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PROCEEDINGS

FOREST RESEARCH IN CHANGING WORLD

RISTO SEPPÄLÄ, IUFRO PRESIDENT

Distinguished chair, dear colleagues, ladies and gentlemen

It is a great privilege and pleasure for me to deliver this keynote address. I thank the organizers of the meeting for inviting me here. In my capacity as IUFRO President I bring the greetings and best wishes of the IUFRO Board to all of you. The Board has recognized that Research Group "Impacts of air pollution on forest ecosystems" and its Working Parties are and have been already for decades among the most active IUFRO Units. These bi-annual meetings demonstrate clearly the continuation of this high level of activity.

The topic of my address is "Forest Research in Changing World". There are many visible trends that affect forest research and create major challenges to us, especially because world around us is changing at an increasing speed. In the following, I touch some of these trends and challenges.

Many of the recent changes are connected with research funding. For example, national public funding of traditional forest research institutions is decreasing in most countries. At the same time, the number of students enrolled in forestry faculties is declining. Many forestry faculties and forestry schools have even ceased to exist.

The decreasing budgets of conventional forest research institutions and decline of traditional forestry education do not necessarily mean that there is now less money available for forest-related research and education than earlier. In fact, investments in research have been growing at the global level but the structure of funding has changed considerably. Funding has become more international, and the share of public funding has decreased. For example, in OECD countries already two thirds of investments in research and development come from non-governmental sources.

The growing share of private funding is partly causing a business-like environment also in academic and other non-profit research organizations. Consequently, the business rules of private funding are increasingly used in public sector. Such words as commercialization and privatization have become almost slogans in many government research organizations, even in government-funded universities. This has already led to a very result-oriented short-term thinking. As a consequence, long-term basic research and also quality of research are in danger.

Neglecting basic research will sooner or later lead to a situation in which applied research also suffers because the ability to react to rapidly changing research needs will weaken. It is understandable that industry and other private funders want to invest mainly in short-term applied research, but this means that the role of public funding in basic research should be stronger than it is now.

Personally, I am not very worried about the decline of the budgets of the traditional forest research institutions because the amount of money used for forest-related research as a whole has not declined. More and more research especially in new priority areas takes place outside conventional forest research centres. This means that the conventional centers have lost their monopoly on forest research. In the long run and from the viewpoint of the whole forest research, this can be even a positive development.

Although institutional changes in forest research are mainly connected with the structure of research funding we might face serious problems also with the amount of funding if we are not able to react properly and promptly to the needs of our clients. In many countries the forest research community has not been very successful in this respect. The lesson is that in order to survive we researchers must pay more attention to the communication between us and the policy makers and other users of our research results. The users often represent those who make decisions on research funding, and their dissatisfaction with us researchers and our findings can have a negative impact on our research budgets. Therefore, science/policy interface is becoming increasingly important to the wellbeing of research communities.

There seem to be "cultural" differences that create obstacles between the research community and the user community: researchers tend to stress facts, while the decision-makers tend to focus more on values. If we researchers want to get our message across, we must learn how to deal with values. In addition, we have to remember that research is only one policy instrument among many others.

IUFRO as an organization has tried to respond to the challenges of science/policy interface by establishing a special Task Force on this topic. In the next IUFRO World Congress, to be held in Brisbane, Australia, on 8-13 August, 2005, the Task Force will present its findings and give advice how to improve the partnership among researchers, policy-makers and society.

I am looking forward to seeing you all in Brisbane where also Research Group 7.04 will be active in organizing sessions.

INTERACTION OF OZONE AND GRASS COMPETITION ON ABOVE- AND BELOWGROUND GAS EXCHANGE IN PONDEROSA PINE

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Tropospheric O_3 has been shown to affect the physiology and growth of a wide range of herbaceous and woody plant species in the United States and Europe (U.S. EPA, 1996). However, it is difficult to identify the role of ozone stress on plant growth in the field because of other interacting stresses, such as plant competition, which are present in natural ecosystems. Since all possible combinations of stresses and conditions that exist in nature cannot be studied or fully characterized, it is important to understand the mechanisms of plant response to stress to extrapolate responses to greater spatial and temporal scales (Heck et al., 1998).

The goal of the present study was to characterize the effect of grass competition and O_3 stress on ponderosa pine seedling growth and physiology. Two year old ponderosa pine (Pinus ponderosa Laws.) seedlings were planted with 3 densities of blue Wildrye grass (Elymus glaucus Buckl.) in modified open-top chambers (Andersen et al., 2001). One seedling was transplanted into each of nine cells in competition boxes nested within 8 open top chambers (Fig.1). Each cell within the grid was approximately 53 X 53 X 50 cm, with a 15 cm border row. Blue wildrye grass seeds were sown at one of three densities (0= no grass; 1= 32 plants m^{-2}; 2= 88 plants m^{-2}) along with individual pine seedlings within each cell, and a duff layer was placed on top of the grass seeds. Watering was done when cells dried to between 5 and 10% soil moisture by volume.

Soil respiration (SR) and foliar gas exchange was measured monthly during the summer months of the 3-year study using a LiCor 6200 portable photosynthesis system. At final harvest all tissues were dried at 60° C to constant weight. Needle, stem, and fine root tissues were analyzed for P, K, S, Ca, Mg, Mn, Fe, Cu, Zn, Mo, and B (McQuaker et al., 1979) using ICP. Total nitrogen and carbon content were determined with a Carlo Erba EA1108 Elemental Analyzer (C.E. Elantech, Inc., Lakewood, NJ) by standard procedures. The experiment employed a split-plot design with two concentrations of ozone (90 ppm-hr cumulative exposure over each growing season; charcoal-filtered-air control) comprising whole plot treatments and three grass densities comprising split-plot treatments (Clark et al., 1995). Each grass treatment was replicated three times within each open-top chamber, and four chamber replicates were used for each ozone treatment (8 total chambers, 72 sub-plots).

Grass competition significantly reduced ponderosa pine biomass over the experiment at both planting densities. In the absence of grass competition, O_3 did not significantly reduce ponderosa pine growth. However, at the highest level of grass competition, O_3 significantly reduced ponderosa pine biomass below that of grass competition alone, resulting in a significant interaction. O_3 did not affect grass biomass.

SR varied significantly during the season, mainly the result of changing soil temperatures. There was a general pattern of higher SR in O_3 treatments during the first and second exposure seasons, although differences were seldom significant. During the third exposure season, SR was not different between O_3 and control treatments. Soil organic matter (SOM) increased in ozone treated plots compared to control plots early in the study, but by the end there were no significant differences in SOM between O_3 treatments. Soil moisture did not vary significantly among treatments.

Foliar gas exchange was similar among treatments until the end of the third season when differences were observed in 1- year old foliage. A significant interaction was observed such that photosynthesis in 1- year old foliage decreased with grass competition in O_3 treated seedlings, while controls showed an increase in photosynthesis with grass competition. Photosynthesis patterns were positively related to needle nitrogen, which also showed a significant interaction between O_3 and grass competition. Soil nitrogen levels were similar among treatments and could not explain the observed differences in needle nitrogen.

The results suggest that ponderosa pine seedlings are more susceptible to O_3 when growing in competition with blue wild-rye grass than when growing alone. Significant O_3 effects were first observed below ground, followed by changes in photosynthesis and growth by the end of the study. The significant interaction between O_3 and grass competition appears to affect nitrogen uptake and/or retention in ponderosa pine seedlings. The results indicate that root and soil processes may change relatively quickly in response to elevated O_3 concentrations, sometimes before above-ground changes become evident.

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RESPONSES OF MOSSES FROM THREE DIFFERENT HABITATS TO ENHANCED LEVELS OF UV-B RADIATION

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Levels of UV-B radiation not only increased around the Antarctic, but also in the Northern hemisphere at both temperate and higher latitudes (Madronich et al., 1998). The studies on the effects of UV-B mainly concentrated on higher plants, while in recent years some research groups focused on the response of UV-B on bryophytes as well (Gehrke, 1998; Lud et al., 2002; Niemi et al., 2002; Searles et al., 2002.). In contrast to higher plants, bryophytes depend on the air humidity to be in a physiologically active state. Direct repair of occurred damage e.g. through UV-B might therefore not always be possible when plants are dry (Gehrke, 1999). The circumstances of the habitat in which mosses occur might be important in protection against and adaptation to solar UV-B radiation.

In a greenhouse experiment we studied the differences in the response to UV-B radiation in bryophyte species from different habitats. We selected nine moss species from three different habitats (Table 1) and compared growth, DNA damage and UV-B absorbing compounds under four different levels of UV-B radiation and their UV-A control treatments.

Table 1: The nine different species exposed to different levels of UV-B radiation in this experiment and some characteristics of the habitat they originate from.

MOSS SPECIES	**HABITAT**	**CONDITIONS**	
		Wet (+) /Dry (-)	Solar exposure
Syntrichia ruralis	Dunes	-	+
Campylopus introflexus	Dunes	-	+
Polytrichum juniperinum	Dunes	-	+
Mnium hornum	Forest	+/-	-
Dicranum scoparium	Forest	+/-	-
Polytrichum formosum	Forest	+/-	-
Sphagnum recurvum	Bog	+	+
Sphagnum magellanicum	Bog	+	+
Polytrichum commune	Fen	+	+

We hypothesised that moss species which are generally not directly exposed to solar UV-B radiation (such as forest species) would be more sensitive to the UV-B, while species from open and wet habitats (bogs and fens) might be better adapted to UV-B radiation, since they are in a physiological active state thereby able to repair occurred damage directly. The dune species are often in a dry state when solar radiation is high, but they are directly exposed to solar UV-B radiation. Occurred damage cannot directly be repaired. Alternatively mosses in a dry state may avoid suffering from UV-B induced (DNA) damage. We therefore expect this group of species to show an intermediate response to UV-B radiation.

Preliminary results showed that UV-B radiation caused growth reduction in some species, while DNA damage increased with increasing UV-B levels in most species. Further analyses of the data are necessary to elucidate if UV-B sensitivity is related to habitat origin.

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DIAGNOSTICS IN BEECH EXPOSED TO CHRONIC FREE-AIR O_3 FUMIGATION: III. BIOCHEMICAL LEAF RESPONSES TO OZONE UPTAKE OF ADULT TREES

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In order to study and quantify the biochemical leaf response to ozone different biochemical and physiological investigations were carried out. The investigations were implemented on leaves of 60 years old beech trees exposed to doubled ozone concentrations for three years at „Kranzberger Forst" under free air conditions (Werner and Fabian, 2002). Sun and shade leaves of branches of five ozone treated (2 x O_3) and five trees in ambient air (1 x O_3) were analyzed for soluble sugars (glucose, fructose, sucrose) and starch using the method of Moore et al. (1997) in combination with HPLC techniques. Five single leaves of each branch were analyzed separately in order to get information on the distribution pattern of all carbohydrates within single branches. The samples were taken in May, June, July, September and October in 2003 on sunny days between 11:00 and 13:00. In July additional leaf samples were collected for a diurnal assessment.

The deviation of soluble carbohydrates and starch contents was unexpected low within branches. As expected sun leaves contained much higher values than shade leaves. The concentrations of analyzed sugars was correlated with the direction within the tree canopy. The highest concentrations of soluble sugars were found in leaves located in east, south and west crown position. However, the lowest values were found in leaves of the north position. In contrast starch was detected mainly in the northern part of the canopy.

A strong ozone effect was detected for starch. The concentration of sun and shade leaves was significantly lower in leaves of ozone treated canopies as compared to control trees. The first starch data for the diurnal assessment clearly indicate that ozone and control leaves behave different. For the soluble carbohydrates some significant differences in concentrations were found between the treatments and their position in the sun and shade crown at some of the time points within the seasonal course.

The data will be complemented with the activities of RubisCO and PEPC in order to understand the influence of the chronical ozone treatment on leaf physiology. In addition, gene expression of these two enzymes will be studied to get information on their regulation under different O_3 regimes.

Apart from that beech leaves were used for photosynthesis measurements and in parallel for biochemical analysis in order to compare CO_2-fixation under field conditions with enzyme activities of RubisCO and PEPC as well as with carbohydrate contents. The same approach was done with leaves of the cuvette experiments.

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EFFECTS OF ELEVATED UV-B RADIATION AND GROUND-LEVEL OZONE CONCENTRATION ON CO_2 DYNAMICS IN FEN ECOSYSTEMS AND ULTRASTRUCTURE OF PEATLAND PLANTS

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Elevated UV-B radiation and ground-level ozone concentration have been shown to have remarkable effects on structure and function of plants and ecosystems. Still long-term effects of UV-B and ozone on peatland ecosystem are largely unknown in spite of the great importance of northern peatlands as sinks of atmospheric carbon dioxide and their sensitivity to environmental changes.

In our three years' study (2003-2005) we are determining the long-term effects of the elevated UV-B radiation and ground-level ozone concentration on CO_2 dynamics of the fen ecosystems and ultrastructure of Eriophorum species and mosses. In the UV-B experiment on an oligotrofic flark fen in Sodankylä (Northern Finland, 67°22'N, 26°38'E) the study plots are exposed to constant supplement (46 %) of UV-B radiation. The ozone exposure is conducted at an open field facility in Kuopio, Central Finland (62°13'N, 27°35'E), using peatland microcosms cored into PVC tubes from an oligotrofic low-sedge Sphagnum papillosum pine fen (Mekrijärvi, 62°47'N, 30°56'E). The microcosms are exposed to twofold ozone concentration compared to monitored background concentration.

In the first summer, the elevated UV-B radiation did not have significant effect on the ecosystem's gross carbon dioxide uptake or dark respiration. Instead, the ground-level ozone lowered both the dark respiration and gross carbon dioxide uptake. The ultrastucture results from the first year and preliminary results from the second year will be also shown in the poster.

WAX AND UV-B-ABSORBING COMPOUNDS OF POLYTRICHASTRUM ALPINUM FROM DIFFERENT CLIMATIC REGIONS

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Introduction

Bryophytes are the simplest land plants and therefore central to the study of plant acclimation, particularly the impacts of light and UV radiation. Among mosses, the leaves of Polytrichaceae are the closest functional parallels to the leaves of vascular plants. Polytrichastrum alpinum (Hedw.) G.L. Smith, the mountain hair moss, is an polar alpine species found over large areas (Collins, 1976; Schiavone and Hyvönen, 1993) and a key species of special interest in view of enhanced temperatures and UV-B responses at high latitudes (Gignac, 2001; Huttunen et al., 2004). The species occupies mountain heaths and meadows and, in more southern locations, mainly shady rocks and cliffs.

The aim of the pilot study was to 1) get more detailed information on the chemistry and structure of extant bryophyte cuticles in order to use Polytrichastrum alpinum as a model plant for evergreen responses to polar and subarctic climate change and 2) to determine the extent of climatic adaptation and acclimation with special reference to protective wax structure and chemistry and UV-B-absorbing compounds.

Material and methods

The differences in the protective epicuticular wax structures and UV-absorbing compounds of Polytrichastrum alpinum gametophytes were studied by using herbarium specimens originating from different climatic regions.

The herbarium specimen material (n = 18) of the pilot survey was obtained from northern and southern high latitudes and alpine environments at low latitudes. The morphology of the specimens was indicated as size and silhouette. The wax amount was studied by dipping the samples in chloroform for 15 seconds and by weighing the samples and the wax gain. The acid methanol soluble UV-B-absorbing compounds were measured at different wavelengths within 260 – 340nm.

Results and discussion

Polytrichastrum alpinum is characterized as an obligate indicator of late-lying snow beds and a species that is absent from areas with a mean summer temperature above + 16^{o} C (Gignac, 2001). The morphology ("specific leaf area" or silhouette area) and size of the herbarium specimens in this study were greatly variable. Most gametophytes were narrow, straight and compact with small laminal lamellae leaves, but the samples from Macquarie Island, Tasmania, and South Shetland Islands, Antarctica, were broad and branched. The mean amount of chloroform-extractable surface waxes in young green parts was about 4.3 % of dry weight. The above mean wax amount values were obtained from low latitudes. Waxes have been mainly considered to provide drought protection and UV reflectance, but some wax components, e.g., secondary alcohols and β-diketones, do absorb ultraviolet light. One of the main components of epicuticular waxes in Polytrichum sporophytes is a secondary alcohol nonacosan-10-ol (Neinhuis and Jetter, 1995). The gametophytes of Polytrichastrum alpinum in this study had epicuticular waxes, which resemble ketone nonacosan-10-one structures (Jetter et al., 1996) (Figs. 1a-d). The abaxial sides of the leaf lamella margins were ornamented with low papillae covered with membraneous platelets. The size varied in the lamellae from 5 µm to 15 µm and in the papillae from 2 µm to 5 µm. In some samples the waxes were mixed with fibrous structures. The wax surfaces were water-repellent (Wagner et al., 2003), which should make surfaces self-cleaning, and to tolerate solifluction (Collins, 1976).

The absorbance maxima of the UV-B-absorbing compounds varied between 260 nm, in Faroe Islands, and 285 nm, in boreal samples from Kuusamo, Jäkälävuoma. An absorption peak at 265 nm, was observed in the samples originating from Khabarovsk in eastern Asia and Macquarie Island in Tasmania, South Shetland Islands in Antarctica and Sierra Nevada Yosemite Mountains in North America. The samples from mountainous low latitudes had lower UV absorption than the high-latitude samples, the difference being 1.5 to 3 times the absorbance value. The absorption maxima of the extracted surface waxes equalled those in the whole specimen. The absorption capacity of the surface-associated UV-B-absorbing compounds was about 5 % of total absorbance.

