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Surface ozone influence on native vegetation: results based on ozone visible symptoms and stomatal flux

Ph.D. Thesis

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I declare that this thesis has been fully worked out by me using the cited literature only and that neither this thesis, nor any of the publications attached within, have been submitted for the purpose of obtaining the title of Ph.D., or any other title, at another institution.

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V Praze,

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TABLE OF CONTENTS

ABSTRACT

Regarding the vegetation, the most affected areas by high levels of surface ozone (O_3) are the mountain ridges. Our study has been carried out in the Jizerske hory Mts. High O_3 levels together with the convenient environmental conditions for stomatal conductance could be a threat for the health of recovering ecosystems in this area. The aims of this study was both to assess the influence of O_3 on vegetation in the Czech mountains and to provide recommendations and outlooks for possible future using of relatively new methods (visible symptoms and stomatal O_3 flux modelling) used for O_3 impact assessment on native vegetation in the field; that means physiologically relevant methods for the determination of O_3 influence.

During 2006 and 2007, O_3-like visible symptoms were assessed on the leaves of seven species at four sites. Symptoms on only two species (*Fagus sylvatica* L. and *Rubus idaeus* L.) have been determined as O_3-induced. To our knowledge, it is the first study in the Czech Republic in which the O_3-like symptoms on native plants have been verified by the Ozone Validation Centre for Central Europe.

Our results based on O_3-induced symptoms indicate that ambient O_3 is likely to have a much lower impact than expected, considering the measured O_3 concentrations (measured with passive samplers) and favourable environmental conditions for O_3 uptake. Conclusions based on visible foliar injury assessment in the Jizerske hory Mts. are not clear. Nevertheless, the small amount of visible injury does not have to mean that vegetation is not influenced. A few reasons for the small amount of visible injury have been determined. Visible symptoms, as the first information including biological significance from the area where no similar assessment has been carried out, are helpful for further research and assessment.

After assessment of results from 2006 and 2007 seasons, the study has been focused on *Fagus sylvatica* L. as a symptomatic and an important tree species for mountainous forests and on stomatal O_3 flux modelling. The study concerning O_3 flux was carried out at six sites. At all these sites in altitude between 460 and 962 m.a.s.l. during the period from June to September in 2008, O_3 concentrations and environmental parameters necessary for the accumulated stomatal O_3 flux (AF_{St}) into *Fagus sylvatica* leaves and AOT40 index calculation. At five out of these six sites, visible injury on *Fagus sylvatica* juvenile trees leaves has been observed. Combination of O_3 levels and environmental conditions, though relative air humidity and air temperature significantly limited stomatal conductance, has been sufficient to cause O_3 uptake exceeding critical level (CL) for forest ecosystems.

The $AF_{st}Y$ value (the accumulated stomatal flux of O_3 above a flux threshold of Y = 1.6 nmol. m^{-2}. s^{-1}) ranged between 5.8 and 14.8 mmol.m^{-2} PLA. The CL of stomatal O_3 uptake (4 mmol .m^{-2}) was exceeded at all sites from cca 45% to 270% (160% on average). The conclusions based on AOT40 and AF_{St} are not the same. The CL for AOT40 (5 ppm.h) exceeded at four out of all sites (94% on average). The dependence of the visible injury amount on O_3 indices was significant. AF_{St} has been determined as better predictor of visible injury than AOT40. Nevertheless, to make the generalized conclusion concerning values of O_3 uptake related to visible injury onset is impossible since there are many factors influencing this values.

ABSTRAKT

Oblastí nejvíce postiženou v důsledku působení přízemního ozonu (O_3) jsou, z hlediska zdravotního stavu vegetace, horské oblasti. Naše studie byla realizována v Jizerských horách. Vysoké úrovně O_3 společně s vyhovujícími podmínkami životního prostředí pro stomatální vodivost mohou představovat hrozbu pro zdraví zdejších obnovujících se ekosystémů. Cíli této studie bylo vyhodnotit vliv O_3 na vegetaci v českém pohoří a uvést doporučení a výhledy pro budoucí použití relativně nových metod hodnocení vlivu O_3 na přirozenou vegetaci (viditelné symptomy a modelování stomatálního toku O_3 do rostliny); tedy metod, které jsou relevantní pro fyziologii rostliny.

Viditelné symptomy pravděpodobně způsobeny O_3 byly hodnoceny na sedmi rostlinných druzích na čtyřech stanovištích, a to během vegetačních sezón 2006 a 2007. Pouze na dvou druzích (*Fagus sylvatica* L. and *Rubus idaeus* L.) byly symptomy určeny jako O_3 skutečně vyvolané. Podle našich znalostí se jedná o první studii v České republice, kdy viditelné symptomy O_3 byly ověřeny Centrem pro validaci symptomů O_3 pro střední Evropu (Ozone Validation Centre for Central Europe).

Naše výsledky založené na poškození rostlinných listů poukázaly na menší vliv O_3, pokud přihlédneme ke koncentracím O_3 (změřeny pasivními dosimetry) a k příznivým podmínkám životního prostředí, které jsou důležité pro tok O_3 do rostliny. Závěry založené na výsledcích týkajících se viditelných symptomů v Jizerských horách nejsou jasné. Několik příčin, proč se symptomy vyvinuly v malém množství, byly určeny. Nicméně tyto výsledky obsahující informaci relevantní pro fyziologii rostlin byly užitečné a nápomocné pro naplánování dalšího výzkumu.

Po vyhodnocení výsledků ze sezón 2006 a 2007 se studie zaměřila na symptomatický *Fagus sylvatica*, tedy na druh důležitý pro ekologickou stabilitu horských lesů, a na modelování stomatálního toku O_3 do rostliny. Studie týkající se toku O_3 byla realizována na šesti stanovištích. Na všech těchto stanovištích v nadmořské výšce mezi 460 a 962 m.n.m. byly během období červen-září 2008 měřeny koncentrace O_3 a parametry životního prostředí důležité pro výpočet akumulovaného stomatálního toku O_3 (AF_{St}) do *Fagus sylvatica* a expozičního indexu AOT40. Na pěti stanovištích byly hodnoceny i viditelné symptomy působení O_3 na listech mladého porostu *Fagus sylvatica*. I přes některá omezení stomatální vodivosti v důsledku relativní vzdušné vlhkosti a teploty vzduchu byly podmínky životního prostředí spolu s koncentracemi O_3 dostatečné k tomu, aby kumulativní tok O_3 do *Fagus sylvatica* přesáhl svoji kritickou úroveň (CL).

Hodnoty akumulovaného stomatálního toku O_3 nad prahovou hodnotu Y = 1.6 nmol. m^{-2}. s^{-1} ($AF_{st}1.6$) se pohybovaly mezi 5.8 a 14.8 mmol.m^{-2} PLA. CL pro $AF_{st}1.6$ (4 mmol O_3 m^{-2}) byly tedy na jednotlivých stanovištích překročeny o cca. 45% až 270 % (v průměru o 160 %). Závěry založené na hodnocení pomocí AF_{st} a AOT40 nejsou stejné. CL pro AOT40 (5 ppm.h) byla překročena na čtyřech stanovištích ze šesti, v průměru o 94 %. Závislost množství viditelných symptomů na expozici O_3 byla prokázána jako statisticky významná. AF_{st} byl vyhodnocen jako lepší prediktor pro vývoj viditelných symptomů než AOT40. Nicméně nelze učinit obecný závěr ohledně hodnoty dávky O_3 v rostlině, která by zapříčinila vznik viditelného poškození. Důvodem je několik faktorů, které tuto hodnotu ovlivňují.

1. INTRODUCTION

Brief surface ozone history

Surface ozone (O_3) has been discovered by the Swiss scientist C. F. Schönbein in Basle in 1839 (Schönbein, 1844). It consists approximately 10% of total O_3 in the atmosphere. Increase in surface O_3 concentrations in last years is a considerable environmental problem. O_3 is considered to be the most relevant air pollutant in present, affecting plants in different ways (Krupa et al., 2000). Current levels of surface O_3 are high enough to cause damage to both forest trees and agricultural crops (Reich, 1987; Musselman et al., 2006). The importance of O_3 phytotoxicity increases with higher production of O_3 precursors. The relative role of O_3 is higher either because of sulphur dioxide (SO_2) reduction (Miller and Parmenter, 1967). At present, surface O_3 in the Czech Republic is monitored at 74 automatized monitoring station managed by the Czech Hydrometeorological Institute (CHMI, 2011).

First damage on plant (the banana fruit) has been showed by Gane (1937). American scientists started to focus on air pollution with O_3 in the area of Los Angeles suffering with the summer smog in 1940s. In this area, first damage on agriculture products was observed in 1944 (Middletown et al., 1950). Questions concerning O_3 chemistry and the influence of other compounds of the smog are discussed (Haagen, 1952). Since 1950s, the huge effort concerning O_3 chemistry, laboratory experiments and plant protection has been made. The amount of papers has been published; studies by the American scientist Ruth Ann Bobrov belong to the most important ones (Bobrov, 1952; Bobrov, 1955a; Bobrov 1955b).

First more detailed reports concerning ozone and its influence on forests ecosystems were published in 1980s (e.g. Guderian, 1985; Krause and Prinzt, 1989). Many studies concerning ozone and forests ecosystems (see references in Papers 1–4) have been published so far and they indicate the importance of this issue.

Ozone and plants: oxidative stress

O_3 is believed to enter plant tissue by diffusion through stomata that are opened during the photosynthetic process; therefore, the hours for which the stomata are opened are, in theory, the period when vegetation is the most sensitive to injury (Salardino and Carroll, 1998). Inside the stomatal cavity, gases migrate by molecular diffusion and reach the palisade mesophyll cells, where damage follows (Gerosa et al., 2003). Stomatal O_3 fluxes decrease during the growing season, following the maturation and the senescence of plants (Gerosa et al., 2003). It has not been demonstrated so far that current O_3 levels would be able to damage cuticula and epidermal cells under it (Long and Naidu, 2002). Also O_3 deposition on plant surface has not been demonstrated dangerous for plant health (Ashmore, 2003).

