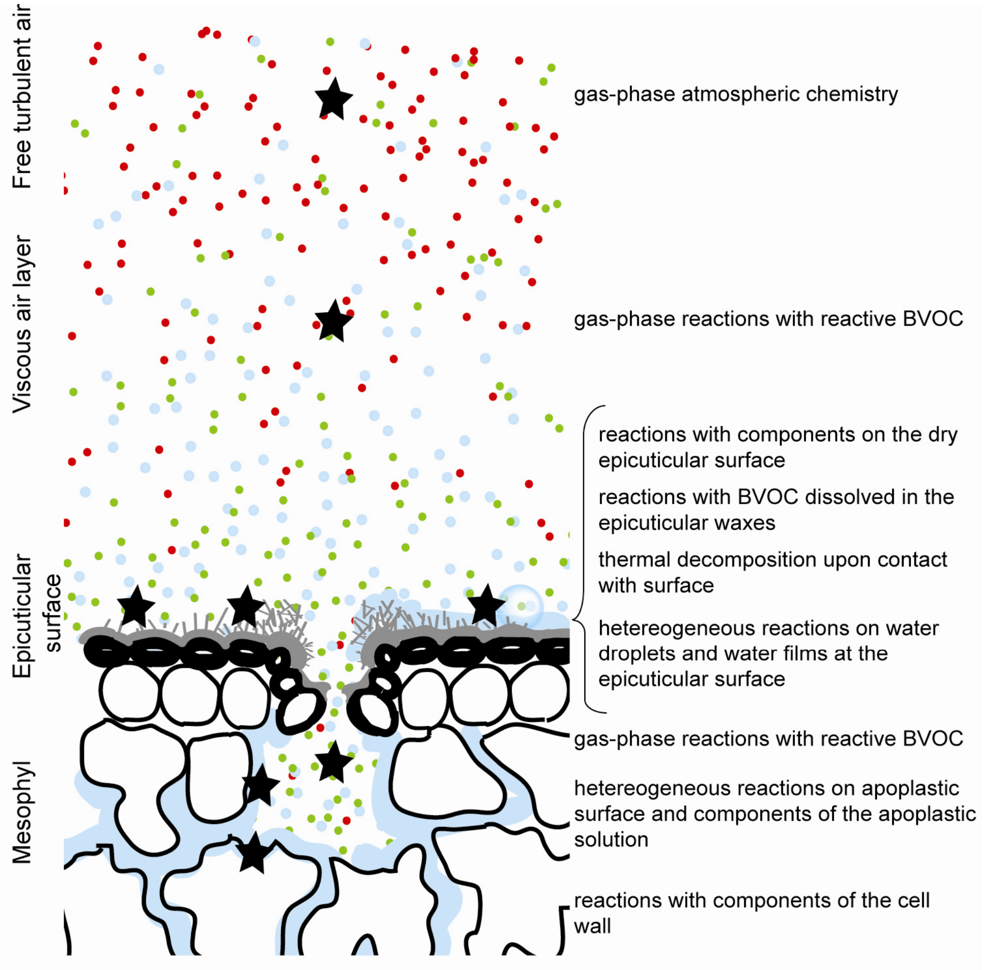


Figure 14. Scheme of the possible location of removal mechanisms at the air-foliage interface, as marked by the stars and detailed on the right hand side. Dots denote gaseous species where red is O_3, blue is water vapour, and green are BVOCs.

thus the lack of surface moisture.

The possible role of BVOC dissolved in the epicuticular waxes could be manifold. They could be a reaction partner for O_3 as well as they could contribute to the formation of surface films (discussed in Paper III).

Our results seem to suggest ozone would be reacting with the foliage surface and the reaction rate would be mediated by the presence of surface wetness. The value that has been interpreted as chemical removal would be related to the compounds present at the surface of the foliage. This composition is likely to change with time. For example, it could depend on the load of aerosol particles deposited on the surface. Aerosol deposition would bring salts and organic acids that would both contribute to the formation of surface moisture and to the composition of the potential solution. The patterns showed in Fig. 15b