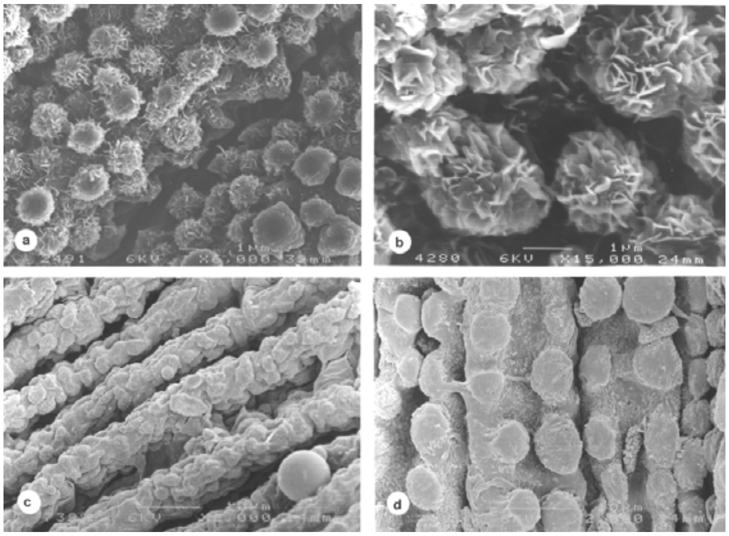

Fig. 1. The abaxial surface waxes of Polytrichastrum alpinum.
a) Papillar structure with nonacosan 10-one waxes (Kuusamo, Jäkälävuoma 66°15’N) bar=1 µm
b) Papillate structure with close-up of nonacosan 10-one waxes (Kuusamo Jäkälävuoma) (bar = 1 µm)
c) Papillate structure from Western Carpatians (49°25’N) (bar =10 µm)
d) Papillate structure from Swalbard (77°01’N) (bar=10 um). Note the differences in the papillate and lamellae structure between the Western Carpatians (c) and Swalbard samples (d).

The ages of the herbarium samples varied between a few months and 29 years, but the effect of sample age on UV-B-absorbing compounds remained equivocal, though the effects of climatic region and global radiation were evident.

As already mentioned, Polytrichastrum alpinum is known as a species favouring cool areas. The differences in wax morphology, UV-B-absorbing screening compounds and specific surface area reflected the adaptation and acclimation to the latitudinal light environment and the continental-maritime features.

The few samples from the southern hemisphere differed from the northern specimens in their low absorption maxima and their robust morphology. Their wax structure, water repellency and chemistry also exhibited exceptional features compared to samples from the northern hemisphere. Hyvönen et al. (2004) suggested in their phylogenetic analyses of Polytrichales that that Polytrichales originate from a common ancestor, and the Polytrichum species represent younger elements of the northern hemisphere, but conclusions whether the common ancestor was found in the north or south should not be made.

Differences in Polytrichastrum alpinum gametophytes revealed three different features: differences in specimen morphology as expected, differences in the ultrastructure of epicuticular waxes and different absorbance maxima of UV-B-absorbing compounds.

Acknowledgements

This preliminary study is a part of a project (financed by the Academy of Finland project no. 173 193 and Thule Institute Northern Issues Programme) titled “UV acclimation in evergreens – mosses as model plants”, which aims to study the species-specific functional responses of arctic and subarctic evergreens. Tiia Taipale, Phil Lic., Ulla Timonen, M.Sc. and Ms. Marianne Kosonen are thanked for technical assistance.

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THE EFFECTS OF WILDFIRE, SALVAGE LOGGING, AND POST-FIRE N FIXATION ON THE NUTRIENT BUDGETS OF A SIERRAN FOREST

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Abstract

The effects fire, post-fire salvage logging, and revegetation on nutrient budgets were estimated for a site in the eastern Sierra Nevada Mountains that burned in a wildfire in 1981. Approximately two decades after the fire, the shrub (former fire) ecosystem contained less C and more N than the adjacent forest ecosystem (Johnson et al., 2004).

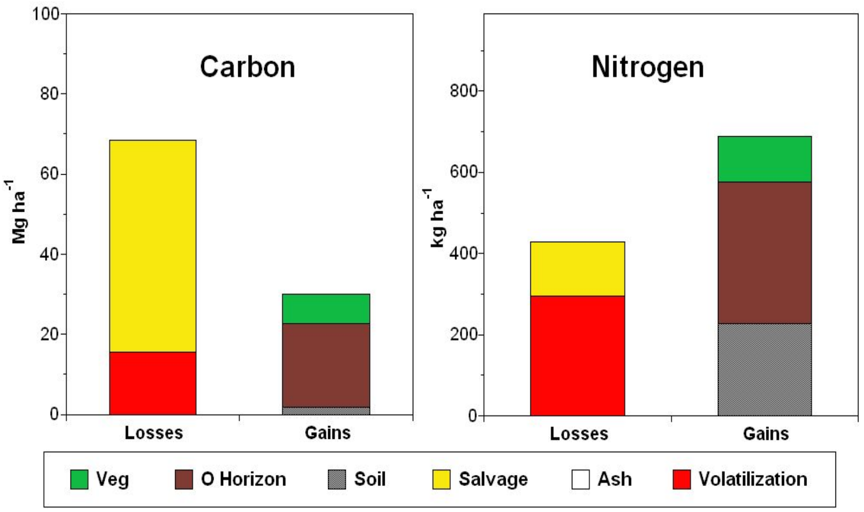

Fig. 1. Estimated losses of C and N by volatilization and salvage logging in the Little Valley fire and gains in soil, O horizon and vegetation over the subsequent two decades. (From Johnson et al., in press)

Reconstruction of pre-fire nutrient budgets suggested that most C was exported in biomass during salvage logging and will not be recovered until forest vegetation occupies the site again. Salvage logging may have resulted in longer-term C sequestration in wood products than would have occurred had the material been left in the field to decay, however. Reconstructed budgets suggested that most N was lost via volatilization during the fire rather than in post-fire salvage logging (assuming that foliage and O horizons were combusted). Comparisons of the pre-fire and present day N budgets also suggested that the lost N was rapidly replenished in O horizons and mineral soils, probably due to N fixation by snowbush (Ceanothus velutinus Dougl.), the dominant shrub on the former fire site.

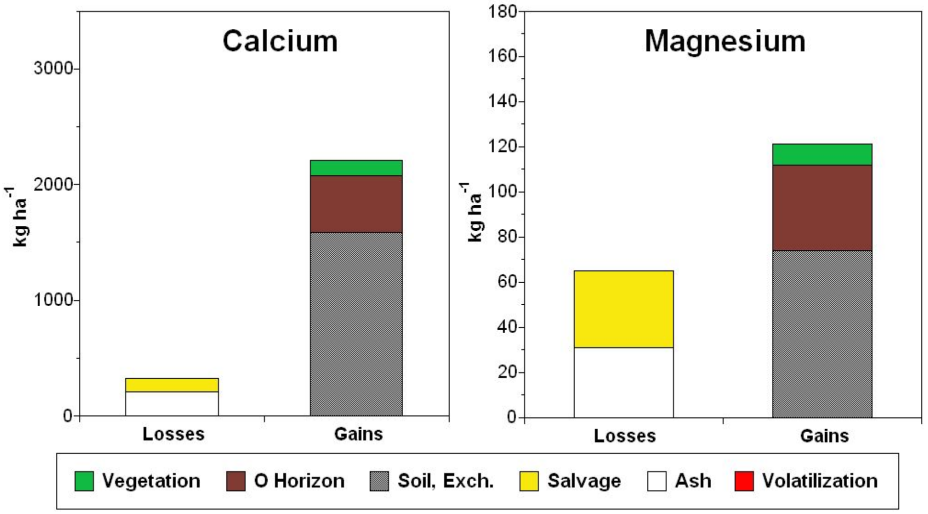

Fig. 2. Estimated conversion of Ca and Mg to ash during the fire, in the Little Valley fire losses by salvage logging and gains in soil, O horizon and vegetation over the subsequent two decades. (From Johnson et al., in press)

There were no significant differences in ecosystem P, K, or S contents and no consistent, significant differences in soil extractable P or S between the shrub and forested plots. Exchangeable K$^+$, Ca^{2+}, and Mg^{2+} were consistently and significantly greater in shrub than in adjacent forested soils, however, and the differences were much larger than could be accounted for by estimated ash inputs (Johnson et al., in press). In the case of Ca, even the combustion of all aboveground organic matter could not account for more than a fraction of the difference in exchangeable pools. We hypothesized that the apparent large increased in soil and ecosystem Ca content resulted from either the release of Ca from non-exchangeable forms in the soil during the fire or the rapid uptake and recycling of Ca by post-fire vegetation.

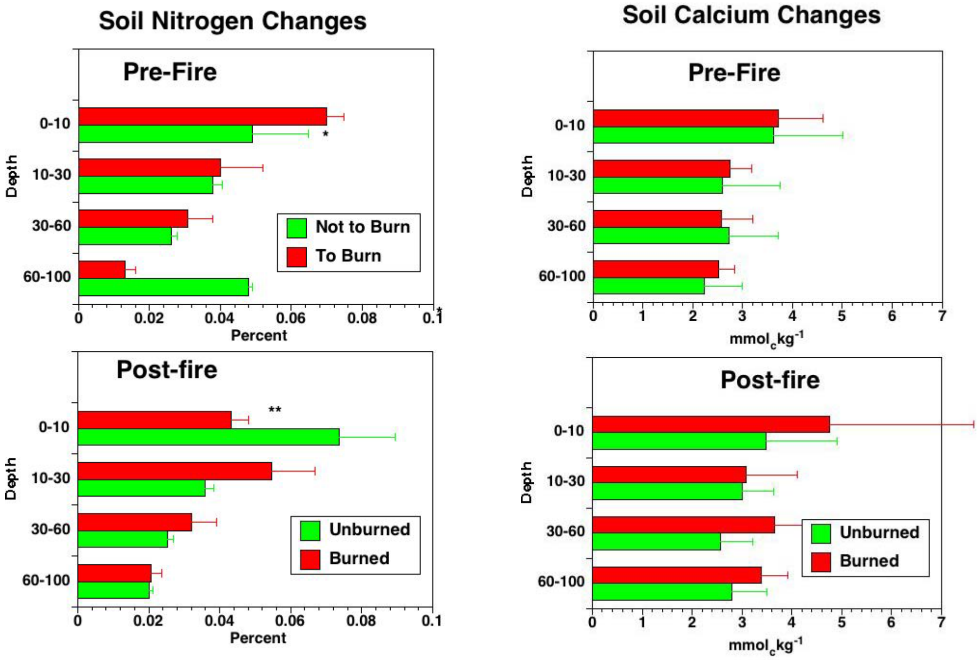

Fig. 3. Changes in soil total nitrogen and exchangeable calcium as a result of the Gondola fire.

We had an unparalleled opportunity to study the before and after effects of a recent wildfire in the Lake Tahoe Basin in Nevada on the same soil type and similar vegetation as at Little Valley. Sixteen replicated plots had been laid out and sampled before the fire in anticipation of conducting prescribed fire and harvesting studies. An accidental wildfire set on 3 July 2002 burned seven of the plots completely, allowing us both before/after and control treatments with which to assess fire effects. Resampling of soils from this fire showed significant soil C and N losses in the A horizons due to fire, but, surprisingly, no statistically significant changes in soil exchangeable Ca^{2+} or Mg^{2+}. Thus, at this stage, the hypothesis that fire caused the decomposition of soil minerals and/or oxidation of soil organic Ca and Mg to explain the apparent large increases in exchangeable Ca^{2+} and Mg^{2+} twenty years after the fire in Little Valley is not supported. Instead, the hypothesis regarding uptake and recycling by post-fire vegetation is supported. Collectively, these results suggest that post-fire vegetation may have a stronger influence on soil carbon, nitrogen, and base cation status than fire itself does.

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OZONE-INDUCED POLYAMINE RESPONSE IN SCOTS PINE

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Introduction

Polyamines (putrescine, spermidine and spermine) are nitrogenous compounds that are ubiquitous in living organisms (Pegg, 1986). Polyamines can occur in plant cells as free forms but also as conjugated fractions, which are covalently linked to low molecular weight (soluble conjugated) or high molecular weight (insoluble conjugated) compounds.

The polyamine metabolism of plants has recently been studied, and one of the objectives is to find out what changes occur in polyamines in plants suffering from stress. Polyamine concentrations have been shown to respond to various stress factors in plants (Bouchereau et al., 1999), and ozone-induced changes in polyamines have also been detected in several coniferous species (e.g. Langebartels et al., 1998). Wellburn and Wellburn (1996) observed a decrease of total polyamines in Pinus taeda in an experiment where young seedlings were passed through short episodic exposures of ozone. On the other hand, there was more putrescine and spermidine in Pinus halepensis after ozone fumigation (Scalet et al., 1995), and Wellburn et al. (1996) also observed an increase of total polyamines (although putrescine declined) due to ozone in Aleppo pine.

We noticed that ozone exposure decreased the concentrations of free putrescine and spermidine in Scots pine trees in the early summer following the first season of ozone exposure (Suorsa et al., 2002) as a carry-over effect of ozone stress. It was hypothesized in our study that this difference will continue to exist after three seasons of ozone exposure and, in addition to this, changes in conjugated polyamines will take place in the ozone-treated pines.

Materials and methods

Ten- to 15-year-old Scots pines (Pinus sylvestris L.) were planted on the experimental field of the University of Oulu in September 1997. The trees were divided into three treatments: 6 trees in OTCs with non-filtered ambient air (NF), 6 trees in OTCs exposed to non-filtered ambient air with supplemental ozone (NF+O_3), and 6 trees on open-field plots (AA). In May, the amount of ozone applied was ambient + 40 ppb, in June ambient + 30 ppb, in July ambient + 20 ppb and in August ambient + 10 ppb. In September, only ambient air was given. The experiment was carried out in the years 1998-2000. The amount of ozone applied followed the natural variation in ozone concentrations in northern Finland, where ozone episodes occur in spring and early summer (Laurila and Tuovinen, 1996). The cumulative O_3 exposure index AOT40 of the experiment was calculated as a sum of the hourly O_3 concentrations above the cut-off of 400 ppb (results presented in Manninen et al., 2003).

The samples for polyamine analyses were collected on the 6th of September, 2000 and on the 30th of May, 2001 (needle year classes 98, 99 and 00). The free, soluble conjugated and insoluble conjugated (PCA-soluble and PCA-insoluble) polyamines were analyzed with HPLC (Merck-Hitachi) as described in detail by Sarjala and Kaunisto (1993). The polyamine data were subjected to an analysis of variance, and the difference between treatments (NF, NF+O_3 and AA) was analysed with Microsoft Excel (Microsoft Office 2000 SR-1 Standard).

Results

Free polyamines

The results of the free polyamine analyses after the first season of ozone exposure were published by Suorsa et al. (2002). We noticed that ozone exposure lowered the level of free polyamines, especially putrescine, and the response took place in the early summer after the first exposure season (June 1999). The analyses were done before the second exposure season began. The free polyamine contents were also lowest in ozone-exposed trees after the third season of ozone exposure (September 2000) in the needle year classes 98 and 99 (Fig. 1), when a significant difference between the treatments was observed in spermidine (needle year class 98, $p=0.026$). In addition, the free polyamines were analysed in the following

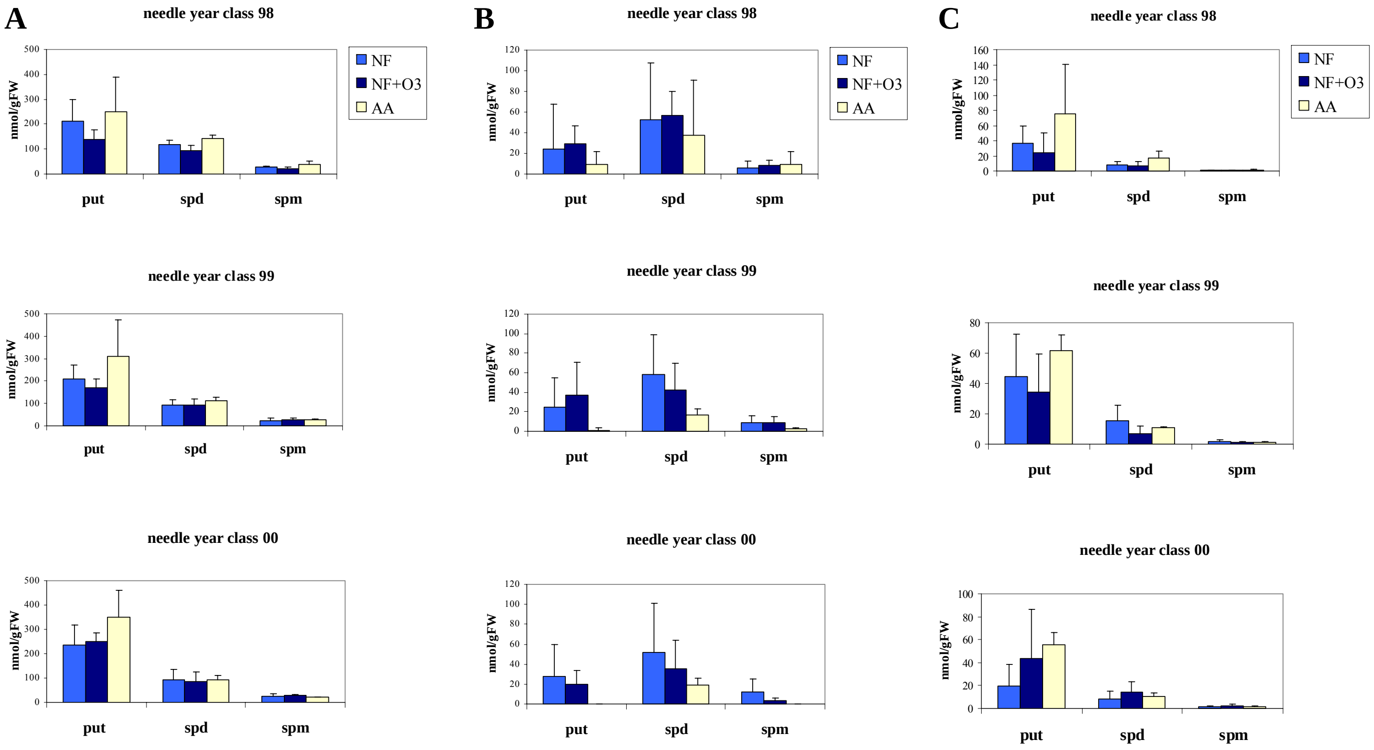

Fig. 1. Free (A), soluble conjugated (B) and insoluble conjugated (C) polyamines in the needles of Scots pine in non-filtered ambient air (NF, n=6), in non-filtered ambient air with supplemental ozone (NF+O_3, n=6), and on open field plots (AA, n=2) in September 2000. put = putrescine, spd = spermidine, spm = spermine.

spring (May 2001), but the contents no longer differed between the treatments (data not shown).