After O_3 entering through the stomata, it diffuses in the apoplast and rapidly decomposes to hydroxyl radical $HO^{\cdot}$, superoxide anion radical O_2^{-}, hydrogen peroxide and other active oxygen species (AOS). These can be detoxified by radical scavengers in apoplast or they can react with proteins or lipids of the plasma membrane (Pell, 1997; Schraudner et al., 1997). First effect of the negative O_3 influence is the loss of cell membrane permeability, the loss of the ability of chemical compound transport and the loss of ion balance in cells (Ashmore, 2003; Skärby et al., 1998).

The process, when the cell structures are being damaged by O_3 molecules, is called ozonolysis, i.e. reaction of O_3 and organic compounds in apoplast. The double bond between carbon atoms is attacked by O_3 molecule and molozonide is formed. Subsequently, all three oxygen atoms are completely inserted between two carbon atoms forming ozonide. Proteins are one of targets of O_3 attack. Especially trypthophan (the pyrrol ring is opened up); cysteine (oxidation of the sulphydryl group) and methionine (disulphide bond development) are sensitive to the O_3 influence (Long and Naidu, 2002).

Unsaturated lipids are also sensitive to the O_3 attack. A set of reactions, known as lipid peroxidation, involves initial attack by free radicals. Oxidation of lipids proceeds via a chain reaction consisting of three phases: initiation, propagation, and termination. LOOHs (lipid hydroperoxides) are results of lipid peroxidation. LOOHs can influence the membrane fluidity or the function of membrane proteins. The peroxidation of lipids is considered as the most damaging process known to occur in every living organism. Membrane damage is sometimes taken as a single parameter to determine the level of lipid destruction under various stresses (Schraudner et al., 1997; Gill and Tuteja, 2010).

According to Pell et al. (1997) or Long and Naidu (2002), O_3 does not penetrate thy cytosol. Nevertheless, stress can increase the radical generation by the cell itself those can damage thylakoid membrane. AOS can partly penetrate thylakoid membranes and a rapid loss of their function follows (Long and Naidu, 2002). Increase oxidative stress relates to normal aging process as there is a decrease in production of some antioxidants in older leaves. Thus, older tissue might be at risk to O_3 influence if oxidative stress is enhanced at a time when detoxifying capacity of plants is being reduced (Pell et al., 1997).

Decreased concentrations of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) are also related to the O_3 influence (Vollenweider et al., 2003). O_3 increases ethylene production (plant hormone) and ethylene induces decreased expression of genes coding for photosynthetic proteins, which code for transcript for the small subunit of Rubisco (Pell et al., 1997). Since Rubisco is central to leaf longevity, the reduced amount of this protein may contribute to the premature leaf senescence and leaf loss (Pell et al., 1997).

Ozone and plants: antioxidative system and damage

Antioxidative system of plant / detoxifying capacity of plant can reduce the influence of O_3. Especially levels of the ascorbic acid in the cell wall is central for the limitation of O_3 flux (Ashmore, 2003). In order to prevent or reduce the O_3 and / or AOS influence, plants produce antioxidative enzymes (mainly peroxidases and reductases), non-enzymatic antioxidants (ascorbic acid, glutathione, a-tocopherols, and polyamines) and secondary metabolites (carotenoids, flavonoids). Plants also produce stress hormone ethylene, which has a few functions in the plant cell – it is associated with the programmed cell death or with leaf abscission and senescence due to reduction of the Rubisco amount (Long and Naidu, 2002; Gill and Tuteja, 2010; Pell et al., 1997).

If the antioxidative system and the protection against the O_3-induced oxidative system are not sufficient, the plant will be damaged (Ashmore, 2003). O_3 may induce damages at cell, organism or ecosystem levels. At the organism level, O_3 can induce visible injury, especially on assimilation organs, reduce the plant growth, reduce the quality and yields of agriculture crops and generally impairs the plant that becomes more sensitive to the pathogen attacks (Stanners and Bourdeau eds., 1995). Plant damages induced by O_3 can be divided into acute and chronic:

Acute damage developed after exposure to high O_3 concentrations for a few hours or days. According to some authors, it is very rare under ambient O_3 concentration in Europe (Innes et al., 2001). During acute exposure, the cell membrane is damaged and the uncontrolled cell death or the hypersensitive response or changes in cell wall structure may be induced (Vollenweider et al., 2003; Pell et al., 1997) and resulted in foliar visible injury (Ashmore, 2003; Salardino and Carroll, 1998). Some of these changes increase the resistance of the leaf to subsequent O_3 exposure (Matyssek and Sandermann, 2003), other decrease photosynthetic and productive capacity (Pell et al., 1997).

Chronic symptoms develop more slowly, within days or weeks following exposure (Kley et al., 1999; Innes et al., 2001). Chlorosis, pigmentation, premature leaf senescence and early leaf fall are typical. Chronic injury usually appears in response to long-term exposure. This type of damage mostly occurs on sensitive plant species in central and southern Europe (Innes et al., 2001). During chronic exposure, there are often no visible injury, but a biochemical changes, decrease in photosynthetic capacity, accelerated senescence of assimilation organs, reduction in growth and yield can occur (Pell et al., 1997; Ashmore, 2003).

2. PASSIVE SAMPLERS

Passive sampler is a relatively simple device used for gases offtake. At the same time, it is prospered from spontaneous diffusion gas transport. Body of passive sampler has a shape of tube or badge where the distance from its bottom to its entrance is the diffusion path (Molín, 2000).

Air quality monitoring is being carried out using automated continuous monitoring stations; however, these are expensive and mostly limited to urban sites. The need for cheaper and/or extensive monitoring to determine exposure of existing forest health monitoring plots, or to identify pollution exposure gradients, or areas of potential impact, has driven the development of passive sampling systems, which can be used in remote areas and need no power supply. The low cost and flexibility of placement for passive sampling systems also make them attractive alternatives for assessing exposures at locations that are difficult to access, such as within the forest canopy. Passive samplers may also be used to identify areas receiving air pollution events, that were previously unknown, and where additional infrastructure for instrumental monitoring may be required (Cox, 2003).

Types and principle of passive samplers

At the present time a number of passive samplers are commercially available for collecting several air pollutants. In general, there are a few types of samplers according to their shapes (Krupa and Legge, 2000):

- Tube sample is characterized by a long diffusion path and small absorption surface. Longer exposition time is necessary when using tubes.
- Badge sample is characterized by a short diffusion path and large absorption surface. It is necessary to use membranes in order to protect inner diffusion space.
- Cartridge samples are opened from both sides. They have shape of a longer tube.

The principle of passive samplers is based on the diffusion of pollutants when the sampled gas from the ambient air diffuse across the sampler volume (Cox, 2003). Passive collection of a given air pollutant is achieved by chemical absorption or by physical adsorption onto a filter medium. This medium is then examined by non-destructive optical methods, or extracted or desorbed to quantify the pollutant of interest (Krupa and Legge, 2000). Rate of pollutant gas absorption/adsorption for a simple diffusion sampler is controlled by the diffusion path length and the internal cross-sectional area of the sampler (Cox, 2003). The absorption/adsorption rate has to be fast and irreversible in order the concentration of the observed pollutant on the sorbent surface will be equivalent to zero (Molín, 2000). Under this condition, the concentration gradient along the diffusion path develops (Hangartner et al., 1996). The concentrations gradient develops the diffusion flow of observed gas into the sampler (Willems and Hofschreuder, 1991).

The unidirectional flow of a gas in the ambient air is given by the first Fick's Law:

$$F = -D\,\frac{dc}{dz}$$

F: the diffusion flux of gas ($\mu g \cdot s^{-1} \cdot m^{-2}$)

D: the diffusion coefficient of gas_1 in gas_2 ($m^2 \cdot s^{-1}$)

c: the concentration of gas_1 in gas_2 ($\mu g \cdot s^{-1}$)

z: the length of diffusion (m)

Absorbents used today for O_3 sampling e.g. nitrite (Koutrakis et al., 1993), indigo (Grosjean et al., 1995), potassium iodide (Kanno and Yanagisawa, 1992) and 1,2-Di(4-pyridyl)Ethylene (Hauser and Bradley, 1966) are used in badge or in tube samplers.

Advantages and disadvantages of passive samplers

On the positive side, excluding the laboratory analysis costs, passive samplers are inexpensive, easy to use in the sense of their preparation and analyse in laboratory and the manipulation in the field, no production of noise. They do not require electricity to operate. Therefore, they are very attractive for use in regional-scale air quality assessments. Passive samplers allow the quantification of cumulative air pollutant exposures, as total or average pollutant concentrations over a sampling duration (Krupa and Legge, 2000).

The disadvantage of using passive samplers is that only average cumulative concentrations, having a low temporal resolution generally inconsistent with definition of air quality standards and exposure indices, can be obtained (Tuovinen, 2002). The influence of environmental conditions on the passive sampler collection rate and therefore the necessity to use co-located samplers with the continuous method of measuring is considered to be another disadvantage (Bytnerowicz et al., 2008; Paper 1). Environmental conditions as the air humidity or wind velocity can influence the diffusion process (Molín, 2000). Under low wind speed, the boundary layer is higher and the diffusion path is longer. The result is underestimation of monitored gas concentrations. On the other hand, under the high wind speed, the diffusion process in samplers is influenced by high turbulence. The shorter diffusion path results in higher monitored gas concentrations. This is the reason to use membranes, especially in badges, and to calculate resistance (conversion) factors (Cox, 2003; Bytnerowicz et al., 2008). Sanz et al. (2004) or Koutrakis et al. (1993) also showed the importance of right using of protecting shelters against rain and mist. In the case of O_3

monitoring, the influence by other factors (air temperature or interference with other gases) is negligible due to the constant O_3 diffusion flow, lower diffusion flow of other pollutants or lower other pollutants concentrations in relatively clean areas (Koutrakis et al., 1993; Grosjean et al., 1995)

Passive samplers using

Passive samplers have been originally developed for the indoor air quality monitoring. Passive samplers are being used to determine the air quality in: (1) the work place; (2) the indoor living environment; and (3) the ambient, outdoor environment including regional-scale air quality. During some past two decades, scientists start to be more interested in using passive sampling systems for quantifying ambient, gaseous air pollutant concentrations, particularly in remote and wilderness areas (Krupa a Legge, 2000).