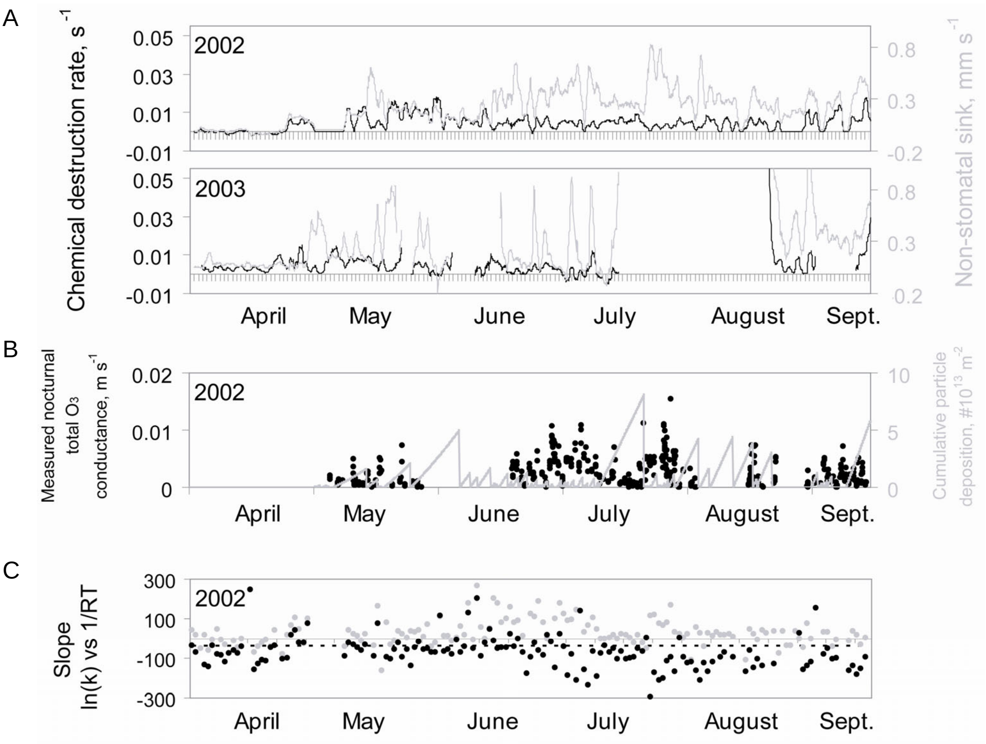


Figure 15. A) The estimated non-stomatal sink and the chemical destruction rate according to Eq 5-6 Paper III, 24 hours running mean of the averaged chamber data for 2002 and 2003; B) An estimation of the accumulated aerosol deposition and the measured nocturnal O_3 deposition. Aerosol load is reset to 0 after a rainfall; C) In black, value of the slope of ln(k) vs. 1/RT, where k is the chemical destruction rate according to Eq 5-6 Paper III. The slope yields –Ea, where Ea is the activation energy of a reaction. The dashed line indicates the value reported in Fowler et al. (2001) as the activation energy of the thermal decomposition reaction of O_3 (~36 kJ mol^{-1}). Data in grey show the values of a slope calculated from the $ln(g_{nsto})$, i.e. from the non-stomatal removal rate that includes also the wetness enhancement.

do not give an obvious answer. The preliminary scrutiny of this data again suggests that the process is not simple. At least it is obvious that estimation of aerosol load would need a more detailed simulation than a simple accumulation that resets at zero upon rainfall.

Nonetheless the results are consistent with a moisture-enhanced removal of O_3 at the surfaces. The idea has been seen counterintuitive by some that considered the low solubility of O_3 to limit and hinder reactions with dissolved compounds. The reply to this is in the reactivity of the O_3 molecule and the renewed presence or reaction partners. The liquid films on the foliage are not pure water so the effective solubility is increased by reactions in the solution, with higher pH favouring the dissolution. Coyle (2005) shows 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. In particular, the presence of NH_3

raises the pH of the solution so that the oxidation of SO_2 is not saturating. Other explanations should be found for solution of low pH, like the films on coniferous needles often are (pH~4). The moisture-enhanced deposition of substances with low Henry law coefficient has been found in other studies (e.g. for PAN in Turnipseed et al. 2006). This points to a lack of understanding of the heterogeneous reactions for oxidants.

Potential reactions behind the non-stomatal sink could also be photochemical reactions mediated by the foliage surface. Rondón et al. (1993) speculated on this possibility from the observation that the non-stomatal portion of the measured flux correlated with radiation. Coyle (2005) has recently proposed photochemistry to play a role both on dry and wet surfaces upon the observation that radiation further enhanced the temperature and RH dependent non-stomatal sink.

Another mechanism would be that destruction of ozone at the surfaces happens by thermal decomposition (Fowler et al. 2001). It means that the O_3 molecules would decompose upon contact with any surface in a rate dependent of temperature. This mechanism would in principle be independent of the characteristics of the surface. This was also considered the basic removal mechanism on dry surfaces in the study of Coyle (2005). The reaction can be examined through calculation of the activation energy (Ea). Fig. 15c shows the values of Ea calculated from the estimation of non-stomatal conductance and the estimation of the chemical destruction rate once the formation of films is taken into account (Paper III). The above-mentioned studies both found a similar value of Ea in their analyses. From our data, the daily value of the estimated Ea falls close to the same value on some occasions, but there are other occasions when the value is clearly different. Again there is a temporal variability that would need further analysis

5.3.1 Competition and relevance of sink location

The toxicological relevant portion of the flux is mostly the stomatal uptake but it does not represent the whole flux generated at the foliage. Being O_3 very reactive, the question arises whether it is at all possible that O_3 can be significantly depleted before it reaches the stomatal aperture or, if passing through the pore, at what depth total depletion occurs. If the O_3 removal rates at inner and outer plant surfaces were of comparable magnitude, it is conceivable that the outer sinks could preclude or diminish the stomatal uptake. Properly located and fast enough reactions could outcompete the diffusive transport and scavenging of O_3 at he inner surfaces.