Soluble conjugated polyamines

The fraction of soluble conjugated polyamines was analysed after the third season of ozone exposure, and the putrescine (needle year classes 98 and 99) and spermidine contents (needle year class 98) were highest in the ozone-exposed trees (Fig. 1), although the difference was not statistically significant. In the following spring (May 2001), the ozone-exposed trees had higher polyamine levels than the NF trees, although the highest levels were measured in the AA trees (data not shown).

Insoluble conjugated polyamines

Ozone exposure lowered the levels of insoluble conjugated putrescine and spermidine in September 2000 (Fig. 1) as well as in May 2001 in the needle year classes 98 and 99, but no significant differences between the treatments were observed.

Discussion

The levels of free polyamines were lowest in the 1-year-old and 2-years-old needles of the ozone-exposed trees after the first season of exposure, indicating a carry-over effect (Suorsa et al., 2002). The level of free polyamines remained low in the ozone-exposed trees after the third season of exposure. The stronger response of the free polyamine pool to ozone compared to that of conjugated polyamines was prominent, because conjugated polyamines are assumed to have scavenging properties comparable to those of the known efficient radical scavengers, e.g. ascorbate (Bouchereau et al., 1999). The decrease of free polyamines may indicate ozone-induced premature senescence in Scots pine. Sandermann (1996) as well as Wellburn and Wellburn (1996) suggested that the induction of either the polyamine- or the ethylene-dominated pathway might represent an important physiological switch to amplify the ozone effects, because ethylene is a plant hormone related to senescence, and the effects of polyamines and ethylene on senescence are thought to be antagonistic.

There was a decreasing trend in net photosynthesis, changes in pigment ratios and a reduction in shoot growth in the ozone-exposed trees in our experiment, which indicates that peak ozone episodes during the early summer are harmful to subarctic Scots pine (Manninen et al., 2003). According to the polyamine results presented here, it seems likely that the potentially protective polyamine pathway is not induced in Scots pine needles due to ozone exposure.

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FUTURE CLIMATE EXTREMES – MODELLING THE EFFECTS ON NORWAY SPRUCE

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Climate extremes affect tree vitality

Climate change will be a challenge to forestry, as not only the mean temperature but also the magnitude and frequency of weather extremes, e.g. storms, temperature backlashes and heat spells, could increase. Both spring frost and summer drought could damage Norway spruce (Picea abies*)*, increasing the risk for attacks by pests and fungal pathogens. Wind-thrown spruce trees are the key-factor for an increasing population of the spruce bark beetle Ips typographus, and the temperature is the key factor controlling the developmental time from egg to mature bark beetle.

Model data and Impact indices

The results presented were based on data from the Hadley Centre regional climate model HadRM3. The control period 1961-1990 was compared with the SRES A2 and B2 scenarios for 2070-2099.

Spring backlashes

The risk for frost damage has been calculated by four different indices, based on the 1/ start of dehardening, 2/ severity of temperature backlash, 3/ timing of bud burst and 4/ cold hardiness level. The difference between the future scenarios and the control period indicated that the risk for frost damage could increase in the southern part of Sweden. The effect became gradually less pronounced towards the north, as the seasonal temperature progression during the sensitive season is faster further north (Jönsson et al., 2004).

Dry spells

The risk for summer drought in Sweden, expressed as the number of dry days during the period July 15th to August 15th, will on average increase by 4-8 days according to scenario SRES A2. This is in accordance with findings by Christensen and Christensen (2004). However, they also pointed out an increased risk for extreme summer precipitation, i.e. flooding.

Storms

Storm damage in Swedish forests seems to have increased during the 20th century, and the highest wind-throw damage per ha was found in southern Sweden. Apart from a possible shift in storm intensity and frequency, the increase in storm damage can be attributed to changes in forest management (Nilsson et al., 2004). According to scenario SRES A2, there might be an increased storm frequency in a zone from northern Germany to southern Scandinavia in relation to the control period (Leckebusch and Ulbrich, 2004) as an effect of a general strengthening of the wind climate, thus increasing the risk for storm felling.

Spruce bark beetle

Today, a second generation of Ips typographus is rarely produced in Sweden. Higher temperatures will increase the risk for a swarming period during late summer, as the spring swarming and the development from egg to bark beetle will be completed earlier (Jönsson, 2004).

Discussion

These results show that climate change could increase the risk of frost damage, drought stress and bark beetle attacks in Norway spruce forests. The impact caused by one stress factor can affect the risk for damage caused by another, enhancing the negative effects by reinforcing feedback loops:

- A severe spring frost causing root and bark damage could render the trees more sensitive to summer drought.

- Long-term summer droughts may reduce the tree's carbohydrate reserves, lowering the defence capacity as well as the ability to sustain repeated cycles of hardening and dehardening caused by temperature fluctuations in early spring.
- Storm could damage tree roots, lowering the water uptake ability and increasing the sensitivity to drought. Wind-thrown spruce trees increase the amount of breeding material for Ips typographus, and a population build-up will increase the risk for attacks on living trees.
- A warmer climate will affect the population dynamic of Ips typographus, increasing the risk for a late summer swarm in the southern Sweden. Whether this will cause a substantial increase in population size or not will depend on autumn temperatures, as the winter mortality of immature bark beetles is very high.

Conclusion

Climate change could increase the risk for forest damage. Further studies aiming to assess the most probable threat in different geographical regions are needed, as the information could be valuable when planning for regeneration of forest stands. In order to reduce uncertainties associated with climate modelling, data from several climate models needs to be evaluated.

Acknowledgement

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ROOT AND LEAF LITTER RESPONSES OF SILVER BIRCHES EXPOSED TO ELEVATED CO_2 AND O_3

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The tropospheric concentrations of CO_2 and O_3 are increasing by 1–2 % per year, and common expectation is that atmospheric CO_2 and O_3 levels will be doubled by year 2100. The prevailing hypothesis is that elevated CO_2 will increase fine root and mycorrhiza growth and soil respiration rates, whereas elevated O_3 is harmful to plants and thus might diminish below-ground carbon allocation. Both elevated CO_2 and O_3 are thought to affect the leaf litter quality by changing the litter C:N-ratio and thus decreasing the subsequent decomposition rates.

In order to study effects of elevated CO_2 and O_3 on soil respiration, fine roots, mycorrhizas and leaf litter decomposition, we exposed initially seven-year-old silver birch trees (Betula pendula Roth) to elevated CO_2 and O_3 alone and in combination in open-top chambers for three growing seasons (1999–2001). In an earlier seedling study, clone 4 and 80 trees used in the present OTC-experiment were determined as ozone-tolerant and ozone-sensitive genotype, respectively. Fine root and mycorrhiza growth was studied with root ingrowth bag (2000–2001) and soil core sampling methods (2002) and soil respiration measurements were conducted with a soil respiration chamber attached to LI-COR 6250 infrared analyzer (1999–2001). In year 2001, cumulative biomass of fungal fruit bodies was determined from chamber treatments. We also performed three litter-bag field experiments (1999–2000, 2000–2001 and 2001–2002) in order to study whether the exposure to elevated CO_2 and O_3 changes the subsequent leaf litter decomposition.

Fine root (≤2 mm) growth in root ingrowth bags was not significantly affected by any of the treatments. However, soil core samples taken after the experiment indicated that in both clones the positive effect of elevated CO_2 on standing biomass of fine roots was diminished under elevated O_3 (EC+EO), this trend being most obvious in clone 4 trees. The total mycorrhizal infection level increased under elevated CO_2 (EC) and elevated O_3 (EO) treatments. Interestingly, elevated O_3 changed the relative proportions of some mycorrhizal morphotypes, e.g. it increased the amount of thick-sheathed and old mycorrhizas and decreased the proportion of thin-sheathed liver-brown mycorrhizas and black mycorrhizas with rhizomorphs. A negative effect of ozone on the cumulative biomass of all fungal fruit bodies increased with time in clone 4 trees especially. In contrast, soil respiration rates were increased due to ozone, while elevated CO_2 increased soil respiration in clone 80 only. Litter-bag studies conducted with leaf litter collected in 1999 and 2000 showed that clone 4 leaves produced under elevated CO_2 had decreased decomposition rates, although this trend did not continue throughout these litter-bag experiments. At the end of the last litter-bag experiment (2001–2002), ozone exposed leaves had significantly lower decomposition rates.

In conclusion, elevated CO_2 effects on fine roots and mycorrhizal morphotypes were relatively small, while elevated ozone changed the proportions of mycorrhizal morphotypes, decreased the biomass of fungal fruit bodies and increased soil respiration. Elevated ozone decreased leaf litter decomposition after third exposure season, whereas the negative effect of elevated CO_2 was observed after first and second exposure season in clone 4 only. Although there was no clear clonal difference in O_3 response, clone 4 was in general more responsive to ozone stress than clone 80, which result is in contrast with the previous seedling study.

EFFECT OF INCREASED UV-B RADIATION ON FLAVONOIDS IN SEEDLINGS OF BETULA PUBESCENS AND B. PENDULA

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Introduction

Increased UV-B radiation due to ozone depletion challenges the plants to increase protection from UV-B. Flavonoids are a means of protection and their concentration can increase in response to elevated UV-B radiation (e.g. Lavola et al., 1997; de la Rosa et al., 2001; Tegelberg et al., 2001). In addition, concentrations of flavonoids may change in response to nutrient availability (e.g. de la Rosa et al. 2001, Keski-Saari and Julkunen-Tiitto 2003). There occur also changes during ontogeny (Julkunen-Tiitto et al., 1996; Tegelberg et al., 2001). We studied flavonol glycosides in seed-germinated birch seedlings at the beginning of their development. We were especially interested in the ability of young seedlings to react to elevated UV-B radiation. Also, we were interested if the level of nitrogen fertilisation affects this ability, and if seedlings of two birch species react similarly to the two environmental factors.

Material and methods

Seeds of two birch species, common white birch (B. pubescens Ehrh.) and silver birch (B. pendula) were germinated and the seedlings grown in the UV experimental field at the Botanical Gardens of the University of Joensuu, Finland (62°36’N, 29°40’E). Experimental design consisted of fifteen lamp frames divided into five blocks. Each block consisted of a constant 50 % increase in UV-B compared to ambient, UV-A control (because of the small increase in UV-A emitted by the lamps) and control with unlit lamps (see Tegelberg et al., 2001 for details). A mixture of unfertilised turf, vermiculite and perlite 7:2:1 was used in the pots. The seedlings were fertilised using Ingestad’s nutrient solution for birch (Ingestad, 1962), except three levels for nitrogen: 3 ppm, 30 ppm and 140 ppm. Under each frame there was one pot of each fertilisation level of each species.

The experiment lasted for two months. Then the seedlings were dried at room temperature and aboveground biomass was measured. The largest leaves of eight seedlings per pot were combined for analyses. Flavonoids were determined from methanol-extracted samples by HPLC, as described by Julkunen-Tiitto et al. (1996). Both birch species contained myricetin 3-galactoside, myricetin 3-glucoside, myricetin 3-arabinoside, myricetin 3-rhamnoside, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-arabinopyranoside, quercetin derivative 1, quercetin 3-rhamnoside and kaempferol 3-rhamnoside. In addition, leaves of B. pubescens had quercetin 3-glucuronide, kaempferol 3-galactoside, kaempferol 3-glucoside, kaempferol 3-glucuronide, kaempferol derivative 1 and B. pendula quercetin 3-arabinofuranoside and quercetin derivative 2. With our method, quercetin 3-glucoside and quercetin 3-glucuronide coincided, and quercetin 3-rhamnoside coincided with kaempferol 3-glucoside and kaempferol 3-glucuronide.

Results and discussion

Neither species nor UV-B had any main effect on the aboveground biomass of the seedlings at the end of the two-month experiment. Fertilisation with different concentrations of N affected biomass significantly. In both species, B. pubescens and B. pendula, growth increased markedly at the highest level of N compared to the lower concentrations of N.

In seedlings of both species, higher concentrations of quercetin 3-galactoside and quercetin 3-rhamnoside were induced by elevated UV-B. However, in B. pubescens quercetin 3-rhamnoside could not be separated from co-occurring kaempferol 3-glucoside and kaempferol 3-glucuronide; and increase in peak area might be due to an increase in any or all of them. In B. pubescens quercetin 3-glucoside+glucuronide and in B. pendula quercetin 3-arabinopyranoside were also induced by elevated UV-B. The results agree with previous results (e.g. Tegelberg et al., 2001) that quercetin glycosides are induced in response to elevated UV-B. Their structure is the most efficient in protection against UV-B. Increase in quercetins might be a fairly universal protective response in deciduous trees to elevated UV-B (e.g. for willows, see Tegelberg et al., 2003), at least during the first year of growth.

In both B. pubescens and B. pendula N affected the concentration of quercetin 3-galactoside so that it was lowest at the highest availability of N. In B. pubescens, myricetin 3-galactoside+glucoside and quercetin 3-arabinopyranoside were also affected. The concentrations were lowest at the highest level of N. No interaction between UV and N was detected.

Summary

Generally, quercetin glycosides were increased by elevated UV-B in both birch species. Quercetin 3-galactoside was affected by both elevated UV-B and nitrogen level in both species.

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DEFOLIATION NEGATIVELY AFFECTS FOREST TREES' ECTOMYCORRHIZAL SYMBIONTS

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Introduction

Most woody plants form ectomycorrhiza (ECM) in their roots with symbiotic soil fungi. Trees can form ECM with several dozens of fungal species at the same time and have several of them on the same fine root. ECM fungi benefit their host trees by providing them with nutrients and water through their extensive external mycelia in the soil. In natural boreal forests almost all root tips are covered with ECM mantle (Taylor et al., 2000) and thus the trees' nutrient uptake is almost completely performed by these symbiotic fungi. ECM fungi are also costly for the host tree, since 10-30% of host tree's primary production is estimated to be allocated to ECM fungi (Finlay and Söderström, 1992).

Defoliation caused by air pollution or herbivory reduces host tree's photosynthesising leaf area, and may thus cause remarkable harm also for ECM associations. Defoliation has been found to either reduce total ECM colonisation (Gehring and Whitham, 1991; Gehring et al., 1997) or have no effect at all (Markkola, 1996; Saikkonen et al., 1999). Although defoliation does not necessarily affect total colonization, it may alter the below ground community structure of ECM fungi (Saikkonen et al., 1999). Defoliation changed ECM morphotypes towards smooth, thin-mantled and assumedly less carbon demanding types in Scots pine roots (Saikkonen et al., 1999). Different cost of symbiosis may play an important role, and it has been suggested that a fungal symbiont with low cost may have a competitive advantage in colonizing the roots of a plant with reduced photosynthetic capacity (Saikkonen et al., 1999).

To study the effects of defoliation on ECM fungi we performed three sets of experiments to study various effects of simulated defoliation on ECM symbionts: 1) Young Scots pine (Pinus sylvestris) trees defoliated in middle of growth season (all old needles clipped off) on two consecutive years; 2) White birch (Betula pubescens) seedlings defoliated with 50% or 100% intensity on one or two consecutive years; 3) Scots pine seedlings defoliated early, middle or late in the growth season (all old needles clipped off) on one, two or three consecutive years. Experiment 1 was done in natural nutrient-poor forest, experiments 2 and 3 at experimental field. All defoliations were performed manually.