Currently, the great interest for passive sampler using in the field is evident and particularly, passive samplers are used for the assessment of the surface O_3 influence on forests (Grosjean and Hirsham, 1992). Passive samplers have become interesting for their possible usage in remote and natural areas for purposes of air pollutants measuring and assessment of their influence on vegetation (Cox, 2003).

To sum up, passive samplers are attractive for purposes of the atmospheric chemistry and the ecological assessment (UNECE, 2000), monitoring in remote areas within the forest canopy, for identifying new areas receiving air pollution events or for O_3 measuring, bioindication and sensitive plant species identification (Blum et al., 1997; Godzik et al., 1997; Cox and Malcom, 1999; Manning et al., 2002; Cox, 2003; Yuska et al., 2003; Sanz et al., 2007; Paper 1; Paper 3; Paper 4).

Currently, there is a great focus on the assessment of the O_3 influence using accumulated stomatal O_3 flux (Paper 4). Passive samplers again seem to be very appropriate device for the cumulated O_3 concentration monitoring following hourly O_3 concentrations modelling (Loibl et al., 1994). Hourly O_3 concentrations are necessary to calculate O_3 flux (Emberson, 2000). Passive samplers have been used for this purpose within a number of studies (Schaub et al., 2007; Baumgarten et al., 2009; Paper 4).

Ogawa passive sampler

Commercially available Ogawa passive samplers (Ogawa Co., USA, Inc., Pompano Beach) were used for the aim of this study (Paper 1–4). The Ogawa sampler has been developed by the Japan engineer Hiroshima Ogawa and his colleagues in 1986. Currently, it is used for the detection of NO, NO_2, SO_2, O_3, NH_3. It can be used as a personal sampler or in environmental outdoor and indoor programs. It has weather shelters that protect it from high wind speeds and moisture. Because they only consumable item for each exposure is the pre-coated collection pad, the Ogawa sampler represents one of the lowest cost devices for accurate measurements of large urban and rural areas (Internet (1)). Assembly of the Ogawa sampler and its protection in the field by the wind shelter is shown in Fig. 2 in Paper 1.

Passive sampler includes two separated nitrite coated filters. Each filter is placed between two stainless steel screens on each side of the solid tube. Nitrite ion is oxidized to nitrate ion during an exposition to ambient O_3. After exposure the filters are extracted with the certain amount of ultrapure water and the nitrate ion amount is determined by ion chromatography. The nitrate ion amount is proportional to ambient O_3 concentration (Koutrakis et al., 1993). The concentrations were calculated using average R_t value for each growing season.

The calculation of O_3 concentration is following (Bytnerowicz, personal communication; Bytnerowicz et al., 2008):

$$Rt = \frac{C_{cont} \times t}{m_1} \qquad\qquad c = \frac{m_2 \times R_t}{t}$$

R_t:	conversion factor ($h \cdot m^{-3}$)
c_{cont}:	O_3 concentration measured with the continuous method ($\mu g \cdot m^{-3}$)
t:	exposition time (h)
c:	ambient O_3 concentration ($\mu g \cdot m^{-3}$) at the site
m_1:	nitrate ion amount determined on exposed filter of co-located sampler minus nitrate ion amount determined on blank sampler filter ($\mu g \cdot filter^{-1}$)
m_2:	nitrate ion amount determined on exposed filter of sampler at the certain site minus nitrate ion amount determined on blank sampler filter ($\mu g \cdot filter^{-1}$)

Passive sampler using seems to be very appropriate and advantageous for O_3 concentrations determination. The results from Ogawa passive samplers show close agreement with the results from continuous monitor and refer to the utility of passive samplers for measuring in the remote areas and for possible O_3 adverse effects on vegetation assessment (Paper 1; Paper 4). Our results concerning their precision (Paper 1; Paper 3) are in agreement with other studies (e.g. Yuska et al., 2003; Bytnewowicz et al., 2002) and they can be recommended for further research. Only the shorter period of their exposure (one week instead of two) would be more appropriate for stomatal O_3 flux calculation (Paper 4).

2. OZONE VISIBLE SYMPTOMS

Surface O_3 is considered to be a very phytotoxic gaseous air pollutant. Its negative impacts at both the cell and the organ level have been shown, mainly as a result of experiments. However, the demonstration of O_3 negative impacts on native plants is not explicit. An assessment of the O_3 impact on vegetation and ecosystems using indicators based on ambient O_3 concentrations (AOT40 index) is insufficient. Assessment techniques based on the internal O_3 dose and on real plant damage are more appropriate. The identification of risk areas in terms of O_3 impacts on native vegetation should not be based exclusively on O_3 exposure indices, as this approach does not account for the environmental and biological variables that affect O_3 uptake and plant injury; the vegetation survey is an important part of such a study (Manning, 2003).

What ozone visible symptoms mean

O_3 (unlike SO_2 pollution) leaves no residue that might be detected by analytical technique. Consequently visible injury on needles and leaves is the only easily detectable evidence in the field and is considered to be a result of oxidative stress (UNECE, 2004a). The identification of O_3 visible symptoms in Western Europe started in the 1990s (Skelly et al., 1999). The first report of probable O_3 injury on native plants in Central and Eastern Europe with an emphasis on the Carpathians has been published by Manning et al. (2002).

The ICP-Forests method concerning O_3 visible symptoms and the assessment of O_3 influence (UNECE, 2004a) was applied for the purposes of this case study. The visible symptoms are characterized by a few typical signs (see below). Visible injury is usually the first indication of the presence of phytotoxic levels of O_3, and its detection is used for monitoring the effects of O_3 over short time periods (Bergmann et al., 1999). O_3 visible symptoms should be used in conjunctions with its measuring to obtain biologically relevant determination of pollution. O_3 symptoms can be also used to indicate air quality in areas where monitoring is not available (Manning and Godzik, 2004).

Ozone visible symptom developing

Foliar injury is a visible manifestation of internal physiological process in leaves (Innes et al., 2001). Visible stippling in broadleaved species result from palisade cells undergoing hypersensitive-like reaction (HR-like). The partial or complete disruption and condensation of cell content, collapse of cell wall, induction of polyphenols and pathogenesis related proteins and programmed cell death restriction to a small group of cells are typical features of HR-like (Vollenweider et al., 2003; Günthardt -Georg and Vollenweider., 2007).

Bronzing symptoms indicate an accumulation of phenolics, often including high content of proanthocyanidins and tannins in palisade mesophyll cells. The condensed tannins represent important mark for validation of O_3 influence. On the other hand, often coloration of leaves – reddening – is not specific O_3 symptom and can also occur due to other stress (light, drought, nutrient, biotic factors). Reddening is a result of the accumulation of anthocyanins (i.e. antioxidants). Although reddening in older leaves and at sun exposed and interveinal leaf parts is typical for O_3, its influence in these cases has to be validated by other O_3-specific cell markers (Vollenweider et al., 2003; Bussotti et al., 2006; Günthardt-Georg and Menard, 2008).

How to recognize ozone-induced foliar symptom in native conditions

The method of visible O_3-like symptom assessment is described in detail in the manual for the assessment of O_3 injury to European forest ecosystems of the ICP-Forests programme (UNECE, 2004a). A few signs are typical for O_3 visible injury and important for their identification (Fig.1). Visible symptom develops on leaves that are well-exposed to sunlight, and middle-aged and older leaves suffer more damage than younger leaves (age effect – Fig. 3), whilst shaded portions (i.e. if two leaves overlap) do not usually show any injury. Overlapped parts of leaf are protected against the developing of symptoms (shade effect – Fig. 2). Visible injury does not normally extend throughout the leaf tissue, visible symptoms are usually confined to the upper leaf surface. Stippling or pigmentation is the most common O_3 symptom on broadleaved species. Coloration (tan, red, purple, brown, bronzing or black) on the upper leaf surface may seem to be uniformly distributed. Closer examination (using hand lens or toward the sun light) show that the symptoms are restricted to certain areas of leaves or they are discrete. Both stippling and discolouration occur only between the veins. Severely injured leaves appear to age faster and drop sooner (UNECE, 2004a; Innes et al., 2001).

Within our study, four out seven species developed O_3-like symptoms (Fig. 2-5); O_3-induced symptoms have been validated on leaves of two species (Paper 3). Their validation has been made in the Ozone Validation Centre for Central Europe (Günthardt-Georg and Menard, 2008). Results based on O_3 visible symptoms, discussion concerning mainly the need of their validation, problems with their identification in the field and general conclusions concerning future using of this method in the conditions of the Czech mountains can be mainly found in Paper 2 and Paper 3.

Nevertheless, the identification of visible symptoms in native conditions can be problematic and misleading conclusions could be drawn. Therefore it is necessary to complete the field identification of visible symptoms with a microscopical validation in order to confirm O_3 as the cause of plant injury (Paper 2).