The scavenging reactions can happen both at the exterior of the foliage or at its inner surfaces. These two reaction sites have different implications for the plant, since the O_3 reacting in the interior of the leaf is the most likely to generate deleterious consequences to plant performance. An interesting implication is that external reactions preventing passage through the stomata could act as defence against O_3 deleterious effects. Would this defence be biologically active, issues of competition for resources between the several physiological functions could be raised (an entirely different context for the word competition).

Paper IV reproduced several situations where the proportion of removal at the outer surfaces could be of equal or larger magnitude than the proportion of removal at the inner surfaces. This happened for example if all surfaces were set with the same characteristics, an expectable result on account of the relatively small area of stomata aperture versus outer surface. Another situation where the proportion of removal outside is larger than inside is the case of small stomatal aperture.

But 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 affect the diffusion through open stomata. There are, however, some possibilities for the outer sinks to represent a real competition to the stomatal flow. 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, but this is in contradiction with the levels of measured ozone flux. There exists the possibility that the very strong sink is 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. 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 oxidised compounds. The existence or relevance of these possibilities remains to be studied further.

5.4 Conditions conductive to maximum uptake

According to our measurements, the highest total flux towards foliage and forest happens when both the plant activity and ambient moisture are high. In light of the analyses, it seems the highest uptake into the interior of the foliage would happen at large stomatal apertures, provided that scavenging reactions located near the stomatal pore are comparably weak or non-existent.

6. CONCLUSIONS

In view of the analysis and results presented in this work I draw the following conclusions, as framed by the initial working questions:

- How to obtain direct measurements of ozone flux in the field by the enclosure gas-exchange technique?

Enclosure gas-exchange techniques such as those commonly used for the measure of CO_2 and water vapour can be applied to the measure of ozone gas-exchange in the field. Through analysis of the system dynamics the occurring disturbances and noise can be identified. In the system used in this study, the possible artefacts arising from the ozone reactivity towards the system materials in combination with low background concentrations need to be taken into account. The main artefact was the loss of ozone towards the chamber walls, which was found to be very variable. The level of wall-loss was obtained from simultaneous and continuous measurements, and was included in the formulation of the mass balance of O_3 concentration inside the chamber.

- What is the relative contribution of the stomatal versus the non-stomatal sinks to the total flux generated by foliage?

The analysis of the field measurements in this study show that the flux of ozone to the Scots pine foliage is generated in about equal proportions by stomatal and non-stomatal controlled processes. Deposition towards foliage and forest is sustained also during night and winter when stomatal gas-exchange is low or absent.

The non-stomatal portion of the flux was analysed further. The pattern of flux in time was found to be an overlap of the patterns of biological activity and presence of wetness in the environment. This was seen to occur both at the shoot and canopy scale. The presence of wetness enhanced the flux not only in the presence of liquid droplets but also during existence of a moisture film on the plant surfaces. The existence of these films and their relation to the ozone sinks was determined by simultaneous measurements of leaf surface wetness and ozone flux. The results seem to suggest ozone would be reacting at the foliage surface and the reaction rate would be mediated by the presence of surface wetness. Alternative mechanisms were discussed, including nocturnal stomatal aperture and emission of reactive volatile compounds.

The prediction of the total flux could thus be based on a combination of a model of stomatal behaviour and a model of water absorption on the foliage surfaces.

The concepts behind the division of stomatal and non-stomatal sinks were reconsidered.

- Is it possible that the reactions in air and on surface prevent the diffusion through the stomata?

This study showed that it is theoretically possible that a sink located before or near the stomatal aperture prevents or diminishes the diffusion of ozone towards the intercellular air space of the mesophyll. This obstacle to stomatal diffusion happens only under certain conditions, which include a very low presence of reaction sites in the mesophyll, an extremely strong sink located on the outer surfaces or stomatal pore.

The relevance, or existence, of this process in natural conditions would need to be assessed further. Potentially strong reactions were considered, including dissolved sulphate, volatile organic compounds, and apoplastic ascorbic acid. Information on the location and the relative abundance of these compounds would be valuable.

- What conditions produce maximum uptake?

The highest total flux towards the foliage and forest happens when both the plant activity and ambient moisture are high.

The highest uptake into the interior of the foliage happens at large stomatal apertures, provided that scavenging reactions located near the stomatal pore are weak or non-existent.

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