Results

We did not found reduction in overall ECM colonization in any of the experiments despite even a severe, repeated defoliation. Yet there is evidence that simulated herbivory has certain negative impacts on ECM fungi. Sporocarp production of ECM fungi around defoliated pines declined in natural, nutrient poor forest (Kuikka et al., 2003). Furthermore, colonisation by ECM morphotypes with relatively high amount of fungal biomass decreased in both birch and pine fine roots (Kuikka et al., 2003; Markkola et al., 2004). Similar trend was also found in Scots pine seedlings (Kuikka et al., unpublished). In accordance with these results, fine root fungal biomass (i.e. ergosterol concentration; indicating live fungal biomass bound to ECM symbionts) decreased in defoliated birch compared to controls (Markkola et al., 2004). The response was non-cumulative, since defoliation affected fungal biomass significantly only when conducted during current season. Defoliation negatively affected also host growth in all experiments.

Apparently it is not beneficial for the host tree to reduce the ECM colonisation even when the carbon gain is limited. Nutritional benefits may be important in sustaining ECM associations despite the reduced carbon availability, especially in the harsh field conditions.

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ACCLIMATION OF THE MOSS PLEUROZIUM SCHREBERI (BRITT.) MITT. *IN SITU* TO ULTRAVIOLET RADIATION

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Introduction

Experiments with increased UV-B have shown that flavonoids and related phenolic compounds increase in higher plants. The other stress factors can affect a great deal to the effectiveness of UV-B radiation (Caldwell et al., 1995). The aim here was to measure responsiveness with acid methanol-extractable UV-absorbing compounds of the moss Pleurozium schreberi (Britt.) Mitt. under enhanced UV treatment *in situ*.

Materials and methods

The modulated UV research field (FUVIRC field) was situated in a dry pine forest with moss and lichen cover in Sodankylä (67ºN). The system maintained the UV-B treatment level at a constant 46 % above the ambient level of UV-B (CIE weighted), corresponding to about 20 % ozone depletion. The samples were collected from three treatments under 15 lamp banks; supplemental UV-B+UV-A (cellulose diacetate filters, transmission down to 290nm), UV-A control (polyester filters, absorbs almost all radiation below 315nm) and ambient control (equal shading). The experiment was started on June 14, 2002. The samples of P. schreberi were collected at 8-9 a.m. on June 5, August 7 and October 9 in 2002 and on May 16, June 6, June 26 and October 1 in 2003 (Fig. 1 and 2 a, b).

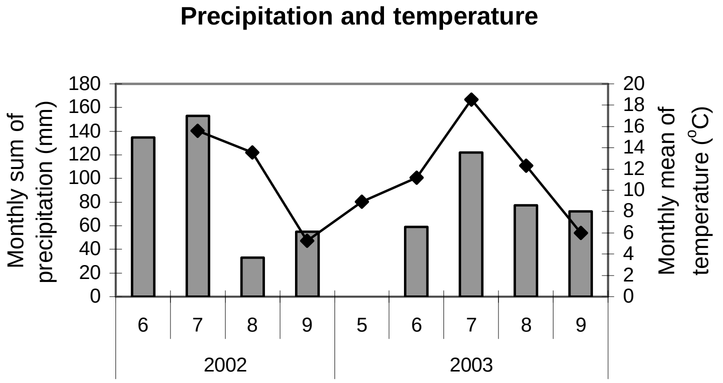


Fig. 1. Precipitation and temperature at Sodankylä summer 2002 and 2003.

The UV-B-absorbing compounds were determined by extracting 5 mg of dried moss material in acidified methanol (79:20:1 v:v:v, methanol:H_2O:HCl). The moss samples were weighed and the top surface area of the samples was measured (n=15). The specific surface area (here called specific leaf area, SLA, mm^2 mg^{-1}) of the samples was calculated by dividing the measured surface area by leaf mass. The absorbance of the extracts were analyzed with a spectrophotometer (Beckman, DU-64) at the wavelengths of 280, 300, 320, 340 and 360nm. The absorbance of UV-B ($A_{280-300}$) and UV-A ($A_{320-360}$) per sample were calculated by summing up the absorbance values at single wavelengths. The content of UV-B-absorbing compounds was expressed as per specific leaf area (SLA). SLA was also used to measure growth.

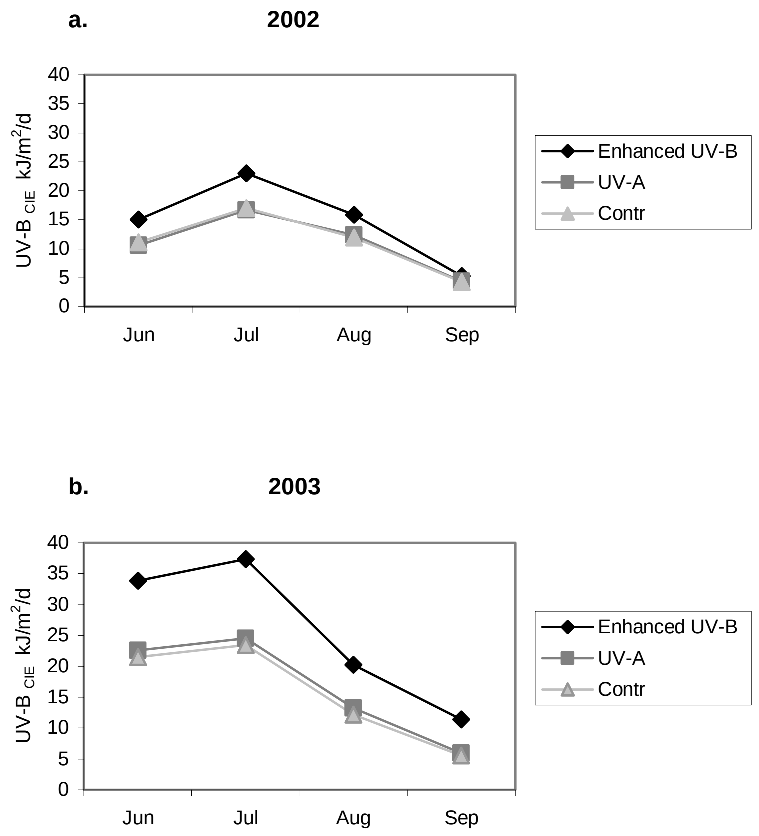


Fig. 2. Dose of UV-B $_{\mathrm{CIE}}$ radiation in the treatments in 2002 (a.) and 2003 (b.).

Results and discussion

The specific leaf area (SLA) was higher in 2002 than in 2003. SLA was significantly higher in the UV-B treatment than in the control area in 2003 (One-way ANOVA F=4.656, p=0.013). There were no differences in 2002. When the treatments were tested separately for each collecting day, there were no differences between the treatments (Fig. 3).

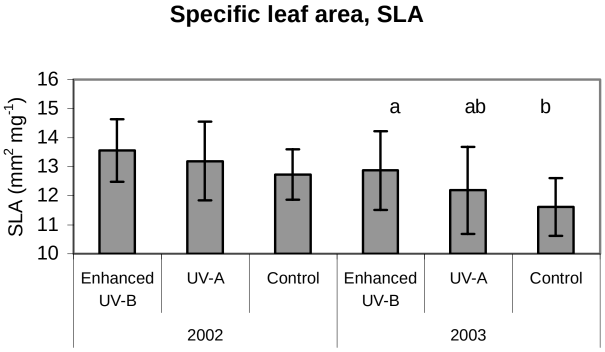
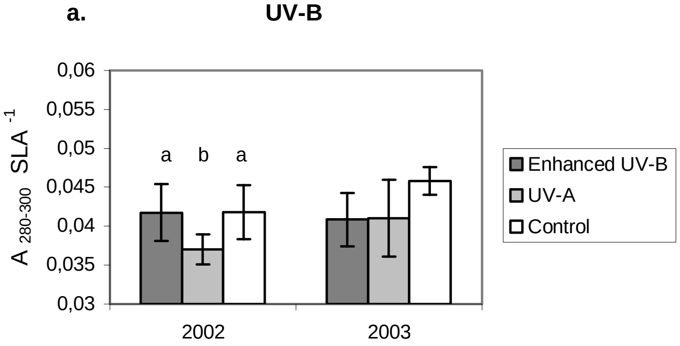


Fig. 3. The specific leaf area of Pleurozium schreberi in 2002 and 2003.

The absorbance of UV-B wavelengths per specific leaf area ($A_{280-300}$ SLA^{-1}) was smaller in the UV-A treatment than in the other treatments in 2002 (One-way ANOVA F=3.949, p=0.027, Fig. 4a.). The absorbance of UV-A wavelengths per specific leaf area ($A_{320-360}$ SLA^{-1} was higher in the enhanced UV-B than in the UV-A treatment in 2002 (F=4.948, p=0.012, Fig. 4b.). No differences were found in 2003. When the treatments were tested separately for each collecting day there were differences between the treatments only in the samples collected on August 7, 2002.

a.



b.

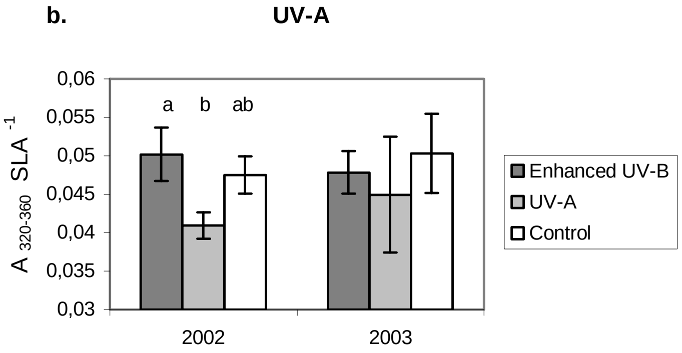


Fig. 4. The total absorbance per specific leaf area, a) $A_{280–300}$ nm SLA^{-1}, b) $A_{320-360}$ nm SLA^{-1}.

Discussion

Experiments with higher plants have shown that SLA increases with decreasing irradiance (Shipley and Almeida-Cortez 2003). In this study, SLA was smaller in every treatment in the year 2003 compared to 2002. SLA was, however, higher in the enhanced UV-B or UV-A compared to control. It has been discovered that the ratio of photo repair wavelengths (UV-$A_{320-400}$ nm and blue light) to damaging wavelengths (UV-B) has an effect on the severity and even the direction of UV-B-induced reactions (Teramura et al., 1980; Gehrke et al., 1996).

Gehrke (1999), among others, presented that elevated UV-B radiation does not have any effect on the mean flavonoid concentration of mosses after short-term exposure. During a two-year experiment we found minor effects. The absorbance of UV-B ($A_{280-300}$ SLA^{-1}) was smaller in the UV-A treatment than in the other treatments in 2002. The absorbance of UV-A ($A_{320-360}$ SLA^{-1}) was smaller in the UV-A treatment than in the UV-B treatment in 2002. The differences in SLA between the treatments have an effect on the results of UV-absorbing compound absorbance per SLA. The differences in absorbance between the treatments leveled off as the experiment continued for a second year, but changes in specific leaf area affected the results during the second year.

Acknowledgements

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MODELLING CRITICAL LEVELS OF OZONE FOR THE FORESTED AREA OF AUSTRIA
MODIFICATIONS OF THE AOT40 CONCEPT

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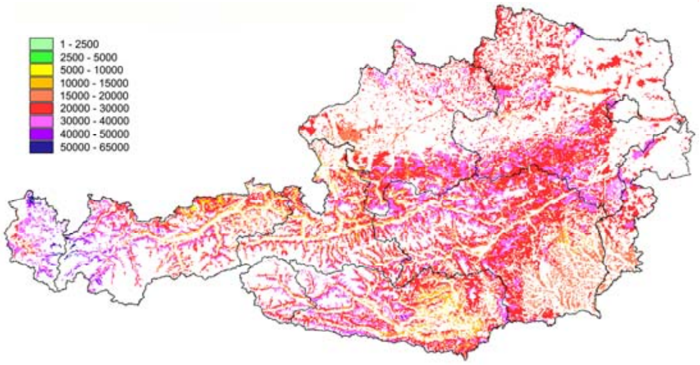
Abstract

The ozone risk impact for trees has been calculated by modelling the AOT40 (UN-ECE 1994; = accumulated exposure over a threshold of 40ppb. Provisional Critical Level for ozone, an AOT40 value of 10 ppm.h) for forest areas based on the Austrian Forest Inventory, ozone measuring data 1993 (120 monitoring sites), meteorological data and an elevation model. However, the **basic AOT40 approach** does not reflect the health status of Norway spruce in Austria.

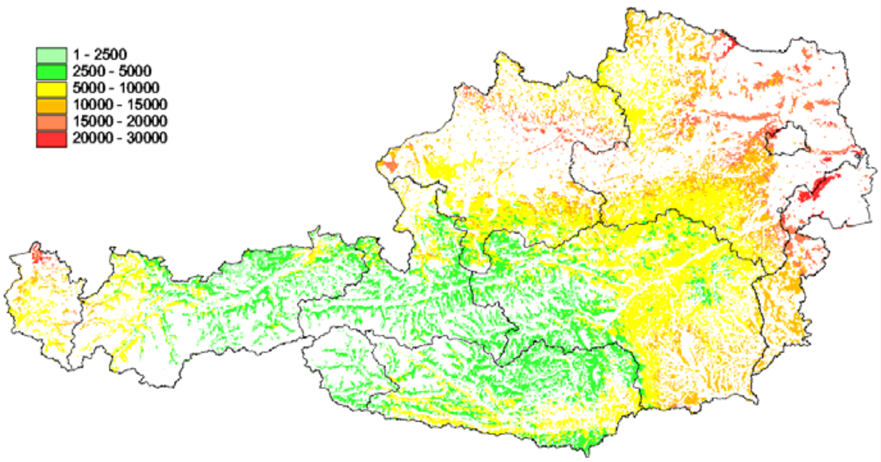


Basic approach: Accumulated ozone exposure exceedances of 40 ppb.

Therefore, additional thoughts are outlined in the following approaches taking into consideration the adaption of trees to the longtime ozone level and the ozone uptake into the sub-stomata cavity.

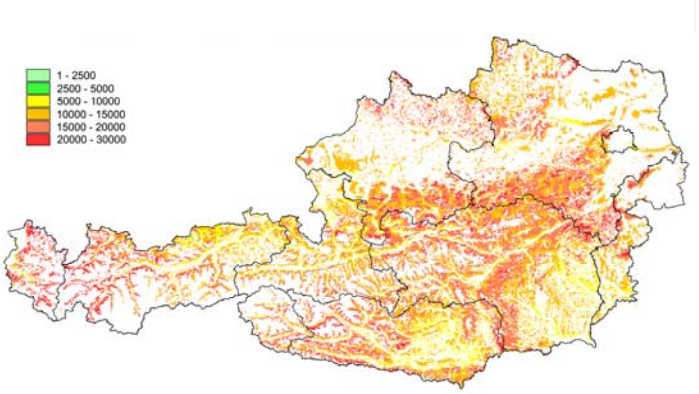
Conclusions: The approaches revealed that a plausible result concerning the ozone impact on spruce forests in Austria could only be reached by combining pre-industrial ozone levels, ozone flux into the spruce needles and the hemeroby of forests.

The **1st approach** modifies the AOT40 based on the assumption that forests have adapted to the pre-industrial levels of ozone, which increase with altitude. Thus the AOT level has to be defined as altitude-dependent gradient instead of a fixed AOT40 threshold.



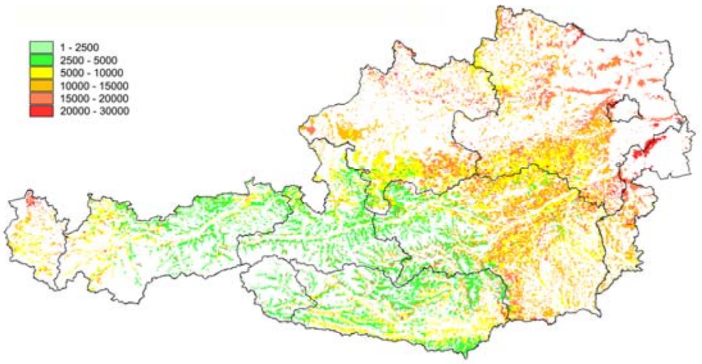
1st approach: Exceedances of an elevation-dependent gradient (32 to 57 ppb.h).

The **2nd approach** modifies the AOT40 according to the ozone concentration in the sub-stomata cavity as response to light intensity and water vapour saturation deficit, which affect stomatal uptake.



2nd approach: Exceedances considering climatic factors that are influencing stomata opening.

The **3rd approach** combines the other two approaches and includes forest hemeroby: The 1st approach (considering pre-industrial ozone levels) has been applied to autochthonous ("natural") forests, the 2nd (ozone uptake) approach to non-autochthonous ("altered") forests which have not been adapted to higher ozone levels at higher altitudes.



3rd approach: AOT elevation gradient applied for "natural forests" and climate influence on stomata opening for "altered forests.