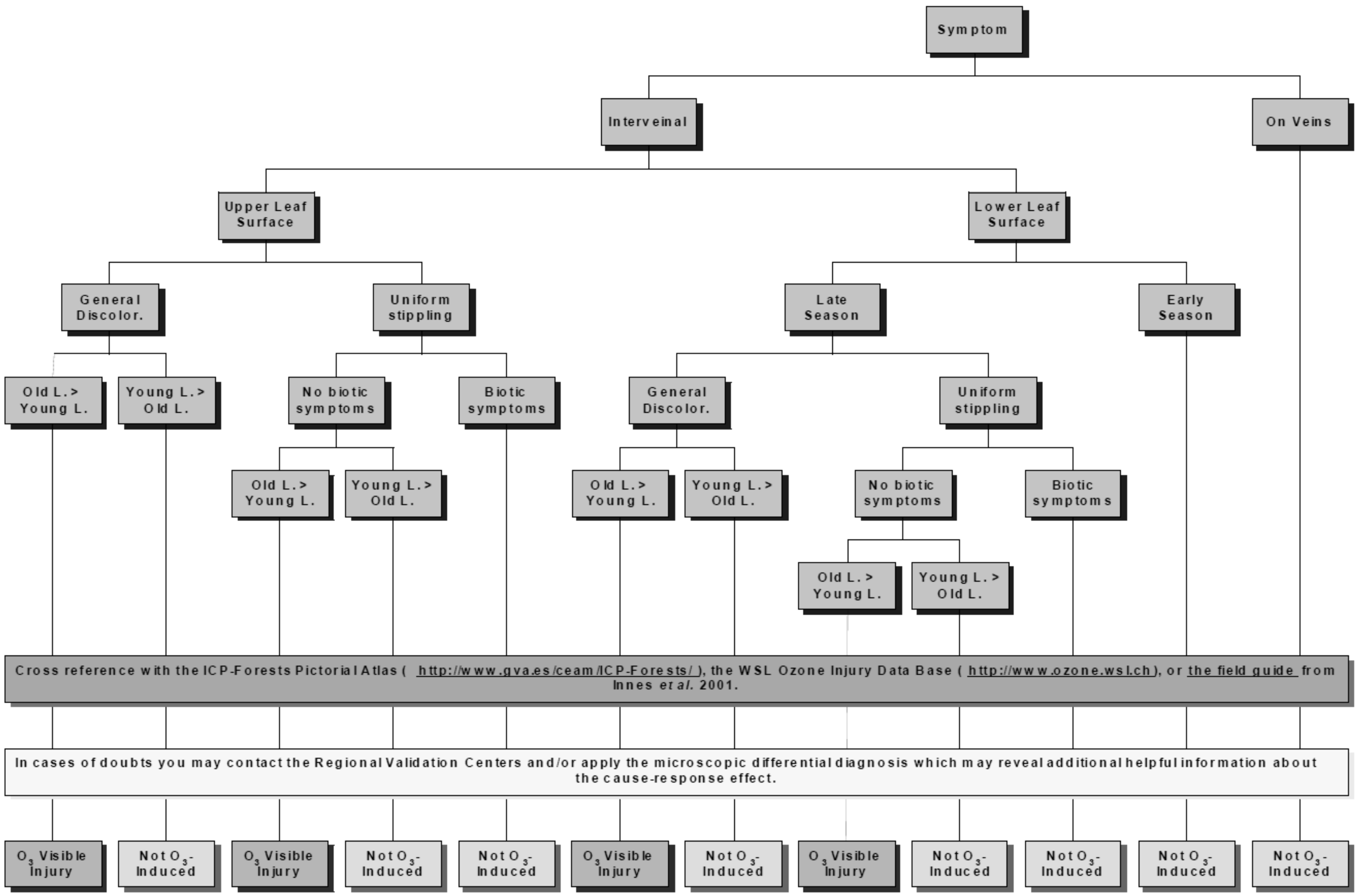

Fig. 1 Flowchart for the diagnosis of ozone symptoms on broad-leaf species (source: Innes et al., 2001)

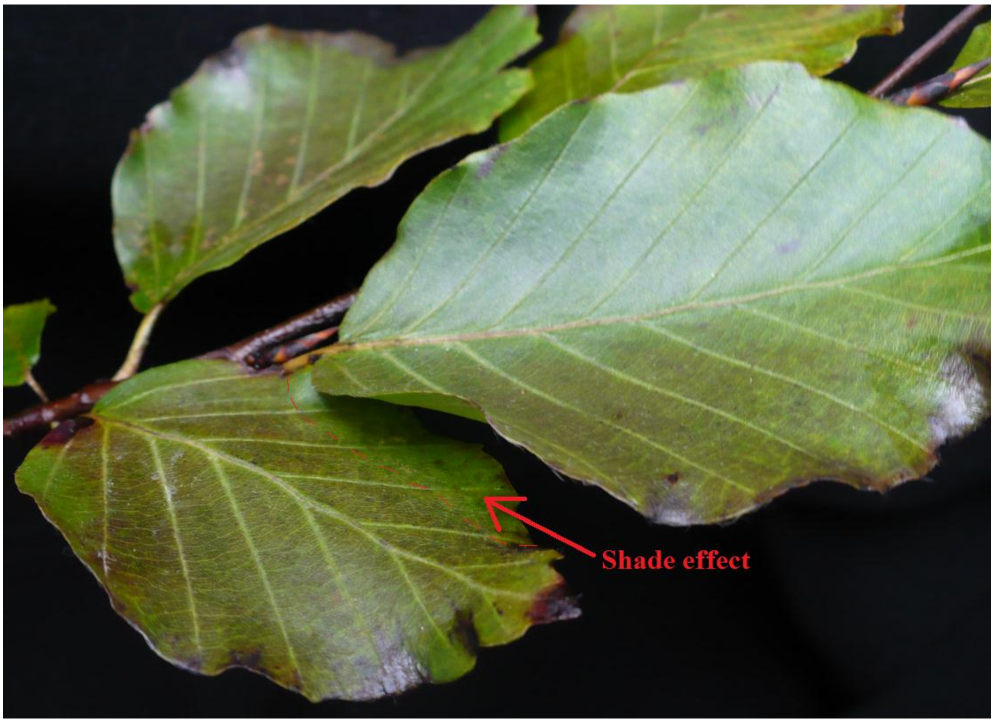

Fig. 2 Shade effect developed on overlapped part of *Fagus sylvatica* leaf (the Jizerske hory Mts., 15th September 2008). Symptoms on *Fagus sylvatica* have been validated as ozone-induced (photo: author).

Fig. 3 Age effect – localisation of reddening on older leaf of *Rubus idaeus* (the Jizerske hory Mts., 15th September 2007). Symptoms on *Rubus idaeus* have been validated as ozone-induced (photo: author).

Fig. 4 Reddenning on *Geranium sylvaticum* leaf. This often ozone-like symptom has not been validated as ozone-induced by the Ozone Validation Centre for Central Europe (photo: author).

Fig. 5 Reddenning on *Cirsium heterophyllum* leaf. This often ozone-like symptom has not been validated as ozone-induced by the Ozone Validation Centre for Central Europe (photo: author).

How to assess ozone symptoms in the field

The assessment proceeded in the framework of the LESS plots (Light Exposed Sampling Site, 2 m ×1 m) which were established in the vicinity of the O_3 measurement device. O_3 foliar injury was assessed at the light-exposed edge of the forest closest to the measurement device, within a maximum radius of 500 m. The edge was divided into a number of LESS plots corresponding to the length of the edge of the forest, the southern exposed edge of the forest being preferred (UNECE, 2004a). The leaves of symptomatic species supplied as voucher samples were sent to the Ozone Validation Centre for Central Europe for their O_3-like symptoms to be validated.

For the establishment of the LESS, the following procedure is to be applied (UNECE, 2004a):

1) Identify an area (A) (500 m radius) centred around the meteorological open-field monitoring station where passive O_3 samplers are installed (M).

2) Identify all the light exposed forest edges within A.

3) From those, choose the forest edge closest to M.

4) Determine the start point and measure the length of the selected forest edge and virtually identify a 1 m width area along them. You now have an *x* m long and 1 m width transect.

5) Calculate how many possible 2 x 1 m non-overlapping quadrates fit into the selected forest edge area by dividing the *x* m long transect by 2. The 2 m long edge of the rectangular quadrate lies along (parallel) the forest edge. The total number of nonoverlapping quadrates is our target population.

6) Select your sampling quadrates, which will represent the respective LESS:

a) On a paper, number all the possible non-overlapping quadrates. For practical reasons, start from the point closest to M and label each quadrate assigning a code 0, 2, 4, 6, 8, 10 ..., n which means the distance of the beginning of each quadrate from the beginning of the selected forest edge.

b) Extract randomly *n* quadrates and compile a list randomly selected, nonoverlapping quadrates. Replace any extraction, i.e. put again the extracted number in the “basket”. If you extract the same number again, repeat this step until you “draw” a different number.

7) At the end, you will obtain a list of *n* codes. Each code is a 2 x 1 m quadrate within the LESS; the codes will give you the distance of the beginning of each quadrate from the beginning of the previously determined start of the forest edge. Now you care ready for the field to install the LESS.