ACID DEPOSITION IN MIXED DECIDUOUS FORESTS IN THAILAND

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Abstract

A study on acid deposition in mixed deciduous forests in Thailand was conducted during 2001-2003 in Nongrawiang plant genetic conservation centre in Nakhon Ratchasima province, and at Khon Kaen University in Khon Kaen province. It was part of a joint project between the Royal initiative projects and the Khon Kaen University. The study objectives were to compare acid deposition, chemical components in stemflow, throughfall and rainfall, chemical components in fresh leaves of the dominant tree species (Xylia xylocarpa), and biomass in this forest type.

The results showed that the pHs of the rainfall at Nongrawiang in 2001, 2002, and 2003 were 6.09, 5.43, and 4.00, respectively, and the electrical conductivities (ECs), were 0.09, 0.14, and 0.29 dS/m, respectively. The respective pHs were 6.92, 6.35, and 5.25, and the ECs 0.02, 0.03, and 0.03 dS/m at Khon Kaen. The pHs of stemflow, throughfall and rainfall were lower in 2003 than in 2002 and 2001 in Nakhon Ratchasima and Khon Kaen. Analysis of the rainwater chemical components in 2001-2003 indicated that NH_4, NO_3, and SO_4 were higher in throughfall in Khon Kaen than in Nakhon Ratchasima, and Cl in stemfall was also higher in Khon Kaen than in Nakhon Ratchasima. The contents of N and P on fresh leaves of Xylia xylocarpa were similar over the three years on both sites. However, the content of for Ca, Mg, and S were higher in 2003 than in 2002 and 2001. The biomass results showed more biomass in Khon Kaen

than in Nakhon Ratchasima, *i.e*, 7.99, 7.36, and 9.56 tonnes/ha/yr in Khon Kaen and 6.16, 3.26, and 5.81 tonnes/ha/yr in Nakhon Ratchasima, in 2001, 2002, and 2003, respectively.

In conclusion, considering pHs alone, according to the results, there were more acid deposition at Nongrawiang in Nakhon Ratchasima than in Khon Kaen, and there was more acid deposition in 2003 than in 2002 and 2001.

Introduction

Acidifying atmospheric deposition has increased dramatically around the world due to the industrial development. In response to this, several monitoring programmes have been set up in Thailand. One of these programmes was directed on forest ecosystems, to study the effects of the acid compounds on soil, water and plants. The acid compounds affect mainly plant metabolism, growth and morphology. A study on acid deposition in a mixed deciduous forest in Thailand started in the year 2001 and completed in 2003. The objectives of this study were 1) to investigate the acidity of stemflow, throughfall and rainfall in northeastern Thailand, 2) to assess the effect of being aware of acid rain and pollution on vegetation, 3) to determine the rates of acidity that can affect vegetation and living organisms.

This study would provide further information on the effects of acid deposition on mixed deciduous forest in Nakhon Ratchasima and Khon Kaen provinces, northeastern Thailand. It also would provide guidelines for presenting and taking precautions against acid deposition due to the industrial estate in both provinces and Thailand.

Materials and Methods

Data were collected in two mixed deciduous forest sites. One site was at the Nongrawiang plant genetic conservation centre in Nakhon Ratchasima province, and another was at the Khon Kaen University (near the chemical furnace of Sri Nakarin hospital) in Khon Kaen province, Northeastern Thailand. These monitoring sites were located in the mixed deciduous forests in semi-rural and semi-urban areas, respectively. The soil profiles were dark reddish brown at the A and B horizons on both sites. The dominant tree species was Xylia xylocarpa, which is typically present on this type of forest. Analysis on changes and acid deposition on plants was taken by sampling fresh leaves from the dominant trees, installing stemflow, throughfall and rainfall equipments to determine the chemical elements, acidity of rainfall was investigated, and litter fall was also collected twice a month to determine the change in biomass in relation to the changes in the environment. Details of the rainwater analysis, fresh leaf analysis, soil analysis, and biomass determination are described below.

Analytical equipment and methods for rainwater and soil monitoring were analysed as follows:

Parameters	Equipment/methods
a) Moisture Content	Dry oven, balance
b) pH (H_2O) and pH (KCL)	Glass electrode
c) Electric Conductivity	Conductivity cell, glass electrode
d) Exchangeable Base Cations (Ca, Mg, K and Na)	AAS (CH_3COONH_4-Extraction) Titration (KCl- Extraction)
e) Exchangeable Acidity	Ibid.
f) Exchangeable Al, H	Calculation (as sum of
g) Effective Cation Exchangeable Capacity (ECEC)	exchangeable Cations) Volumetric calcimeter
h) Carbonate Content (for calcareous soil)	Titration (Walkley-Black
i) Total Carbon Content	method) or CN-analyzer Titration (Kjeldahl method) or
j) Total Nitrogen Content	CN-analyzer Spectrophotometry (Bray-1 test)
k) Available Phosphate	Turbidimetry, IC IC (preferably with suppressor),
l) Chloride, Nitrate, Sulfate	Spectrophotometry
m) Phosphate, Nitrite, Fluoride	IC, Spectrophotometry (indophenol blue) AAS with Graphite Furnace, ICP
n) Ammonium	Emission IC
o) Heavy Metals, Aluminium	
q) Organic Acids	

According to preventing the error of the chemical analysis results between rainwater and soils, which were different in morphological characteristics, it was used to separate equipment for analysing.

Rainwater analysis

Rainwater from stemflow of four Xylia xylocarpa tree stems per site, as well as throughfall, was collected from four points under the crown canopy. Throughfall was based on 4 replications and rainfall was based on only one replication at the open area. For the rainfall, a rain gauge was set in an open place within the study sites. The gauge was located more than 100 m from the heavy traffic road and 50 m from tall trees and houses at the Nongrawiang plant genetic conservation centre in Nakhon Ratchasima province. It was located on the deck of the building at Khon Kaen University in Khon Kaen province. At both sites, stemflow was measured by setting a trap on dominant Xylia tree-trunks. A vinyl tube with a plastic container at one end was attached to the trunk to collect stemflow water. The tubes were installed carefully so as not to injure the stem cambium, and no adhesive was used except for silicone. The throughfall gauges were placed in the crown canopy of the trees in order to collect throughfall water.

The pH and electrical conductivity (EC) were immediately measured every time it rained at 08:00 hr on the site. If the pH and EC could not be determined at the site on the same day, the rainfall water would be brought to the site offices and stored in the refrigerator then pH and EC were measured within 7 days. One liter of each rain sample was used to determine the chemical properties in the laboratory. The stemflow, throughfall, and rainfall of the first rainfall of each month of the year were analysed for pH, EC, Sodium, (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Nitrate (NO_3), Chloride (Cl), Ammonium (NH_4), and Sulphate (SO_4) in the laboratories at the Watershed Research group in National Park, Wildlife and Plant Conservation Department and at the Soil and Water Conservation group in Land Development Department which both are in Bangkok. The methods of analysing were mentioned above.

Fresh leaf analysis

Fresh leaves were examined for Nitrogen and Sulphur contents in order to evaluate the nutrient and pollution levels and dry deposition of Sulphur dioxide. (Studies of areal and seasonal variations of Sulphur content are necessary for evaluating air pollution.) P, K, Ca, Mg, Cl, and Fe were also determined at the Department of Soil Science in Kasetsart University. Approximately 500 g of fresh leaf samples from the top crown or outer canopy of the plot were collected. To obtain a more representative sample of the area, leaves from more than one tree were mixed. Only mature leaves growing in sunny positions were selected. The leaf samples were then dried in the oven at 75-80°C for two days, weighed, and then ground into small (less than 0.5 mm) particles. About 100 g of the particles were placed in a plastic bag and sent away for analysis of Sulphur content (mg/100 g) by the LECO Sulphur analyser.

Soil analysis

Each studied site, soil profile, soil horizon at levels 0 – 10, 10 – 20, 20 – 40 and 40 – 60 cm were collected to determine the pH, EC, CEC, OM, OC, N, P, K, Ca, Mg, Na and SO_4^{2-}. The standard methodology was used in the laboratory to be analysed.

Biomass determination

Ten litter traps measuring 1 m x 1 m were set up at each site. Litter fall of the mixed deciduous forest was collected twice a month to estimate the amount of annual dry-weight biomass (tonnes/ha/yr).

Results and Discussion

Rainwater analysis

Acid deposition was high at the beginning of the rainy season, and the first rainfall after a dry spell, due to more pollutants in the air. The pHs were 4.03 and 3.71 at the Nongrawiang Plant Genetic Conservation Centre in Nakhon Ratchasima and 5.16 and 3.31 at Khon Kaen University in Khon Kaen province, in March and May 2003, respectively. March and May are part of the dry season in Thailand. The average pH values for every rainfall for the whole year are given ine Table 1. These results show that rainwater was quite acidic in Nakhon Ratchasima (*e.g.,* the rainfall pH was 4.00 in 2003).

Table1.
Average acidity (pH) and electrical conductivity (EC, dS/m) of the rain water in Nakhon Ratchasima and Khon Kaen sites in 2001-2003.

2001						
Site	Stemflow		Throughfall		Rainfall	
	pH	EC	pH	EC	pH	EC
Nakhon Ratchasima	6.22	0.67	6.18	0.20	6.09	0.09
Khon Kaen	6.46	0.12	6.59	0.05	6.92	0.02
2002						
Site	Stemflow		Throughfall		Rainfall	
	pH	EC	pH	EC	pH	EC
Nakhon Ratchsima	5.54	0.89	5.58	0.29	5.43	0.14
Khon Kaen	6.17	0.21	6.34	0.08	6.35	0.03
2003						
Site	Stemflow		Throughfall		Rainfall	
	pH	EC	pH	EC	pH	EC
Nakhon Ratchasima	5.33	0.43	5.50	0.20	4.00	0.29
Khon Kaen	6.25	0.16	6.07	0.06	5.25	0.03

For chemical components of rainwater in 2001 – 2003, the NH_4, NO_3, and SO_4 were higher in througfall in Khon Kaen than Nakhon Ratchasima, and Cl in stemfall was higher in Khon Kaen than in Nakhon Ratchasima. The Na, K and Ca were highest in 2002 compared to the year 2001 and 2003 on both sites (Tables 2-4).

Fresh leaf analysis

The contents of N and P in fresh leaves of Xylia xylocarpa were similar over three years on both sites. The Ca, Mg and S were higher in 2003 than 2001 and 2002 on both sites. (Tables 5-7). The N deposition from NH_3 or NH_4^+ forms may have enhanced the plant growth (Leith et al., 2001). The N and S content in leaves of Xylia xylocarpa were more than the N and S content of teak leaves (Luangjame et al., 2001), almost double the amount.

Litter fall biomass estimation

The biomass results showed more biomass in Khon Kaen than in Nakhon Ratchasima provinces, *i.e.,* 7.99, 7.36 and 9.56 tonnes/ha/yr in Khon Kaen and 6.16, 3.26 and 5.81 tonnes/ha/yr in Nakhon Ratchasima in 2001, 2002, and 2003, respectively (Table 8). The amount of biomass in mixed deciduous forest was different in time and location as same as biomass of the teak plantation (Luangjame et al., 2001).

Soil analysis

The pHs and C/N ratios of the soils were not different at the different soil levels of both sites. The soil was slightly acidic (pH ~ 5) but it was still in good condition and not showing any acidity symptoms (Table 9). However, the effects of this on soil might be dependent on the time, and thus the effects on the vegetation may show up in the future.

Table 2. The means of the laboratory chemical properties of rainwater (ppm) in Nakhon Ratchasima and Khon Kaen provinces in 2001.

Categories	Nakhon Ratchasima			Khon Kean		
	Stemflow	Throughfall	Rainfall	Stemflow	Throughfall	Rainfall
Acidity (pH)	6.22	6.18	6.09	6.46	6.59	6.92
Electrical conductivity (EC, dS/m)	0.67	0.20	0.09	0.12	0.05	0.02
Sodium (Na^+)	0.96	0.73	0.31	0.76	0.57	0.36
Potassium (K^+)	12.85	10.75	1.30	16.49	9.45	1.14
Ammonium (NH_4^+)	1.81	1.89	0.60	1.00	2.06	0.07
Calcium (Ca^+)	9.03	6.71	1.34	7.78	7.19	4.36
Magnesium (Mg^+)	7.02	4.61	0.41	4.71	1.91	0.43
Chloride (Cl^-)	5.18	4.49	2.39	14.68	4.19	3.60
Nitrate (NO_3^-)	2.09	1.65	0.32	3.53	2.79	0.90
Sulphate (SO_4^{2-})	4.72	5.02	2.87	11.52	8.21	2.55

NB Averaged from 4 replications per parameter and in several months per year

Table 3. The means of the laboratory chemical properties of rainwater (ppm) in Nakhon Ratchasima and Khon Kaen provinces in 2002.

Categories	Nakhon Ratchasima			Khon Kean		
	Stemflow	Throughfall	Rainfall	Stemflow	Throughfall	Rainfall
Acidity (pH)	5.54	5.58	5.43	6.17	6.34	6.35
Electrical conductivity (EC, dS/m)	0.89	0.29	0.14	0.21	0.08	0.03
Sodium (Na^+)	39.14	25.36	8.86	9.66	9.50	8.84
Potassium (K^+)	15.32	13.21	9.41	44.01	34.42	9.94
Ammonium (NH_4^+)	1.71	1.16	1.92	3.24	3.39	1.33
Calcium (Ca^+)	12.16	11.33	10.12	20.43	18.71	15.72
Magnesium (Mg^+)	4.38	3.13	1.20	11.20	9.54	1.22
Chloride (Cl^-)	9.76	5.62	2.85	13.53	5.96	4.50
Nitrate (NO_3^-)	2.15	2.01	0.74	2.12	3.29	3.97
Sulphate (SO_4^{2-})	3.14	2.75	0.72	9.20	6.08	2.54

NB Averaged from 4 replications per parameter and in several months per year

Table 4. The means of the laboratory chemical properties of rainwater (ppm) in Nakhon Ratchasima and Khon Kaen provinces in 2003.

Categories	Nakhon Ratchasima			Khon Kean		
	Stemflow	Throughfall	Rainfall	Stemflow	Throughfall	Rainfall
Acidity (pH)	5.33	5.50	4.00	6.25	6.07	5.25
Electrical conductivity (EC, dS/m)	0.43	0.20	0.29	0.16	0.06	0.03
Sodium (Na^+)	1.72	2.32	1.93	1.72	1.54	1.74
Potassium (K^+)	1.91	0.80	0.68	1.64	1.00	0.70
Ammonium (NH_4^+)	1.88	1.59	0.62	1.53	1.65	1.42
Calcium (Ca^+)	6.71	2.59	1.38	4.95	6.41	1.73
Magnesium (Mg^+)	7.38	1.75	1.06	3.27	2.43	0.86
Chloride (Cl^-)	10.54	3.21	3.48	14.45	5.36	7.52
Nitrate (NO_3^-)	2.06	1.63	0.97	2.69	2.23	2.28
Sulphate (SO_4^{2-})	2.03	2.64	1.51	6.11	3.50	3.00

NB Averaged from 4 replications per parameter and in several months per year

Table 5. Chemical analysis of fresh leaves of Xylia xylocarpa in Nakhon Ratchasima (NR) and Khon Kaen (KK) provinces in June 2001.

No	% in dry matter							In ppm dry matter
	N	P	K	Ca	Mg	S	Cl	Fe
NR1	2.08	0.11	0.41	0.08	0.22	0.04	0.08	133
NR2	2.22	0.11	0.43	0.06	0.17	0.05	0.07	101
NR3	2.36	0.12	0.37	0.06	0.16	0.06	0.09	131
NR4	2.38	0.11	0.58	0.08	0.18	0.06	0.11	123
Average	2.26	0.11	0.45	0.07	0.18	0.05	0.09	122
KK1	2.52	0.16	1.12	0.11	0.14	0.07	0.10	122
KK2	2.71	0.15	0.96	0.14	0.18	0.07	0.09	172
KK3	2.48	0.16	0.88	0.15	0.19	0.09	0.08	133
KK4	2.10	0.12	0.86	0.10	0.19	0.07	0.10	181
Average	2.45	0.15	0.96	0.13	0.18	0.08	0.09	152

Table 6. Chemical analysis of fresh leaves of Xylia xylocarpa in Nakhon Ratchasima (NR) and Khon Kaen (KK) provinces in June 2002.

No	% in dry matter							In ppm dry matter
	N	P	K	Ca	Mg	S	Cl	Fe
NR1	2.26	0.12	1.26	0.81	0.19	0.03	0.74	129
NR2	2.62	0.13	1.25	0.38	0.19	0.04	0.81	156
NR3	2.64	0.13	1.03	0.37	0.17	0.06	1.04	132
NR4	1.92	0.12	1.03	0.26	0.13	0.03	0.86	207
Average	2.36	0.13	1.14	0.46	0.17	0.04	0.86	156
KK1	2.61	0.13	1.02	0.58	0.13	0.03	0.53	135
KK2	2.48	0.12	0.84	0.65	0.14	0.04	0.53	81
KK3	2.40	0.13	1.09	0.52	0.14	0.03	0.60	168
KK4	2.10	0.09	0.82	0.56	0.14	0.04	0.50	73
Average	2.40	0.12	0.94	0.58	0.14	0.04	0.54	114

Table 7. Chemical analysis of fresh leaves of Xylia xylocarpa in Nakhon Ratchasima (NR) and Khon Kaen (KK) provinces in June 2003.

No	% in dry matter							In ppm dry matter
	N	P	K	Ca	Mg	S	Cl	Fe
NR1	2.56	0.12	1.06	0.95	0.86	0.52	0.23	137
NR2	2.66	0.13	1.13	0.91	0.82	0.57	0.27	139
NR3	2.68	0.13	1.10	0.90	0.81	0.58	0.29	137
NR4	2.71	0.11	1.13	0.91	0.83	0.53	0.26	133
Average	2.65	0.12	1.11	0.92	0.83	0.55	0.26	136
KK1	2.42	0.11	0.93	0.96	0.88	0.53	0.23	136
KK2	2.52	0.10	1.03	0.90	0.80	0.50	0.20	139
KK3	2.21	0.10	0.89	0.93	0.81	0.56	0.25	137
KK4	1.93	0.09	0.93	0.87	0.83	0.60	0.26	140
Average	2.27	0.10	0.95	0.92	0.83	0.55	0.24	138

Table 8. Biomass of mixed deciduous forests at Nong Rawiang Plant Genetic Conservation Centre in Nakhon Ratchasima (NR) province and Khon Kaen University in Khon Kaen (KK) province in 2001-3 (tonnes/ha/yr).

Site	2001	2002	2003
NR	6.16	3.26	5.81
KK	7.99	7.36	9.56

Table 9. Soil chemical and physical properties of Nong Rawiang Plant Genetic Conservation Centre in Nakhon Ratchasima (NR) and Khon Kaen University in Khon Kaen (KK) provinces

Soil profile (level/cm)	EC, µS/cm Soil:H_2O (1:1)	pH (1:1)	pH (Dist.) (1:2.5)	pH (KCl) (1:2.5)	% OM	% OC	% Total N	C/N	P ppm.	CEC meq /100g soil	SO_4^{2-} ppm.	Ex. Mg meq/100 g soil	Ex. Ca meq/100 g soil	Ex. Na meq/100 g soil	Ex. K meq/100 g soil	BS (Base Saturation) (%)
NR A	33.00	5.70	5.10	4.80	1.15	0.67	0.06	11.67	1.09	5.90	0.04	0.2869	0.9100	0.0428	0.0574	21.9847
NR B1	27.00	5.40	5.00	4.60	0.45	0.26	0.02	11.82	1.15	3.45	0.08	0.0328	0.1700	0.0409	0.0415	8.2667
NR B2	23.00	5.50	5.00	4.60	0.21	0.13	0.01	11.36	1.04	3.74	0.02	0.1221	0.5900	0.0436	0.0154	20.6176
NR (0-10)	34.00	5.72	5.50	4.80	1.39	0.81	0.07	11.62	1.99	5.24	0.04	0.2508	0.9060	0.0388	0.0734	24.2176
NR (10-20)	30.00	5.30	5.24	4.61	0.56	0.33	0.04	9.50	1.74	3.51	0.13	0.0836	0.3220	0.0388	0.0401	13.8034
NR (20-40)	27.40	5.24	5.12	4.54	0.42	0.24	0.02	11.71	1.20	3.60	0.04	0.0838	0.4300	0.0363	0.0347	16.2444
NR (40-60)	25.40	5.40	5.24	4.56	0.35	0.20	0.05	9.52	1.64	3.71	0.04	0.1274	0.5620	0.0377	0.1079	22.5067
KK Ab	35.00	5.60	5.50	4.80	2.35	1.36	0.12	11.56	2.01	9.50	0.05	0.5025	2.7500	0.0367	0.2628	37.3895
KK B1	44.00	5.20	4.90	4.40	1.20	0.70	0.06	11.65	2.78	5.33	0.02	0.1377	0.8710	0.0291	0.2282	23.7523
KK B2	25.00	6.10	5.85	5.10	0.41	0.24	0.02	11.77	1.17	4.61	0.07	0.4352	1.0600	0.0353	0.1709	36.9067
KK (0-10)	49.6	5.10	5.08	4.50	1.39	0.81	0.07	11.57	2.55	6.13	0.07	0.2297	0.8860	0.0369	0.1266	20.8679
KK (10-20)	29.80	5.12	5.12	4.50	0.55	0.32	0.09	9.53	1.56	4.12	0.05	0.0590	0.5660	0.0372	0.1992	20.9078
KK (20-40)	34.00	5.40	5.40	4.64	0.56	0.32	0.03	11.44	1.71	3.59	0.06	0.1349	0.5740	0.0387	0.0814	23.0919
KK (40-60)	33.00	5.44	5.56	4.72	0.41	0.24	0.02	11.44	1.21	5.04	0.05	0.2433	0.6800	0.0374	0.0955	20.9563

Conclusions and Recommendation

The 3-year results showed slight acidity of rainwater in the mixed deciduous forests in Nakhon Ratchasima province. The pHs were lower at Nongrawiang plant genetic conservation centre in Nakhon Ratchasima province than Khon Kaen university in Khon Kaen province, for both stemflow and throughfall. From pHs averaged records, acidity increased from the year 2001 through 2003 on both sites. This could be interpreted as a warning sign for the industrial estate on acid deposition, especially more so in Nakhon Ratchasima province than in Khon Kaen province. More attention should be apid on acid deposition in Nakhon Ratchasima province. The dominant tree, Xylia xylocarpa, of the mixed deciduous forest that sheds leaves in dry season, showed no symptom of damages from acidity on both sites. Biomass was less in Nakhon Ratchasima than in Khon Kaen province at all three years of observation, presumably because of less precipitation and soil fertility in Nakhon Ratchasima.

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“IMAGING-PAM” CHLOROPHYLL FLUORESCENCE IN THE FIELD: BRIDGING MICROSCOPIC AND MACROSCOPIC SCALES IN LEAF STRUCTURAL ANALYSIS

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Two-dimensional, meso-scale assessment of chlorophyll fluorescence within the leaf blade by the “Imaging-PAM” technique provides a novel approach of quantifying leaf injury. The approach is being employed within the canopy of the “Kranzberg Forest” research site, making use of scaffolding that allows access to the crowns of 60-year-old and 30 m high beech trees (Fagus sylvatica). By means of a “Free-Air Canopy O_3 Exposure” system, trees are examined for their physiological sensitivity, including formation of macroscopic leaf symptoms, to an enhanced O_3 regime (2 x ambient O_3 levels restricted to < 150 ppb; trees under unchanged, ambient O_3 regime as control).

The analysis is based on false-color images of 17 x 22 mm or 3.5 x 4.5 mm in size which express the performance of chlorophyll fluorescence through quantum yield, electron transport rate (ETR) or non-photochemical quenching. Images of chlorophyll fluorescence assessed on the same leaves throughout the growing season allow to pursue the development of injury and to classify and quantify areas of malfunction in photosynthesis. Comparison of false-color images with real-color photographs of the leaf blade facilitates the detection of incipient leaf responses *prior to* discoloration. Assessment of the light dependence of chlorophyll fluorescence within the analyzed leaf patches provides a tool for quantifying photosynthetic responsiveness.

The extent is demonstrated to which the use of the “Imaging-PAM” analysis of chlorophyll fluorescence in addition to microscopic techniques can foster the differential diagnosis of injury by biotic (e.g. insects) *versus* abiotic stress (e.g. ozone). This aim is pursued in cooperation with the "Ozone Validation Center" (Birmensdorf, Switzerland) towards an integrative validation of physiological and structural stress responses and, hence, strengthened specificity of bioindication.

DIAGNOSTICS IN BEECH EXPOSED TO CHRONIC FREE-AIR O3 FUMIGATION: I. OZONE UPTAKE AND GAS EXCHANGE RESPONSES OF ADULT TREES

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Since spring 2000, 60-year-old beech trees (Fagus sylvatica) have been exposed throughout the growing seasons to an enhanced O_3 regime (2 x ambient O_3 at the site) using a “Free-Air Canopy O_3 Exposure” system at the “Kranzberg Forest” research facility. Trees under the ambient O_3 regime serve as a control. To prevent acute O_3 injury, the experimental regime is confined to a maximum of 150 nl O_3 l^{-1}. Scaffolding and a research crane provide access to the sun and shade crowns of the trees being 27 m in height. Given the ample information on short-term responses of juvenile trees, the study aims at clarifying the sensitivity of adult forest trees to chronic O_3 impact.

For creating a mechanistic basis of quantitative risk assessment, diagnostic findings on O_3 effects are scaled from the leaf to the tree level. Gas exchange and chlorophyll fluorescence are measured simultaneously to assess changes in photosynthetic performance. Leaf injury is quantified by the novel “Imaging-PAM” technique that provides a two-dimensional analysis (patches of 3.7 cm²) of chlorophyll fluorescence as reflected in quantum yield, ETR and non-photochemical quenching. The false-color images can be used as a tool for detecting early leaf responses *prior to* discoloration. Light dependence of chlorophyll fluorescence within the patches is analyzed for quantifying responsiveness. Symptoms detected by “Imaging-PAM” are validated in comparison with microscopic and histochemical assessments (cooperation with “Ozone Validation Centre”, Birmensdorf / Switzerland). CO_2 fixation is assessed by porometry (analysis of light and CO_2 dependence) for parameterizing models that allow scaling (in combination with structural data) to the crown level and the calculation of O_3-affected C gains of the whole tree.

The duration of the assimilation period is determined through phenological observations. During the past five years, the assimilation period became significantly shortened in the sun crowns due to accelerated leaf senescence under the enhanced O_3 regime, with the largest effect occurring in 2000. Similar trends of shade crowns were not significant. Lammas shoots initiated in beech in response to stress appear to support the conception that their high photosynthetic capacity, stomatal conductance and maximum quantum yield may counteract photosynthetic limitations in the foliage of the spring flush. The growing season of 2003 provided the opportunity to analyze the interaction between the exceptional drought conditions and ozone impact. The persisting drought caused the pre-dawn water potential to drop to –1.4 MPa, and stomatal conductance was lowered by more than 50% as compared to previous years, which resulted in decreased ozone uptake.

The consistency of findings is examined in the context of further investigations which are conducted by different partner groups within the joint EU project “CASIROZ – The Carbon Sink strength of beech in a changing environment: Experimental risk assessment by mitigation of chronic ozone impact” (see abstracts Pt. II Then et al., Pt. III Blumenröther et al., and Pt. IV Herbinger et al.).

UV-B EXPOSURE STUDY ON PEATLAND ECOSYSTEM IN NORTHERN FINLAND

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Effects of increasing UV-B radiation on the functioning of the peatland ecosystems have so far received little attention. Short-term microcosm experiments have shown that enhanced UV-B can alter pigment contents and membranes of the mosses (Sphagnum spp.), structure of the cotton grass (Eriophorum vaginatum) and the fluxes of CO_2 and CH_4 between the peat and the atmosphere. To verify the results in a more natural setting, we started a long-term experiment at a natural peatland site in Sodankylä.

In our UV-B experiment on an oligomesotrophic flark fen (Sodankylä 67º22'N, 26º38'E) study plots will be exposed to enhanced UV-B radiation for several growing seasons, started in the beginning of June 2003. The UV-B exposure is applied with a modulated system in which the natural solar UV-B is continuously monitored and a constant proportional (46%) supplement of UV-B is provided by UV lamps. The UV-B experiment consists of a total of 30 plots (120 x 120 cm) randomly allocated to UV-B treatment, UV-A control and ambient control plots (n=10). Through the summer time 2003 methane dynamics was studied using a static chamber method and in the winter time we used so called snow gradient method. All gas samples were analysed in Kuopio by a gas chromatograph equipped with FID. Plant samples were taken for microscopic and pigment analysis. Also membrane permeability tests were made and growth parameters of sedges were monitored.

The results from the first study season under supplement UV-B radiation did not show drastic changes in the functions of the peatland ecosystem.

UV-radiation did not significantly change typical methane fluxes. The highest fluxes 130 mg CH_4 m^2 d^{-1} were measured in the end of July. Methane fluxes followed normal summer time pattern. Snow gradient measurements taken from UV-exposed study plots indicate some changes on methane fluxes. Number of Eriophorum russeolum leaves in study plots increased due to UV-B radiation, but no clear effects on stomatal density was observed. UV-B related pigments will be analysed from the plant samples. The analysis consists both soluble and cell wall related pigments. The results so far confirmed our hypotheses that possible changes on northern peatland ecosystem cannot be seen after single growing season.

A COMPARATIVE ANALYSIS OF THE RESPONSE TO OZONE OF MEDITERRANEAN EVERGREEN PLANT SPECIES

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Abstract

Measurements of leaf gas exchange under saturating light and chlorophyll *a* fluorescence parameters, membrane permeability and relative water content were performed on three Mediterranean species (ivy, laurel and viburnum) exposed to ozone for 28 d (100 ppb, 5 h d^{-1}). At the end of the exposure period, stomatal limitation induced by ozone caused significant decreases of photosynthetic activity. The analysis of fluorescence parameters indicates no effects on the maximum efficiency of PSII photochemistry. The relative water content of the leaves was significantly decreased by ozone in ivy, suggesting the occurrence of drought stress. In viburnum, electrical conductivity of leachates from foliar disks increased in response to the treatment, suggesting an alteration of membrane retention capacity dependent on ozone. The evergreen Mediterranean species investigated in this study may be considered tolerant to realistic ozone levels in terms of visible injury, even if gas exchange parameters were negatively affected already after 1 week of fumigation, at least in ivy and viburnum.

Introduction

Ozone (O_3) is the most widely spread pollutant in Europe and North America (Lorenzini and Saitanis, 2003). There has been a marked increase in its background concentration in the troposphere since the industrial revolution, as a result of increasing emissions of the precursors for O_3

production. There has also been a concurrent increase in the frequency and duration of photochemical episodes and the concentration of O_3 in the troposphere continues to rise at a rate of 1-2 % per annum; a trend expected to continue in to the 21st century (Yunus and Iqbal, 1996).

Ambient levels of O_3 over widespread areas of the industrialized world are already known to be high enough to result in extensive visible injury and to have adverse effects on the growth and photosynthesis of plants in natural and managed communities (Krupa and Manning, 1988). The ecological effects of the presence of O_3 are relevant (see BIOSTRESS project, Biodiversity in herbaceous seminatural ecosystems by global change components, www.uni-hohenheim.de/biostress/index.htm).

In the countries of the Mediterranean basin, because of their peculiar climatic features and the increased industrialization, O_3 can easily reach high concentration in the atmosphere (Nali et al., 2002), and it may have phytotoxic effect both in the spontaneous and cultivated vegetation (Kley et al., 1999). Among the Mediterranean woody species, the evergreen shrubs and trees (*e.g.* Laurus nobilis, Eucaliptus spp., Olea europaea, Quercus ilex) are generally assumed to be quite tolerant to the air pollutants thanks to their sclerophyllic adaptations (Christodoulakis and Fasseas, 1990; Christodoulakis and Koutsogeorgopoulou, 1991; Monk and Murray, 1995; Manes et al., 1998). On the other hand, Pinus (Pinus halepensis Mill. and Pinus pinea L.) have been shown to be sensitive to O_3 and other pollutants (Velissariou et al. 1992; Bussotti et al. 1997). Recently, it has been observed that current-year needles of P. halepensis exhibited a higher ability to activate protective systems against oxidative stress than 1-year-old needles. This might explain why O_3 injury to P. halepensis in the field is always observed in previous-year needles (Barnes et al., 2000). In addition, it was found that O_3 exposure reduced the capacity of protection and recovery against oxidative stress, increasing the sensitivity of P. halepensis to photoinhibition (Alonso et al., 2001). However, there is a general shortage of investigations in the examination of O_3 responses in Mediterranean evergreen plant species. Already Inclán et al. (1999) evidenced the necessity of further researches to characterize the physiological, biochemical and anatomical characteristics that may underline the 'sensitivity' of certain Mediterranean species to O_3.

Despite this supposed tolerance, some of the present authors observed, during a previous study (Lorenzini et al., 1999), that strawberry tree

(Arbutus unedo L., a Mediterranean evergreen sclerophyllous shrub), following the treatment with O_3 (200 ppb, 5 h) developed reddish interveinal stipple-like necrotic lesions on the adaxial surface of mature leaves. The same species exposed for 90 d to O_3 (110 ppb, 5 h d^{-1}), manifested a similar injury, while on laurel (Laurus nobilis L.) and phillyrea (Phillyrea latifolia L.), in the same conditions of fumigation, chlorotic mild foliar mottles were observed (Nali et al., 2004b). We extend our observations here by reporting an experiment investigating the effects on photosynthetic process and membrane leakiness of exposures with O_3 dosages of 100 ppb for 28 d (5 h d^{-1}) on ivy (Hedera elix L.), laurel and viburnum (Viburnum tinus L.). This yielded an *A*ccumulated exposure *O*ver a *T*hreshold of 40 ppb (AOT40) of 1,680 ppb·h over 28 days experiment. Critical levels for protection of forest plants are set at 10,000 ppb·h over six months (*i.e.* an average monthly bulk of 1,667 ppb·h). It is necessary to emphasize that exceeding critical levels is quite common in many Mediterranean countries: in Italy, the threshold of 10,000 ppb·h had already been reached after only eight weeks in 2000 (Nali et al., 2004a).