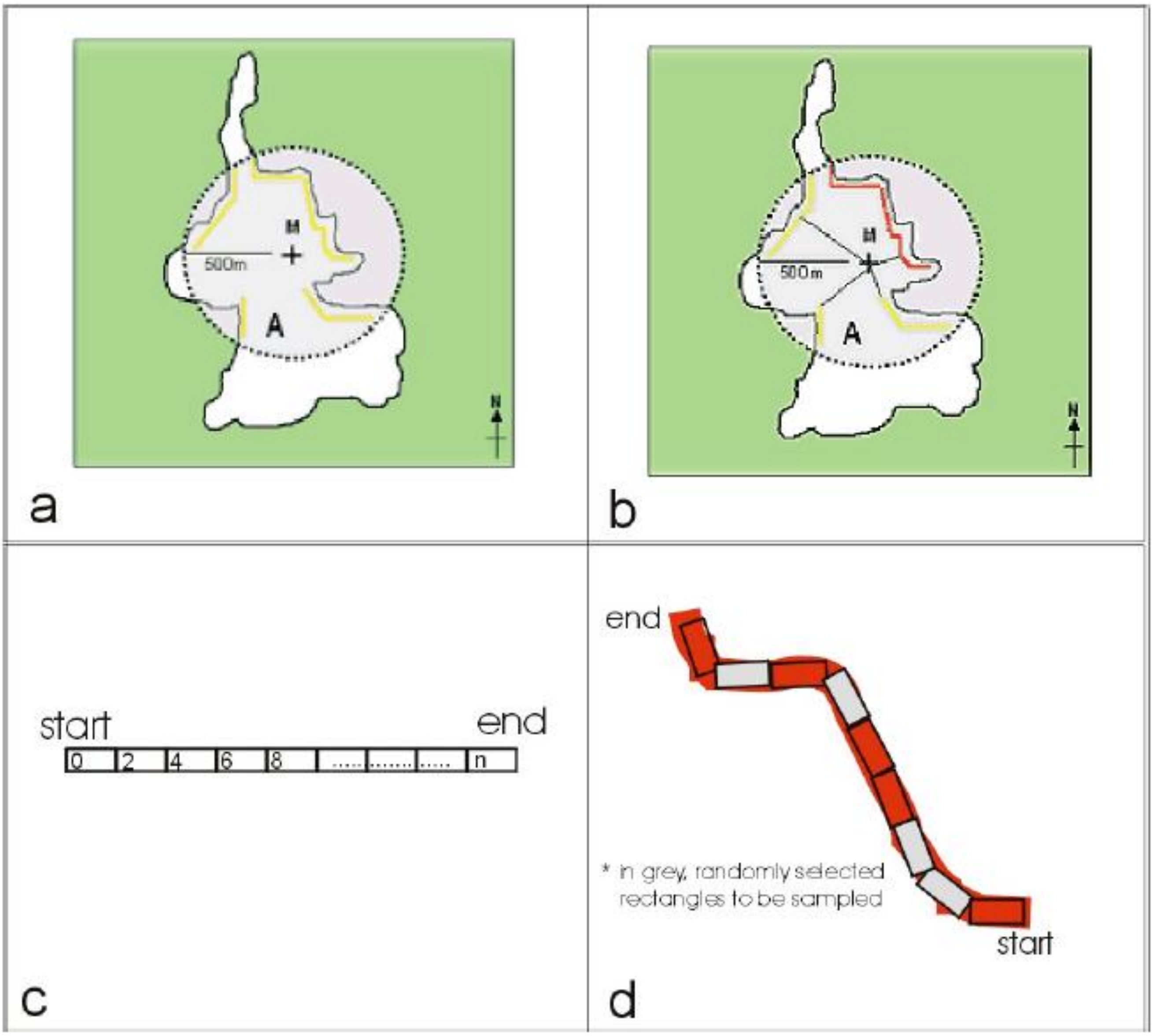

Fig. 6 Procedure for the establishment of a LESS (Source: UNECE, 2004a)

3. OZONE INDICES AND THEIR USING FOR RISK ASSESSMENT

O_3 is generally recognized to be the most relevant pollutant for forests, affecting tree species in different ways. Visible injury, reduction in growth, changes in biomass partitioning, or a higher susceptibility to pathogen attack can be the effect of O_3 influence (Krupa et al., 2000). It is generally accepted that the most severe O_3 effects on plants are caused by O_3 that is taken up through the stomata into the leaf interior (Reich, 1987; Ashmore et al., 2004). Only O_3 molecules, which are absorbed by stomata, are harmful for plants (Dittmar et al., 2005).

From ozone concentration-based approach to ozone flux-based approach

Critical levels for O_3 defined as AOT40 (Accumulated Exposure over a Threshold 40 ppb) was agreed at the workshop in Kuopio, Finland in 1996 (Kärenlämpi and Skärby, 1996). AOT40 is calculated as the sum of the difference between hourly O_3 concentrations and thresholds level 40 ppb for each hour when this threshold value is exceeded (EC, 2002). The approach is based on ambient concentrations, regardless of whether O_3 is actually absorbed by the vegetation causing subsequent damage (Fuhrer, 1997; Gerosa, 2003).

The concentration-based critical level of O_3 for forest trees, has been reduced from an AOT40 value of 10 ppm.h (Kärenlampi and Skärby, 1996) to 5 ppm.h. This value of 5 ppm.h is associated with a 5% growth reduction per growing season for the deciduous sensitive tree species category (beech and birch). It represents a continued use of sensitive, deciduous tree species to represent the most sensitive species under most sensitive conditions. As previously, it should be strongly emphasized that these values should not be used to quantify O_3 impacts for forest trees under field conditions (UNECE, 2004b).

The use of the AOT40 exposure index was introduce for practical reasons, mainly because obtaining its values is quite an easy operation, which only depends on the availability of O_3 concentrations time series. It completely neglects the mechanism by which atmospheric O_3 reaches the plants and penetrates into their tissues causing subsequent damage (Gerosa, 2003).

This approach, exclusively based on O_3 concentration records, quantifies the potential O_3 risk to vegetation, but does not give any information about the physiological uptake processes (Fuhrer and Achermann, 1999; Tuovinen, 2000; Gerosa et al., 2003). AOT40 assumes O_3 concentrations below 40 ppb and night-time exposure to be negligible. Hence, this concept is rather inconsistent with observed forest conditions. In contrast, the flux concept of cumulative O_3 uptake into the leaves has the potential of reflecting a physiologically meaningful internal

O_3 dose experienced by trees. AOT40 should be replaced, therefore, with flux concepts of actual O_3 uptake into leaves. The flux-based approach has the potential of providing physiologically meaningful O_3 doses that are of relevance for the actual O_3 stress experienced by trees, in particular, if combined with measures of detoxification which modifies O_3 impact (Matyssek et al., 2004; Fuhrer, 2003; Nunn et al., 2005).

The unsuitability of AOT40 indices use has been demonstrated by many authors. Emberson et al. (2000) has demonstrated the significant differences in spatial patterns across Europe that resulted from using a flux, rather than an exposure-based approach. Cieslik (2004) at South Europe sites demonstrated high AOT40 values because of high O_3 concentrations due to summertime photochemical O_3 production, whereas corresponding O_3 doses were relatively low, due to vegetation drought and thus reduced stomatal activity. Schaub et al. (2007) have shown that O_3 flux-based approach allow the identification of different spatial and temporal areas and periods as having higher risk to O_3 than those identified using the AOT40 approach. The different results or conclusions based on O_3 effect or flux in comparison to AOT40 have been also shown in the present study (Paper 3, Paper 4).

The development of a physiologically based dose-response relationship for O_3 requires that O_3 exposure should be described as leaf cumulative uptake of O_3 based on O_3 flux estimates (Fuhrer 2000, Karlsson et al., 2003).

Accumulated stomatal ozone-flux and its modelling

The major pathway for O_3 to reach targets within the plant, where it may cause significant effects on the physiology, is via the stomata (Nussbaum, 2003). Molecules penetrate into the leaves mostly through the stomata; the contribution of cuticular deposition is negligible (Kerstiens and Lendzian, 1989; Monteith and Unsworth, 1990) since the cuticula is covered by waxes which generally isolate and thus protect the tissues from external agents. Since stomata constitute the main pathway followed by O_3 molecules leading to plant damage, it is reasonable to assess biomass losses determining the O_3 dose received by the plants, i.e. the time-integrated stomatal O_3 flux (Cieslik, 2004).

O_3 flux through the stomata of leaves found at the top of the canopy is calculated using a multiplicative algorithm based on the methodology described by (UNECE, 2004b) according to Emberson et al. (2000) based on Jarvis (1976). This method provides a way to estimate the accumulated stomatal O_3 flux (AF_{st}) into a single leaf and has been used for the assessment of O_3 influence within the framework of the present study (Paper 3, Paper 4).

The internal O_3 dose, i.e. cumulative O_3 uptake, is calculated over the course of the growing season by multiplying the ambient O_3 concentration by the corresponding stomatal conductance (g_{sto}) to O_3. G_{sto} was calculated using a multiplicative stomatal conductance model (Emberson et al., 2000) based on Jarvis (1976) as a function of species-specific maximum g_{sto} (expressed on a single leaf-area basis), phenology, and prevailing environmental conditions (photosynthetic photon flux density (PPFD)), air temperature, vapour pressure deficit (VPD), and soil water potential (SWP). The stomatal conductance term is calculated using the multiplicative algorithm in Eq. (1) where g_{sto} is the actual stomatal conductance for O_3 and g_{max} is the species specific maximum stomatal conductance (both in mmol O_3 m^{-2} PLA s^{-1}) where PLA is per unit projected leaf area.

The parameters f_{phen}, f_{light}, f_{temp}, f_{VPD} and f_{SWP} are all expressed in relative terms (i.e. they take values between 0 and 1) as a proportion of g_{max}. These parameters allow for the modifying influence of phenology, O_3 and four environmental variables (light (irradiance), temperature, atmospheric vapour pressure deficit (VPD) and soil water potential (SWP)) on stomatal conductance to be estimated. The parameterisation of the model for *Fagus sylvatica* (Table 2 in Paper 4) was made according to UNECE (2004b).

$$g_{sto} = g_{max} * [\min(f_{phen}, f_{O3})] * f_{light} * \max\{f_{min}, (f_{temp} * f_{VPD} * f_{SWP})\}$$

The AF_{st} was calculated as the hourly sum of stomatal O_3 flux through the stomata of sun-exposed leaves of the upper canopy (UNECE, 2004b). The $AF_{st}Y$ value (the accumulated stomatal flux of O_3 above a flux threshold of Y = 1.6 nmol. m^{-2}. s^{-1}) was calculated as the sum of differences between hourly stomatal O_3 flux and a flux threshold Y = 1.6 nmol O_3 m^{-2} PLA s^{-1} (Karlsson et al., 2004). The Critical Level (CL) of $AF_{st}Y$ for *Fagus sylvatica* over one growing season was set provisionally to 4 mmol O_3 m^{-2} PLA (Karlsson et al., 2004; UNECE, 2004b). The Critical Level (CL) of $AF_{st}Y$ was calculated both for 24 hours and for daylight hours (hours with global radiation > 50 W m^{-2}; the same approach is regularly used for AOT40 calculation). The date of exceedance of the CL during the growing season was recorded (Paper 4).

Beech and wheat were selected for this preliminary investigation due to their wide distribution across Europe, and since they are the species upon which the current critical Level I values are based for forest trees and agricultural crops, respectively (Emberson, 2000). *Fagus sylvatica* is the most important broadleaf tree species in Central and Western Europe (Wieser, 2003).

Damage by game and cattle and forest management has led to a strong reduction of ecologically important mixed forest species such *Fagus sylvatica* or *Abies alba* is (Smidt and Herman, 2004). *Fagus sylvatica* trees showed repeated anomalous growth depression since the second half of 1970s, always following years with high O_3 uptake values. Conspicuous and alarming are the increasing O_3 flux levels for *Fagus sylvatica* during last decades (Dittmar et al., 2005). The influence of O_3 on *Fagus sylvatica* has been also observed in the Jizerske hory Mts. (Paper 3). Based on our further result (Paper 4), CL of $AF_{st}Y$ for *Fagus sylvatica* has been exceeded at all sites. O_3 can represent the threat for forests ecosystems, especially for recovering forest those are in the Jizerske hory Mts. Similar situation is probable for other Czech mountains (Paper 4).

Environment and ozone flux

High stomatal activity corresponds to healthy vegetation with strong water supply. When vegetation is dry, stomata are less active and stomatal O_3 uptake is lower, as observed more frequently in Southern Europe (Cieslik, 2004). It is important to note that high concentrations of O_3 are often associated with factors leading to reduced O_3 flux, such as VPDs (Grünhage et al., 1997).

Soil water availability is confirmed to be an important factor for the onset of O_3-induced symptoms, because it delays injury appearance and development by limiting stomatal conductance (Gerosa et al., 2009; Orendovici-Best, 2008). Role of humidity expressed both by water supply from the soil and by air humidity, is important. High values of these two parameters cause higher stomatal O_3 fluxes increasing the risk of O_3 uptake by plant tissues (Cieslik, 2009). The surface O_3 fluxes followed a daily cycle, with maximum around noon and low values during night time. The same O_3 concentration observed during morning and afternoon corresponds to different values of flux, which are lower in the afternoon. High O_3 concentrations are thus less harmful than the same ones in the morning (e.g. Gerosa, 2003, Paper 4).

Furthermore, the highest O_3 concentrations in areas such as South Europe occur during seasons when non-watered vegetation suffer with low content of soil water, resulting in reduced stomatal conductance followed by low O_3 flux. It is thus clear that an ozone-flux based, rather than O_3-exposure based assessment, could provide an improved estimate of the relative degree of risk of O_3 damage to vegetation (Emberson, 2000).

At low altitudes, transpiration is mainly limited by water availability. In contrast, at high altitude sites, temperature and radiation are the main controlling factors of transpiration. Water supply is sufficient most of the time, even during the summer months. Consequently, transpiration is high during the warm and sunny periods while the O_3 concentrations are often especially high, too. This explains high values of O_3 fluxes in high altitudes (Dittmar et al., 2005). The mountain ridges are the most harshly affected by these high levels of O_3 pollution and trees and vegetation there are the most exposed to O_3 (Sicard et al., 2011). Thus, high O_3 concentrations do not necessarily cause large damage when stomata are closed because of dryness, and it is not a priori evident that in a hot summer with high O_3 concentrations like in 2003, vegetation suffer from O_3 stress more than in a cooler and humid year (Keller et al., 2007). In the Jizerske hory Mts., combination of actual O_3 levels and local environmental conditions (even VPD significantly limited stomatal conductance) has been sufficient enough to cause O_3 uptake exceeding CL for forest ecosystems (Paper 4).

Many authors have suggested that foliar injury can provide indirect information about O_3 exposure. Nevertheless, values of O_3 dose, when the visible injury developed found by many authors are very different (Matyssek et al., 2004; Baumgarten et al., 2000; Nunn et al., 2005; Gerosa et al., 2009; Paper 4). Reasons for this fact are discussed more in detail in Paper 4. One of them is detoxifying capacity of plant as the visible injury is a result of the balance between O_3 uptake and this capacity (Matyssek et al., 2004). In order to be effective, a harmful O_3 dose, i.e. the balance between the rate of ozone uptake by the foliage and the rate of ozone detoxification should be obtained by these models (Fuhrer and Booker, 2003). For future, this should be reflected as the new Model of Ozone Deposition and Detoxification that is now being developed (Tuzet et al., 2011).

4. Aims of the study

The aims of this study was both to assess the influence of ozone on vegetation in the Jizerske hory Mts. and to state recommendation and outlooks for possible future using of relatively new methods used for ozone impact assessment on native vegetation in the field.

Therefore, the concrete aims of our study were:

a) to validate ozone-like visible symptoms on native plant species in the typical Czech mountains

b) to assess the usefulness of the method of ozone-like visible foliar injury on native plants growing in situ

c) to gather local input data concerning ozone concentration and environmental conditions important for ozone stomatal flux / uptake modelling

d) to determine O_3 uptake to native *Fagus sylvatica* in the field and assess ozone uptake values with regard to its critical level of accumulated stomatal flux

e) to determine the limitation of stomatal flux due to the local environmental conditions

f) to assess if visible foliar injury can generally provide indirect information about O_3 uptake.

5. List and content of the thesis papers

Paper 1

Matoušková L., Hůnová I., 2007: Measurement of surface ozone concentrations using Ogawa passive samplers in the Novohradske hory Mts. during the 2004 and 2005 vegetation periods, Acta Universitatis Carolinae Environmentalica 21: 162–174

We describe the application of the Ogawa passive samplers in the field in the Paper 1. Calculation of ozone concentrations measured with passive samplers, principle, experiences, the advantages and disadvantages of using and the assessment of passive sampler precision based on results from two growing seasons (2004 and 2005) in the Novohradske hory Mts. are described. Our findings are compared to and discussed with other authors. Recommendations for future use of passive samplers are presented. Experiences obtained within the frame of this study have been adopted in the field of the Jizerske hory Mts. for three growing seasons.

Paper 2

Matoušková L., Novotný, R., Hůnová I., Buriánek, V., 2010: Visible foliar injury as a tool for the assessment of surface ozone impact on native vegetation: a case study from the Jizera Mountains. Journal of Forest Science 4: 177–182.

Paper 2 presents the results of ozone-like and ozone-induced symptoms on common plant species in the Jizerske hory Mts. Surface ozone is considered to be a very phytotoxic gaseous air pollutant. Its negative impacts at both the cell and the organ level have been shown, mainly as a result of experiments. However, the demonstration of ozone negative impacts on native plants is not explicit. An assessment of ozone impact on vegetation and ecosystems using indicators based on ambient ozone concentrations is insufficient and assessment techniques based on internal ozone dose and on real plant damage are more appropriate. Such a possible technique is the mapping of ozone visible symptoms due to ozone influence. The ICP-Forest method concerning ozone visible symptoms and the assessment of ozone influence were applied for the purposes of this case study. The visible symptoms are characterized by a few typical signs. Nevertheless, the identification of visible symptoms in native conditions can be problematic and misleading conclusions could be drawn. Therefore it is necessary to complete the identification of visible symptoms with a validation in order to confirm ozone as the cause of plant injury.

PAPER 3

Hůnová I., Matoušková L., Srněnský R., Koželková K., 2011. Ozone influence on native vegetation in the Jizerske hory Mts. of the Czech Republic: results based on ozone exposure and ozone-induced visible symptoms. Environmental Monitoring and Assessment. In Press. doi: 10.1007/s10661-011-1935-8.

Ozone levels in the Jizerske hory Mts. measured at 13 sites by diffusive samplers during the 2006 and 2007 vegetation seasons are presented. A significant ozone gradient per 100 m difference in altitude between 370 and 1,100 m.a.s.l. was recorded. High-resolution maps of phytotoxic potential were developed. The AOT40 threshold (5 ppm h) was exceeded over the entire area with the highest levels exceeding this threshold in the upper portions of the mountains. Ozone visible injury was evaluated at four of the monitoring sites on seven native plant species. Four species showed ozone-like symptoms, two of which (Rubus idaeus and Fagus sylvatica) were confirmed as ozone-induced. Our results concerning ozone-induced visible symptoms indicate that ambient ozone is likely to have a much lower impact on the Jizerske hory Mts. vegetation than expected, considering the measured ambient ozone exposures and favourable environmental conditions for ozone uptake. Nevertheless, the small amount of visible injury does not have to mean that vegetation is not influenced. Similarly to many other authors, for next growing season we decided to follow so far the newest method for ozone influence assessment, i.e. accumulated stomatal ozone flux modelling.

PAPER 4

Matoušková L., Hůnová I. Stomatal ozone flux and visible leaf injury in native juvenile trees of Fagus sylvatica L. A field study from the Jizerske hory Mts., the Czech Republic. *Manuscript prepared for submission.*

The study was carried out at six sites in the Jizerske hory Mts. At all these sites in altitude between 460 and 962 m.a.s.l. during the period from June to September in 2008, O_3 concentrations and environmental parameters were measured and the accumulated stomatal ozone flux (AF_{St}) and AOT40 index were calculated. At five out of these six sites, visible injury on Fagus sylvatica L. juvenile trees leaves has been observed. Combination of actual O_3 levels and environmental conditions, though relative air humidity and air temperature significantly limited stomatal conductance, has been sufficient enough to cause O_3 uptake exceeding critical level (CL) for forest ecosystems. The CL of stomatal O_3 uptake (4 mmol .m^{-2}) was exceeded at all sites from cca 45% to 270% (160% on average). The conclusions based on AOT40 and AFSt are not the same. CL of 5 ppm.h for AOT40 exceed at four out of all sites (94% on average). The dependence of the visible injury on O_3 indices was significant. AF_{St} has been determined as better predictor of visible injury than AOT40.

6. CONCLUSIONS

It is possible to obtain sufficient information about O_3 concentrations in the field using passive samplers. Based on these data together with other measurements, it is possible to assess the O_3 influence on vegetation.

O_3 concentrations are highly variable in time and space and depend strongly on altitude. Generally O_3 levels increase with increasing altitude, although high O_3 levels can be present also in lower situated areas. Except O_3 concentrations, it is necessary to take into account also other environmental conditions influencing O_3 flux to plants.

Ozone-induced symptoms have been confirmed on two out of seven species. Visible ozone symptoms may provide us with information about the harmful effects of ozone, but they have to be validated. The results can be interpreted only under this condition.

Conclusions based on visible foliar injury assessment in the Jizerske hory Mts. are not clear. Nevertheless, as the first information including biological significance from the area where no similar assessment has been carried out, they are helpful for further research and assessment.

To make the generalized conclusion concerning values of O_3 uptake related to visible injury onset is impossible since there are many circumstances influencing this values (plant detoxification capacity, soil conditions, genetic variability etc.).