Material and methods

Plant material

One-year-old plants (average height 0,4 m) of ivy, laurel and viburnum were selected by phenotypical homogeneity. The material, obtained by vegetative propagation (ivy) or from seeds of a single mother plant (laurel and viburnum), was grown in pots containing a fertilized compost of peat, perlite and natural soil under a shade awning until the time of the fumigation. All containers were regularly provided with optimal water supply by means of an automatic drip irrigation system. Plants were pre-adapted to greenhouse conditions a week before the O_3 treatment.

O_3 fumigation

Fumigation was performed in 2 Perspex chambers, each measuring 0.90 *x* 0.90 *x* 0.65 m, continuously ventilated with charcoal-filtered air (two complete air changes/min). Ozone was produced by electric discharge with an air-cooled generator (Fischer 500, Zurich, CH), supplied with pure oxygen, and was mixed with the inlet air entering the fumigation chambers. The concentration of O_3 at plant height was continuously monitored with a photometric ML8810 analyzer (Monitor Labs, San Diego, USA). More details are reported elsewhere (Lorenzini et al., 1994).

The target dose was 100 ppb (for O_3, 1 ppb = 1.96 µg m^{-3}, at 20°C and 101.325 kPa) for 28 d (5 h d^{-1}, from 09.00 to 14.00, solar time.) in form of a square wave. Control plants were exposed only to charcoal-filtered air in two Perspex chambers. Seven individuals for each species were used and randomly allocated in each fumigation chamber. Totally, 14 plants for each treatment (ozone and filtered air) were used.

Ecophysiological measurements

The experimental protocol included non-destructive (photosynthetic activity and related parameters) and destructive measurements (membrane leakiness).

The CO_2 and water vapour exchange of leaves were measured in an open infra-red gas-exchange system (CIRAS-1 PP-System, Stotfold, UK) equipped with a Parkinson leaf chamber able to clamp single leaves. Details are reported in Castagna et al. (2001). Measurements were performed at ambient CO_2 concentration (340-360 ppm), 80% R.H. and leaf temperature was maintained at 26±0.4°C, while the chamber was illuminated by a quartz halogen lamp. The light-saturated rates of photosynthesis (Amax) were measured at 800 µmol $m^{-2}s^{-1}$. The calculation of the intercellular CO_2 concentration (Ci) was based on the equations described in von Caemmerer and Farquhar (1981). Amax, stomatal conductance to water vapour (Gw) and Ci were determined at 7, 15, 21 and 28 days of fumigation on four recently mature fully expanded leaves per plant. All measurements were conducted from 09:00 to 15:00 h solar time.

Modulated chlorophyll a fluorescence measurements were made with a PAM-2000 fluorometer (Walz, Effeltrich, Germany) on the same leaves used for gas exchange at the end of the fumigation for a total of five replicates. For details see Degl'Innocenti et al. (2002). Prior to the measurements, the attached leaves were dark-adapted for 35 min in leaf-clips. Values for maximum fluorescence (F_m) and ground fluorescence (F_0) from the fluorescence induction curve, were used for the calculation of the F_v/F_m ratio. The saturation pulse method was used for the analysis of quenching components (Schreiber et al., 1986). Determination of photochemical quenching, q_P, and non-photochemical quenching, q_{NP}, were calculated as defined by Schreiber et al. (1986).

Electrical conductivity (EC) and relative water content (RWC) were determined, according to Guidi et al. (2001), in leaf discs (∅ 1 cm) excised from recently mature fully expanded leaves avoiding the midrib.

Statistics

All experiments were repeated three times and a minimum of five plants per treatment were used in each experiment for the analysis. Data were examined using Student's *t*-test ($P \leq 0.05$) to compare the differences due to O_3 fumigation. Where appropriate, angular transformation of raw data was performed.

Results and Discussion

Even if the plants never showed visible injury attributable to O_3 effects, their physiological parameters proved to be significantly affected by the fumigation, and the response of the three species turned out to be different.

Figure 1 reports the trend of some parameters during successive readings. It clearly shows how in ivy and viburnum a depression of CO_2 assimilation rate at light saturation level (Amax) (about -30 and -55%, respectively, at the end of the treatment) was due to the fumigation. In laurel, O_3 caused a 16 and a 20% inhibition of CO_2 uptake after 15 and 21 days of exposure, while at the end of the experiment not significant differences were found between control and treated plants. In ivy, stomatal conductance to water vapour (Gw) was strongly affected by O_3 (-52, -33, -35 and -50% at 7, 14, 21 and 28 days of exposure, respectively). Although a unforeseen conductance variability in carchoal filtered laurels was observed, in treated material Gw was significantly lower in the first 21 days of fumigation; at the end of the experiment no differences were found.. In viburnum a reduction of this parameter was observed at the end of fumigation. During the fumigation, intercellular CO_2 concentration (Ci) was not affected significantly (*data not shown*).

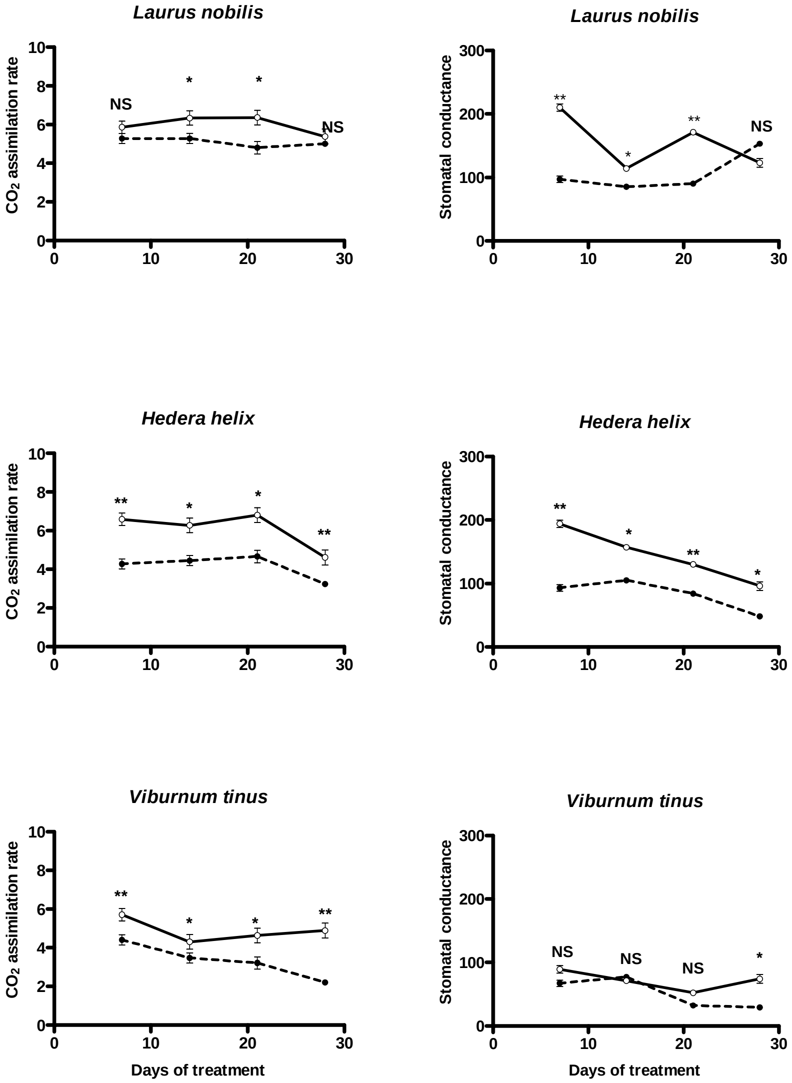

Figure 1. – CO_2 photoassimilation rate (μmol CO_2 $m^{-2}s^{-1}$) and stomatal conductance (mmol H_2O $m^{-2}s^{-1}$) in leaves of Mediterranean species exposed to chronic O_3 (100 ppb for 5 h day^{-1} for 28 days) (closed circles). Control was represented by plants kept in charcoal-filtered air (open circles) for the same period. The gas exchange parameters were determined at saturating light, 345 ppm CO_2, 21% O_2, 26°C and 80% RH. Each value is the mean of 15 replicates. Bars indicate the standard deviation of the mean. For each time the difference between control and ozonated leaves is reported following the Student't test. NS: $P>0.05$; *: $P<0.05$; **: $P<0.01$.

The ground and maximum fluorescence were not affected by O_3, with the exception of a slight reduction in laurel (-15%, in comparison to control; Table 1). The F_v/F_m ratio significantly changed in viburnum (-5%, in comparison to control). Photochemical quenching coefficient q_P was similar in control and treated leaves of all species, while an increase in non-photochemical quenching coefficient was observed following O_3 treatment. It is know that non-photochemical quenching (q_{NP}) represents a non-radiative dissipation of excitation energy as heat and can be a photoprotective strategy (Horton et al., 1996).

Table 1. Chlorophyll fluorescence parameters determined at the end of the exposure to chronic O_3 concentration (100 ppb for 5 h day^{-1}). The experiments was carried out for 28 days for all species. Controls were represented by plants maintained in filtered air and in the same environmental conditions. Each value represents the mean of 15 replicates.

	F_0	F_m	F_v/F_m	q_P	q_{NP}
Laurus nobilis					
Control	164	851	0.8060.770	0.771	
Ozonated	159	722	0.799	0.781	0.814
P	*NS*	*	*NS*	*NS*	*
Hedera helix					
Control	203	896	0.772	0.810	0.279
Ozonated	198	870	0.7720.740	0.675	
P	NS	NS	NS	NS	**
Viburnum tinus					
Control	127	637	0.830	0.860	0.354
Ozonated	133	641	0.7900.840	0.400	
P	NS	NS	**	NS	*

*For each species the last row indicate the significance of the difference between control and ozonated leaves following the Student't test. NS: $P>0.05$; *: $P<0.05$; **: $P<0.01$.*

Twenty-eight days of O_3 exposure significantly decreased the water and solutes retention capacities of membranes, as reflected by relative water content (RWC) (-12%) and electrical conductivity (EC) (+10%) values in ivy and viburnum, respectively (Table 2). On the contrary, RWC and EC were not affected by O_3 in laurel leaves.

Table 2. Electrical conductivity (EC; %) and relative water content (RWC; %) determined at the end of the exposure to chronic O_3 concentration (100 ppb for 5 h day^{-1}). The experiments was carried out for 28 days for all species. Controls were represented by plants maintained in filtered air and in the same environmental conditions. Each value represents the mean of 15 replicates.

	EC	RWC
Laurus nobilis		
Control	58.3	93.8
Ozonated	58.4	94.2
P	*NS*	*NS*
Hedera helix		
Control	30.1	72.0
Ozonated	28.0	63.4
P	NS	*
Viburnum tinus		
Control	15.5	84.9
Ozonated	17.0	84.7
P	**	NS

*For each species the last row indicate the significance of the difference between control and ozonated leaves following the Student't test. NS: $P>0.05$; *: $P<0.05$; **: $P<0.01$.*

From a general analysis of the data, we can observe that realistic O_3 concentration affected physiological parameters in evergreen Mediterranean shrubs also in the absence of visible injuries. Actually, other authors have reported significant reduction of growth of other Mediterranean species even without macroscopical effects (Inclan et al., 1999).

The negative effect on Amax was associated with reduced Gw in ivy and viburnum which may therefore be indicated as a primary limiting factor to

CO_2 assimilation. Taking into consideration that in these species Ci did not change during the entire experimental period, it can be postulated that stomatal, but also mesophyllic limitation occurred. Data from chlorophyll *a* fluorescence analysis confirmed this hypothesis only for viburnum. Indeed, in this species a significant reduction in the F_v/F_m ratio was observed to indicate an alteration in the photochemical efficiency of PSII. Different was the behaviour in ivy where a strong increase in non photochemical quenching coefficient was observed and it is probably responsible to the unaffected F_v/F_m ratio. However, the stomatal limitation of the photosynthetic process is frequently documented in species growing in the Mediterranean basin (Lorenzini et al., 1999; Soldatini et al., 1998).

Laurel leaves, however, presented a different picture, where the slight reduction of Amax being completely recovered at the end of the experiment. Different was the picture in laurel in which the slight reduction of Amax observed after 15 and 21 days of O_3 treatment, was completely recovered at the end of the experiment when also Gw showed values similar to those of the controls. A similar response was observed also when laurel leaves were exposed to a single photochemical episode (Lorenzini et al., 1999). On the other hand, the tolerance to pollutants shown by laurel had been reported by previous authors (Christodoulakis and Fasseas, 1990). Nali et al. (2004) reported that the inhibition of CO_2 fixation in this species is related to the stomatal component.

From a physiological point of view, the response to O_3 of investigated species was not uniform and the mechanisms involved appeared to be different. In laurel an evident mechanism of acclimation was observed, wich is absent in viburnum and ivy. Taking into account that these shrubs species grow in the same habitat, this phenomenon might be useful in the Mediterranean ecosystems where several abiotic stresses co-occur. This has been already demonstrated by Bombelli and Gratani (2003) for three me evergreen Mediterranean shrubs species: Q. ilex, P. latifolia and Cistus incanus.

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GAS EXCHANGE AND OZONE VISIBLE INJURY IN MEDITERRANEAN EVERGREEN BROADLEAVED SEEDLINGS

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Abstract

The Mediterranean evergreen broadleaved shrubs, Phillyrea latifolia, Arbutus unedo and Laurus nobilis, were exposed to 0 (charcoal-filtered), 55 (ambient) or 110 (2 x ambient) ppb O_3, 5 h each day. After 90 days, gas exchange and extent of visible injury were recorded on the same leaves. Symptoms attributable to O_3 did not exceed 0.2, 1, and 7.5% of the sampled leaf area in P. latifolia, L. nobilis, and A. unedo, respectively. Despite visible injury increased with increasing O_3 doses, elevated variability among individual plants prevented statistical significance. Ambient O_3 did not modified net photosynthesis, while 2 x ambient O_3 impaired it in A. unedo and mainly L. nobilis. Photosynthesis was not correlated with visible injury. Instantaneous WUE was adversely affected by 2 x ambient O_3 level, and significantly decreased with increasing visible injury. The response was mediated by an O_3 effect on transpiration rather than net photosynthesis. As WUE sensitivity to O_3 damage overlapped with the constitutional adaptability to drought P. latifolia > A. unedo > L. nobilis, impairment of leaf water control is suggested as a main mechanism of O_3 injury in these species.

Introduction

Tropospheric ozone (O_3) concentrations have been rising since pre-industrial times, in both developing and developed countries, and are projected to further increase (Fowler et al., 1999). The phenomenon is pronounced in regions with high photochemical activity, such as the Mediterranean basin (Butkovic et al., 1990; Lorenzini et al., 1994), where O_3 can cause visible symptoms both in natural and cultivated vegetation (Fumagalli et al., 2001; Nali et al., 2002). At a rural site in Tuscany (Gabbro, central Italy), an average hourly O_3 concentration of 54 ppb (daylight hours; for O_3, 1 ppb = 1.96 $\mu g\ m^{-3}$ at 20°C and 101.325 kPa) and peaks up to 103 ppb have been recorded in springs 2000 to 2003 (M. Chini, pers. comm.).

The Mediterranean climate shows a strong seasonality in water availability and temperature (Aschman, 1973). Irradiance and temperature are high during the summer, but precipitation is minimal. Winters are wet and mildly cold. Accordingly, the dry, hot and cloudless summer, with its high evaporative demand, is the most stressful period for the local flora. High irradiance (about 3,000 sunshine hours per year; Aschman, 1973), elevated O_3 levels, and summer drought characterise Mediterranean-type ecosystems as highly oxidative environments. Evergreen broadleaved forest is the climax vegetation in Mediterranean environments (De Lillis, 1991). Evergreen sclerophylls (e.g. Arbutus unedo and Phillyrea latifolia*)* avoid excessive water loss by deep roots and coriaceous leaves. Evergreen lauriphylls (e.g. Laurus nobilis) avoid water stress by occuping wet and shaded sites.

In the last decade, considerable interest has been devoted to O_3 visible injury at the leaf level (Chappelka et al., 1999, 2003; Sanz et al., 2001; Innes et al., 2001; Smith et al., 2003; Novak et al., 2003). As attention has primarily focused on pre-visual effects on leaf physiology, visible injury consequences on leaf functionality have been little investigated, both in crop (Citrullus lanatus and Cucumis melo, Fernandez-Bayon et al., 1993; Phaseolus vulgaris, Kanoun et al., 2001; Nicotiana tabacum, Saitanis et al., 2001) and in forest species (Pinus jeffreyi, Patterson and Rundel, 1995).

Cause-effect relationships between ozone, visible injury and growth losses have not been validated yet. Inclan et al. (1999) did not find a clear relationship between the development of visible injury and effects on plant growth or accumulated biomass in several Mediterranean evergreen species. For forests, the provisional critical level for ozone in Europe is an AOT40 of 10 ppm h over six months during daylight hours (UN-ECE, 1996). The aim to estimate actual growth loss in connection with ecological factors is the so called Level II approach (Fuhrer et al., 1997).