Environmental conditions (relative air humidity and temperature) even though significantly limiting the stomatal conductance together with O_3 levels have been sufficient enough to cause O_3 uptake to exceed critical levels for *Fagus sylvatica*. Influence of ambient O_3 on beech in the typical Czech mountain is evident regarding actual critical levels for accumulated O_3 stomatal uptake.

Even under favourable soil moisture conditions, i.e. the most important determinant for conductance, the conclusions based on AOT40 and AF_{St} are not the same. AOT40 is not suitable indicator for negative O_3 influence. The flux-based approach is better predictor of the O_3 risk than the exposure-based approach.

Fagus sylvatica is the convenient bioindicator for its verified symptoms, the relative wide spread in the Czech mountains, its importance for the mountainous forests ecology stability and the possibility to model the ozone flux into its leaves. The future research should be focused on this species, e.g. long-term research concerning the influence of ozone on the tree growth is more than demanded.

7. REFERENCES

Ashmore M. R. (2003): Surface Ozone Effects on Vegetation. In: Holton JR., Curry JA. and Pyle JA. eds.: Encyclopedia of Atmospheric Sciences. Academic Press, Elsevier Science, Ltd., London. 1663–1671 p.

Ashmore M., Emberson L., Karlsson P.E., Pleijel H. (2004). New directions: a new generation of ozone critical levels for the protection of vegetation in Europe. Atmospheric Environment 38: 2213–2214.

Baumgarten M, Huber Ch., Büker P., Emberson L., Ditrich H.-P., Nunn A.J., Heerdt Ch., Beudert B., Matyssek R. (2009). Are Bavarian Forests (southern Germany) at risk from ground-level ozone? Assessment using exposure and flux based ozone indices. Environmental Pollution 157: 2091–2107.

Baumgarten M., Werner H., Häberle K. -H., Emberson L. D., Fabian P., Matyssek R. (2000). Seasonal ozone response of mature beech trees (Fagus sylvatica) at high altitude in the Bavarian forest (Germany) in comparison with young beech grown in the field and in phytotrons. Environmental Pollution 109: 431–442.

Bergmann E., Bender J., & Weigel H.-J. (1999). Ozone threshold doses and exposure–response relationships for the development of ozone injury symptoms in wild plant species. New Phytologist 144: 423–435.

Blum O., Bytnerowicz A., Manning W., Popovicheva L. (1997). Ambient tropospheric ozone in the Ukrainian Carpathian Mountains and Kiev region: detection with passive samplers and bioindicators plants. Environmental Pollution 98: 299–304.

Bobrov RA. (1952). The effect of smog on the anatomy of oat leaves. Phytopathology 42: 558–563.

Bobrov RA. (1955a). The Leaf Structure of Poa annua with Observations on its Smog Sensitivity in Los Angeles County. American Journal of Botany 42: 467–474.

Bobrov RA. (1955b). Use of Plants as Biological Indicators of Smog in the Air of Los Angeles County. Science, New Series 121: 510–511.

Bussotti F., Cozzi A., & Ferretti M. (2006). Field survey of ozone symptoms on spontaneous vegetation. Limitations and potentialities of the European programme. Environmental Monitoring and Assessment 115: 335–348.

Bytnerowicz A., Arbaugh M., Schilling S., Frączek W., Alexander D. (2008). Ozone distribution and phytotoxic potential in mixed conifer forests of the San Bernardino Mountains, southern California. Environmental Pollution 155: 398–408.

Bytnerowicz A., Godzik B., Fraczek W., Grodzinska K., Krywult M., Badea M., et al. (2002). Distribution of ozone and other air pollutants in forests in the Carpathian Mountains in central Europe. Environmental Pollution 116: 3–25.

CHMI, 2011. Air Pollution in the Czech Republic in 2010. http://portal.chmi.cz/files/portal/docs/uoco/isko/grafroc/grafroc_CZ.html. Accessed 20th October 2011.

Cieslik S.A. (2004). Ozone uptake by various surface types: a comparison between dose and exposure. Atmospheric Environment 38: 2409–2420.

Cieslik S.A. (2009). Ozone fluxes over various plant ecosystems in Italy: A review. Environmental Pollution 157: 1487–1496.

Cox R.M. & Malcom J.W. (1999). Passive ozone monitoring for forest health assessment. Water, Air and Soil Pollution 116: 339–344.

Cox R.M. (2003). The use of passive sampling to monitor forest exposure to O_3, NO_2 and SO_2: a review and case studies. Environmental Pollution 126: 301–311.

Dittmar Ch., Pfaffelmoser K., Rötzer T., Elling W. (2005). Quantifying ozone uptake and its effects on the stand level of common beech (Fagus sylvatica L.) in Southern Germany. Environmetal Pollution 134: 1–4.

EC (2002). Directive 2002/3/EC of the European Parliament and the Council of 12 February 2002 relating to ozone in ambient air. Official Journal of the European Communities L 67/14.

Emberson L.D., Ashmore M.R., Cambridge H.M., Simpson D., Tuovinen J.-P. (2000). Modelling stomatal ozone flux across Europe. Environmental Pollution 109: 403–413.

Fuhrer J., (2000). Introduction to the special issue on ozone risk analysis for vegetation in Europe. Environmental Pollution 109, 359–360.

Fuhrer J. & Achermann B. (1999). Critical levels for ozone. Level II. Environmental documentation 115, Swiss Agency for Environment, Forest, and landscape, Bern, Switzerland pp. 333.

Fuhrer J. & Booker F. (2003). Ecological issues related to ozone: agricultural issues. Environment International 29: 141–154.

Fuhrer J., Skärby L., Ashmore M.R. (1997). Critical levels for ozone effects on vegetation in Europe. Environmental Pollution 97: 91–106.

Gane R. (1937): The respiration of bananas in presence of ethylene. New Phytologist 36: 170–178.

Gerosa G., Cieslik S., Ballarin-Denti A. (2003). Micrometeorological determination of time-integrated stomatal ozone fluxes over wheat: a case study in Northern Italy. Atmospheric Environment 37: 777–788.

Gerosa G., Marzuoli R., Desotgiu R., Bussotti F., Ballarin-Denti A. (2009). Validation of the stomatal flux approach for the assessment of ozone visible injury in young forest trees. Results from the TOP (transboundary ozone pollution) experiment at Curno, Italy. Environmental Pollution 157: 1497–1505 .

Gill S.S. & Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48: 909–930.

Godzik B. (1997). Ground level ozone concentrations in the Krákow region, southern Poland. Environmental Pollution, 98: 273–280.

Grosjean D. & Hisham M.W.M. (1992). A passive sampler for atmospheric ozone. Journal Air Waste Management Association 42: 169–173.

Grosjean D., Williams EL., Grosjean E. (1995). Monitoring ambient ozone with a network of passive samplers: a feasibility study. Environmental Pollution 88: 267–273.

Grünhage L., Jäger H.-J., Haenel H.-D., Hanewald K., Krupa S. (1997): PLATIN (Plant-ATmosphere INteraction) II: Co-occurrence of high ambient ozone concentrations and factors limiting plant absorbed dose. Environmental Pollution: 51–60.

Günthardt-Goerg, M. S., & Menard, T. (2008). Validation of ozone symptoms on leaves from the Jizerske hory. Birmensdorf: WSL.

Günthardt-Goerg, M. S., & Vollenweider, P. (2007). Linking stress with macroscopic and microscopic leaf response in trees: New diagnostic perspectives. Environmental Pollution, 147: 467–488.

Guderian R. (1985). Air pollution by photochemical oxidants. Formation, transport, control, and effects on plants. Springer, Berlin 346.

Haagen – Smith AJ. (1952): Investigation on injury to plants from air pollution in the Los Angeles Areas.Plant Physiology 27: 18–34.

Hangartner M., Kirchner M., Werner H. (1996). Evaluation of passive methods for measuring ozone in the European Alps. Analyst 121: 1269–1272.

Hauser, T.R. & Bradley, D.W. (1966): Specific spectrophotometric determination of ozone in the atmosphere using 1,2-Di-(4-pyridyl) ethylene. Analytical Chemistry 38: 1529–1532.

Innes J.L., Skelly J.M., Schaub M. (2001): Ozone and broadleaved species: a guide to the identification of ozone- induced foliar injury. Birmensdorf, Eidgenössische Forsc- hungsanstalt WSL, Haupt, Bern, Stuttgart, Wien 136.

Internet (1). http://www.ogawausa.com/passive.html. Accessed 3rd October 2011.

Jarvis P.G. (1976). The interpretation of the variation in leaf water potential and stomatal conductance found in canopies in the field. Physiological Transactions of the Royal Society of London, Series B: Biological Sciences 273: 593–610.

Kanno S. & Yanagisawa Y. (1992). Passive ozone/oxidant sampler with coulometric determination using I2/nylon-6 charge-transfer complex. Environmental Science and Technology 26: 744–749.

Kärenlampi L. & Skärby L. eds. (1996). Critical Levels for Ozone in Europe: Testing and Finalizing the Concepts. University of Kuopio, Kuopio. 363 p.

Karlsson P.E., Uddling J., Braun S., Broadmeadow M., Elvira S., Gimeno B. S., Le Thiec D., Oksanen E., Vandermeiren K., Wilkinson M., Emberson L. (2004). New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone. Atmospheric Environment 38: 2283–2294.

Karlsson P.E., Uddling J., Skärby L., Wallin G., Selldén G. (2003). Impact of ozone on the growth of birch (Betula pendula) saplings. Environmental Pollution 124: 485–495.

Keller F., Bassin S., Ammann Ch., Fuhrer J. (2007). High-resolution modelling of AOT40 and stomatal ozone uptake in wheat and grassland: A comparison between 2000 and the hot summer of 2003 in Switzerland. Environmental Pollution 146: 671–677 .

Kerstiens G. & Lendzian K.J. (1989). Interactions between ozone and plant cuticles. 1. Ozone deposition and permeability. New Phytologist 112: 13–19.

Kley D., Kleinmann M., Sanderman H., Krupa S. (1999). Photochemical oxidants: state of the science. Environmental Pollution 100: 19–42.

Koutrakis P., Wolfson J.M., Bunyaviroch A., Frohlich S.E., Hirano K., Mulik J.D. (1993). Measurement of ambient ozone using a nitite-coated filter. Analytical Chemistry 65: 209–214.

Krause G.H.M. , Prinz B. (1988). Current Knowledge of Ozone on Vegetation/Forest Effects and Emerging Issues. Studies in Environmental Science 35: 45–55.

Krupa S.V. & Legge A.H.Y (2000). Passive sampling of ambient. gaseous air pollutants: an assessment from an ecological perspective. Environmental Pollution 124: 173–178.

Krupa S., McGrath M.T., Andersen C.P., Booker F., Burkey K.O., Chappelka A.H., Chevone B.I., Pell E.J., Zilinskas B.A. (2000). Ambient ozone and plant health. Plant Disease 85: 4–12.

Loibl W., Winiwarter W., Kopsca A., Zueger J., Baumann R. (1994). Estimating the spatial distribution of ozone concentrations in complex terrain. Atmospheric Environment 28: 2557–2566.

Long SP. & Naidu SL. 2002: Effects ox oxidants at the biochemical, cell and physiological levels, with particular reference to ozone. In: Bell JNB and Treshow M eds.: Air Pollution and Plant Life. John Wiley & Sons, Chichester.

Manning W.J., Godzik B., Musselman R. (2002): Potential bioindicator plant species for ambient ozone in forested mountain areas of Central Europe. Environmental Pollution 119: 283–290.

Manning W. J. (2003). Detecting plant effects is necessary to give biological significance to ambient ozone mon- itoring data and predictive ozone standards. Environmental Pollution 126: 375–379

Manning W. J., & Godzik B. (2004). Bioindicator plants for ambient ozone in Central and Eastern Europe. Environmental Pollution 130: 33–39.

Matyssek R., Wiesner G., Nunn A.J., Kozovits A.R., Reiter I.M., Heerdt C., Winkler J.B., Baumgarten M., Häberle K.-H., Grams T. E. E., Werner H., Fabian P., Havranek W.M. (2004). Comparison between AOT40 and ozone uptake in forest trees of different species, age and site conditions. Atmospheric Environment 38: 2271–2281.

Matyssek R. & Sandermann H., (2003). Impact of O_3 on trees: an ecophysiological perspective. Progress in Botany 64: 350–404.

Middletown JT., Kendrick JB. & Schwalm HW (1950): Injury to herbaceous plants by smog or air pollution. Plant Dis. Reporter 34: 245–252.

Miller P.R. & Parmenter J.R. (1967). Effects of ozone injury to ponderosa pine. Phytopathology 57: 822.

Molín R. 2000: Pasivní dozimetr nové konstukce. Část 1. Teoretické vztahy. Meteorologické zprávy 53: 89–92.

Monteith, J.L., Unsworth, M., 1990. Principles of Environmental Physics 2nd Edition. Arnold, London.

Musselman R.C., Lefohn A.S., Massman W.J., Heath R.L. (2006). A critical review and analysis of the use of exposure- and flux-based ozone indices for predicting vegetation effects. Atmospheric Environment 40: 1869–1888.

Nunn A.J., Kozovits A.R., Reiter I.M., Heerdt C., Leuchner M., Lütz C., Liu X., Löw M., Winkler J.B., Grams T.E.E., Häberle K.-H., Werner H., Fabian P., Rennenberg H., Matyssek R. (2005). Comparison of ozone uptake and sensitivity between a phytotron study with young beech and a field experiment with adult beech (Fagus sylvatica). Environmental Pollution 137: 494–506.

Nussbaum S., Remund J., Rihm B., Mieglitz K., Gurtz J., Fuhrer J. (2003). High-resolution spatial analysis of stomatal ozone uptake in arable crops and pastures. Environmental International 129: 385–392.

Orendovici-Best T., Skelly J.M., Davis D.D., Ferdinand J.A., Sabate J.E., Stevenson R.E. (2008).
ozone uptake (flux) as it relates to ozone-induced foliar symptoms of Prunus serotina and Populus maximowizii × trichocarpa. Environmental Pollution 151: 79–92.

Pell E.J., Schlagnhaufer C.D., Arteca R.N. 1997: Ozone induced oxidative stress: Mechanism of action and reaction. Physiologia Plantarum 100: 264–273.

Reich, P.B. (1987). Quantifying plant response to ozone: a unifying theory. Tree Physiology 3: 63–91.

Salardino D.H. & Carroll J.J. 1998: Correlation between ozone exposure and visible foliar injury in ponderosa abd Jeffrey pines. Atmospheric Environment: 3001–3010

Sanz M.J., Calatayud V., Sanchez-Pena G., 2004: Ozone concentrations measured by passive sampling at the Intensive Monitoring Plots of South Western Europe. In: Ferreti M., Sanz M.J., Schaub M. (Eds.): Ozone and the forests of South-West Europe, 51–74 pp.

Sanz M.J., V. Calatayud, G. Sánchez-Peña (2007). Measures of ozone concentrations using passive sampling in forests of South Western Europe. Environmental Pollution 145: 620–628.

Schaub M., Emberson L., Büker P., Kräuchi N. (2007). Preliminary results of modeled ozone uptake for Fagus sylvatica L. trees at selected EU/UN-ECE intensive monitoring plots. Environmental Pollution 145: 636–643.

Schönbein C.F. (1844): On the production of ozone. Abstracts of the Papers Communicated to the Royal Society of London 5: 508.

Schraudner M., Langebartels C. & Sandermann H. 1997: Changes in the biochemical status of plant cells induced by the environmental pollotant ozone. Physiologia Plantarum 100: 274– 280.

Sicard P., Dalstein-Richier L., Vas N. (2011). Annual and seasonal trends of ambient ozone concentration and its impact on forest vegetation in Mercantour National Park (South-eastern France) over the 2000–2008 period. Environmental Pollution 159: 351–362.

Skärby L., Ro-Poulsen H., Wellburn F.A.M, Sheppard L.J. 1998: Impacts of ozone on forests: a European perspective. New Phytologist 139: 109–122.

Skelly J.M., Innes J.L., Savage J.E., Snyder K.R., Vanderheyden D., Zhang J., Sanz M.J. (1999). Observation and Confirmation of Foliar Ozone Symptoms of Native Plant Species of Switzerland and Southern Spain. Water, Air, & Soil Pollution 116: 227–234.

Smidt S. & Herman F. (2004). Evaluation of air pollution-related risks for Austrian mountain forests. Environmental Pollution 130: 99–112.

Stanners D. & Bourdeau P. eds. (1995). Europe´s Environment: The Dobris Assessment. EEA, Copenhagen. 676 p.

Tuovinen J.P. (2002). Assessing vegetation exposure to ozone: is it possible to estimate AOT40 by passive sampling? Environmental Pollution 119: 203–214.

Tuovinen, J.P. (2000). Assessing vegetation exposure to ozone: properties of the AOT40 index and modifications by deposition modelling. Environmental Pollution 109: 361–372.

Tuzet A., Perrier A., Loubrt B., Cellier P. (2011). Modelling ozone deposition fluxes: The relative roles of deposition and detoxification processes. Agricultural and Forest Meteorology 151: 480–492.

UNECE (2000). Manual on Methods and Criteria for Harmonized Sampling. Assessment. Monitoring and Analysis of the Effects of Air Pollution on Forests. Part X.A.: Monitoring of Air Quality. International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests. United Nations Economic Commision for Europe (UN ECE) Convention on Long-range Transboundary Air Pollution.

UNECE (2004a). Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the ef fects of air pollution on forests. Part X/B. Assessment of Ozone Injury. http://www.icpforests.org/Manual.htm. Accessed 1 November 2010.

UNECE (2004b). Mapping Manual 2004. Manual on the methodologies and criteria for modelling and mapping critical loads and levels and air pollution effects, risks and trends. Chapter 3: Mapping Critial Levels for Vegetationhttp://www.icpmapping.org. Accessed 1 November 2010.

Vollenweider P., Ottiger M., Günthardt-Goerg M.S. (2003). Validation of leaf ozone symptoms in natural vegetation using microscopical methods. Environmental Pollution 124: 101–118.

Wieser G., Hecke B., Tauzs M., Häberle K. H., Grams E. E. G., Matyssek R. (2003). The influence of microclimate and tree age on the defense capacity of European beech (Fagus sylvatica L.) against oxidative stress. Annals of Forest Science 60: 131–135.

Willems J.J.H & Hofschreuder P. (1991). A passive monitor for measuring ammonia. In: Allegrini I., Febo A., Perrino C. (eds.) Field intercomparsion exercise of ammonia and ammonium measurement. Commision of the European Communities: 56–63 p.

Yuska D.E., Skelly J.M., Ferdinand J.A., Stevenson R.E., Savage J.E., Mulik J.D., Hilnes A. (2003). Use of bioindicators and passive sampling devices to evaluate ambient ozone concentrations in north central Pennsylvania. Environmental Pollution 125: 71–80.

THESIS PAPERS

PAPER 1

Matoušková L., Hůnová I., 2007: Measurement of surface ozone concentrations using Ogawa passive samplers in the Novohradske hory Mts. during the 2004 and 2005 vegetation periods, Acta Universitatis Carolinae Environmentalica 21: 162–174

PAPER 2

Matoušková L., Novotný, R., Hůnová I., Buriánek, V., 2010: Visible foliar injury as a tool for the assessment of surface ozone impact on native vegetation: a case study from the Jizera Mountains. Journal of Forest Science 4: 177–182.

PAPER 3

Hůnová I., Matoušková L., Srněnský R., Koželková K., 2011. Ozone influence on native vegetation in the Jizerske hory Mts. of the Czech Republic: results based on ozone exposure and ozone-induced visible symptoms. Environmental Monitoring and Assessment. In Press. doi: 10.1007/s10661-011-1935-8.

PAPER 4

Matoušková L., Hůnová I. Stomatal ozone flux and visible leaf injury in native juvenile trees of Fagus sylvatica L. A field study from the Jizerske hory Mts., the Czech Republic. *Manuscript prepared for submission.*