Biomass production is an integrating parameter that will only be affected as a result of damage to metabolic and physiological processes (Broadmeadow, 1998). Since trees and evergreen species are long-living species, it is not easy to gather sufficient reliable and comparable data on final biomass production. Since CO_2 assimilation is the main source of biomass production, it seems appropriate to consider photosynthetic capacity as an indicator of the risk for growth reduction.

We exposed three evergreen broadleaved species representative of the Mediterranean vegetation, L. nobilis, A. unedo and P. latifolia, to zero (control), ambient (55 ppb), and doubled (110 ppb) O_3 concentration, yielding an accumulated exposure over a threshold of 40 ppb (AOT40) of 0, 15.8 and 31.5 ppm h, respectively. Our aim was to study the leaf-level relationship between gas exchange and O_3 visible injury in these species.

Materials and methods

Plant material and O_3 exposure

Pot-sown, two-years-old seedlings of L. nobilis, A. unedo and P. latifolia were purchased from a local commercial nursery. Twenty-one plants per species (1 plant per pot) were selected by phenotypical homogeneity, and pre-adapted to greenhouse conditions two weeks before O_3 exposure. To test the effect of spring exposure to O_3, the experiment lasted from March to May. Exposure was carried out in three cabins inside a greenhouse, supplied with charcoal-filtered air, at ambient light (40% lower than the irradiance outside the greenhouse), temperature (23 ± 2°C), and relative humidity (75 ± 5%). Plants were rotated daily inside each cabin and watered once a week until field capacity throughout the experiment. Ozone was produced by a generator (Fischer, Zurich, Switzerland) supplied with pure, dry oxygen. Ozone concentration was continuously monitored with a PC-controlled photometric analyzer (Monitor Labs mod. 8810, San Diego, CA, USA). The exposure regime was a square wave of 55 or 110 ppb O_3 from 09:00 to 14:00 (GMT). Control plants were maintained in charcoal-filtered air (negligible levels of O_3, never exceeding 5-8 ppb). Before O_3 exposure, three mature fully expanded leaves per plant were selected on seven plants per species and per treatment, as 4th-5th leaf from each shoot tip. A 2.5-cm^2 circle, corresponding to the cuvette clamp size, was drawn by an indelible marker on the central portion of each leaf, across the rib. This leaf part was chosen because of the limited size of most P. latifolia leaves. For each species, preliminary gas exchange measurements did not show significant variations among cabins and plants. Analyses were carried out after 90 days of exposure.

Gas exchange measurements

Measurements were expressed on a projected leaf area basis. Net photosynthesis (A), stomatal conductance to water vapour (g_s), transpiration (T), and the internal CO_2 concentration (Ci) were measured at noon, using O_3-free air, with an infra-red gas analyzer (CIRAS-1 PP-Systems, Herts, UK) equipped with a Parkinson leaf chamber controlling leaf temperature (25°C), leaf-to-air vapour pressure difference (2.0 ± 0.2 kPa), light (800 μmol m^{-2} s^{-1} PAR) and CO_2 concentration (365 ppm). Instantaneous WUE was assessed both as A/T, and as A/g_s.

Visible injury survey

Ozone symptoms in A. unedo were validated by comparison with the literature (http://www.gva.es/ceam/ICP-forests/; Sanz et al., 2001), while no report for L. nobilis and P. latifolia was found (see also http://www.ozone.wsl.ch). Symptoms by fungi or insects were not detected, but yellow (L. nobilis and P. latifolia) or reddish (A. unedo) spots were present on the upper blade of leaves in filtered air, too. To avoid misundertandings in assigning visible injury to O_3, any discoloration inside the marked circles was traced on transparencies. The traces were quantified by means of an image analysis system (Windias 1.5, Delta-T Devices, Cambridge, UK) and expressed as percent relative to the circled area, namely leaf area.

Statistical analysis

The statistical unit was the single leaf. Percent of visible injury were arcsine transformed. After testing that data distribution was gaussian, all variables were tested for autocorrelation and those that were significantly correlated were removed. Therefore, the two-way (O_3 treatment x species) multivariate analysis of variance (MANOVA) considered only net photosynthesis and extent of visible injury. Wilk's lambda was used to test the significance of MANOVA. For each species, a one-way analysis of variance (ANOVA) was used to assess the effect of O_3 treatment on each variable. Comparison among means used the HSD test of Tukey ($p<0.05$). Linear correlations were applied to test the relationship between the extent of visible injury and each variable in the 55 and 110 ppb treatments. Leaves free of symptoms were discharged. All analyses were performed by Statistica 5.1 for Windows.

Results

MANOVA results show significant effects of both O_3 treatment and species, not of their interaction (Table 1). Symptoms attributable to O_3 were observed and consisted in chlorotic and irregular-edged mottles in L. nobilis and P. latifolia, and in dark-red stipplings in A. unedo. All symptoms were interveinal and on the upper leaf surface only. In all species, the mean visibly injured area did not exceed 7.5% of the sampled leaf area (Fig. 1). In L. nobilis, symptoms progressively increased with the O_3 dose (+48% at 110 compared to 55 ppb), but the elevated data variability did not allow statistical significance (Table 2). In A. unedo, symptoms at 110 ppb were 4 times higher than those at 55 ppb. Even this species displayed an elevated variability among leaves and especially among individuals, that prevented statistical significance (Table 2). In the 110-ppb treatment, two plants only showed widespread symptoms (up to 30-60% of the leaf area) on most of the leaves, including the surveyed ones. The other plants showed symptoms on less of 10% of the leaf area and several leaves were still free of symptoms. In P. latifolia, symptoms never exceeded 0.2% of leaf area. Despite they doubled in both the O_3 treatments compared to the controls, the increase was not statistically significant (Table 2).

Table 1. MANOVA results for the effects of ozone treatment (filtered air, 55 or 110 ppb, 5 h d^{-1}, 90 days) and species (Laurus nobilis, Arbutus unedo, Phillyrea latifolia) on net photosynthesis and extent of visible injury. D.f. (H) and d.f. (E) represent the degrees of freedom for the hypothesis and error sum of squares cross product matrices, respectively. Symbols indicate: * = $p<0.05$; *** = $p<0.001$; ns = $p>0.05$.

Source	d.f. (H)	d.f. (E)	*Wilks' lambda*	Level of significance	
Ozone treatment	4	246	0.9163	0.0290	*
Species	4	246	0.7948	<0.0001	***
Ozone x Species		246	0.9534	0.6534	ns

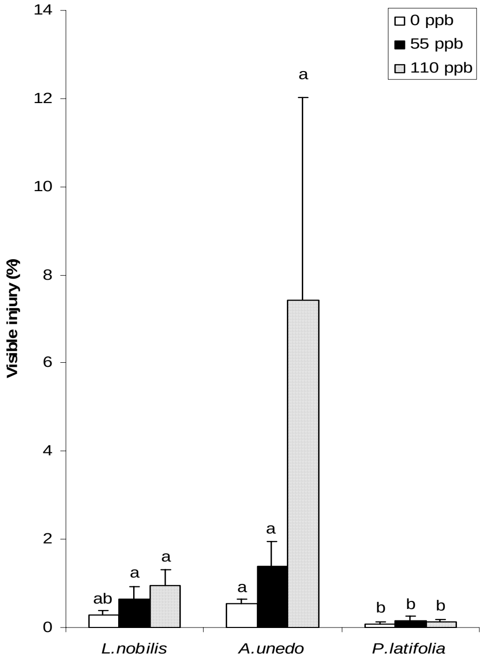

Fig. 1. Visible injury (as percent of surveyed leaf area + SE) in Mediterranean evergreen seedlings (Laurus nobilis, Arbutus unedo, Phillyrea latifolia) exposed to 0, 55, or 110 ppb O_3 (5 h d^{-1}) for 90 days. Different letters indicate significant differences among bars (arcsine-transformed values, Tukey test, $p<0.05$, N=21).

Ozone affected net photosynthesis in L. nobilis and A. unedo (Table 2), even if a significant reduction in assimilation was recorded only in the 110-ppb treatment compared to the controls in filtered air (Fig. 2). Stomatal conductance was impaired only in L. nobilis at 110 ppb (Fig. 2). Substomatal CO_2 concentration was unaffected (Table 2). Transpiration decreased and increased in L. nobilis and P. latifolia, respectively, at 110 ppb compared to the controls (Fig. 2). Instantaneous water use efficiency, both in terms of A/T and of A/g_S, was reduced in P. latifolia, as usual only at 110 vs. 0 ppb (Fig. 3), while it was not significantly affected in L. nobilis (Table 2). A. unedo reduced its A/T ratio in the 110 ppb treatment compared to the controls, while A/g_s was unchanged (Table 2, Fig. 3).

Table 2. Level of significance from one-way ANOVA of the effect of ozone treatment (filtered air, 55 or 110 ppb, 5 h d^{-1}, 90 days) on extent of visible injury (Inj), net photosynthesis (A), stomatal conductance (g_s), internal CO_2 concentration (C_i), transpiration (T), and instantaneous water use efficiency assessed as A/T and A/g_s in all the leaves (with and without visible injury). N=21. Symbols indicate: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; ns = $p>0.05$.

Variable	**Species**		
	Laurus nobilis	Arbutus unedo	Phillyrea latifolia
Inj	0.0503	0.4348	0.7072
	ns	ns	ns
A	0.0073	0.0485	0.7580
	**	*	ns
g_s	0.0040	0.6882	0.0786
	**	ns	ns
C_i	0.3784	0.8417	0.6505
	ns	ns	ns
T	0.0009	0.1954	0.0007
	***	ns	***
A/T	0.0534	0.0002	<0.0001
	ns	***	***
A/g_s	0.1743	0.1606	<0.0001
	ns	ns	***

The relationship between gas exchange and O_3 visible injury was studied in symptomatic leaves from both the O_3 treatments. A positive correlation was found for transpiration in A. unedo ($T = 1.55 + 0.71Inj$, $R^2 = 0.50$, p = 0.0011**). This effect was strong enough to affect the transpiration vs. visible injury relationship when data from all the three species were cumulated ($T = 1.27 + 0.41Inj$, $R^2 = 0.17$, p = 0.0074**). WUE from all species together significantly decreased with increasing visible injury ($A/T = 4.77 - 0.32Inj$, $R^2 = 0.10$, p = 0.0406*; $A/g_s = 0.08 - 0.32Inj$, $R^2 = 0.10$, p = 0.0431*).

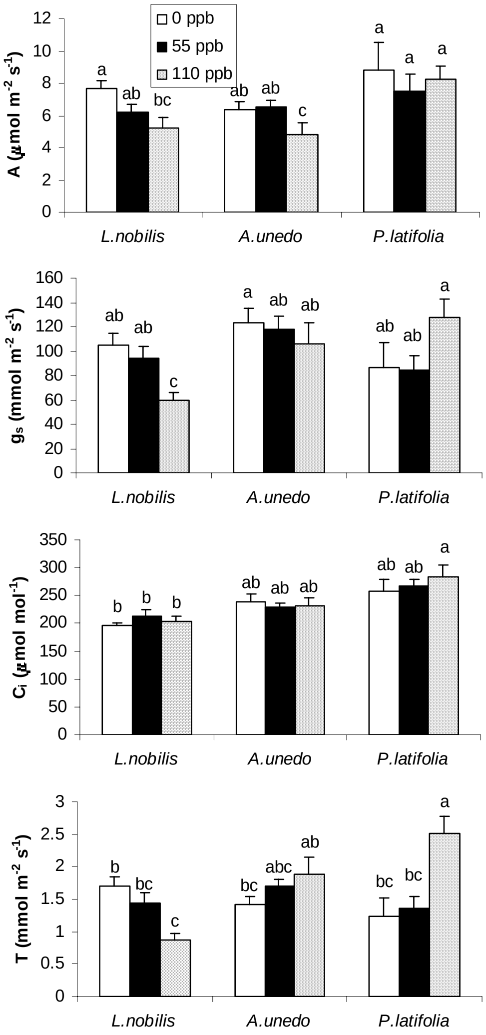

Fig. 2. Net assimilation (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration (T) + SE in leaves (including visible-injured and asymptomatic leaves) of Mediterranean evergreen seedlings (Laurus nobilis, Arbutus unedo, Phillyrea latifolia) exposed to 0, 55, or 110 ppb O_3 (5 h d^{-1}) for 90 days. Different letters indicate significant differences among bars in each graph (Tukey test, $p<0.05$, N=21).

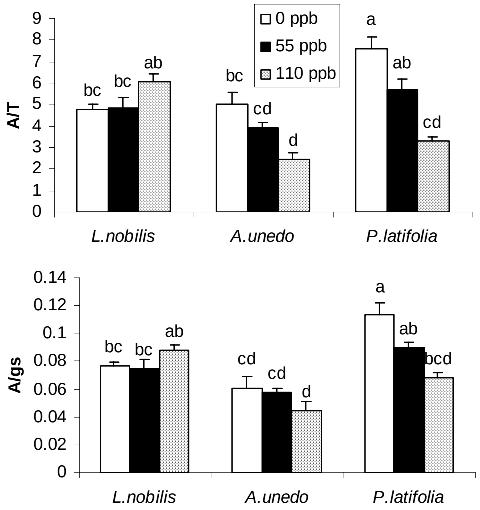

Fig. 3. Instantaneous water use efficiency expressed as net assimilation (A) versus transpiration (T), and as net assimilation (A) versus stomatal conductance (g_s) + SE in leaves (including visible-injured and asymptomatic leaves) of Mediterranean evergreen species (Laurus nobilis, Arbutus unedo, Phillyrea latifolia) exposed to 0, 55, or 110 ppb O_3 (5 h d^{-1}) for 90 days. Different letters indicate significant differences among bars in each graph (Tukey test, $p<0.05$, N=21).

Discussion

Symptoms characteristic of O_3 injury have not been previously identified in L. nobilis and P. latifolia. This is not surprising, as our high value of AOT40 (31.5 ppm h), corresponding to more than three times the critical level for forest trees (UN-ECE, 1996), injured less than 1% and 0.2% of the surface leaf area in L. nobilis and P. latifolia, respectively. Experimental conditions, such as watering during fumigations, limited pre-adaptation period, and sudden and prolonged increase in O_3 concentrations, would have been expected to exacerbate the appearance of visible O_3 injury respect to plants exposed to the same pollutant dose under field conditions (Günthardt-Goerg et al., 1993, 1997). Conversely, the O_3 exposure was not enough to significantly increase visible injury in comparison to the controls in filtered air. Great variations in the sensitivity of single individuals prevented statistical significance, despite symptoms doubled in the ozone-treated P. latifolia, and were on the border of significance for L. nobilis ($p=0.0503$), that showed increasing injury with increasing O_3 doses. Ozone foliar injury strongly depends on the sensitivity of individual plants (Patterson and Rundel, 1995; Lee et al., 1999; Chappelka et al., 1999, 2003; Innes et al., 2001), even if the causes of this variation and its ecological significance are currently unknown (Davison et al., 2003). Great variability prevented statistical significance also in A. unedo, despite symptoms increased with the O_3 doses. Ozone symptoms have been already described in A. unedo (Sanz et al., 2001; Lorenzini et al., 1999; Skelly et al., 1999; Sanz and Millan, 2000). A microscopical investigation on symptomatic leaves of ozonated A. unedo seedlings detected active responses located primarily in the epidermis and consisting mainly in accumulation of tannins (Bussotti et al., 2003). Tannins filling the epidermal cell walls were found also in ozonated Fraxinus excelsior (Günthardt-Goerg et al., 2000). We speculated if this defense reactions reflect overall leaf functionality, particularly gas exchange ability.

The treatment at ambient O_3 concentrations (55 ppb) did not significantly affect any of the gas exchange parameters in any species, in comparison with the controls in filtered air. Even if recovery during the O_3-free hours over the fumigation period cannot be excluded (Nali et al., 1998), present ambient O_3 levels during Mediterranean springs are suggested to be not harmful for the gas exchange performances of native sclerophyll seedlings, in agreement with results from chlorophyll *a* fluorescence (Paoletti et al., 2004).

The treatment at doubled O_3 concentrations (110 ppb) reduced net photosynthesis and stomatal conductance in L. nobilis and A. unedo (even if g_s reduction in the latter species was not statistically significant). This suggests that A and g_s were still tightly coupled, and confirms that O_3-induced limitations in the photosynthesis of these species are primarily controlled by stomata (Paoletti et al., 2004; Nali et al., 2004), adversely to that reported in deciduous broadleaved trees (Matyssek et al., 1991; Clark et al., 1996; Paoletti et al., 2002). A further confirmation that g_s and A acclimated in tandem was the relative insensitivity of the internal CO_2 concentration. As measurements were recorded at constant ambient CO_2 concentration (C_a), the ratio C_i/C_a - that reflects any change in the relationship between g_s and A (Sage, 1994) - was similar among treatments inside each species.

Surprisingly, the 110-ppb treatment did not affect A and g_s in P. latifolia, i.e. the species that showed the highest gas exchange rates and then the highest potential O_3 flux into the leaf. Detoxification of absorbed O_3 by an active antioxidant pool has been proposed to explain P. latifolia tolerance to O_3 in terms of photosynthetic gas exchange (Nali et al., 2004). Therefore, it is confirmed that these evergreen broadleaved shrubs were tolerant to realistic O_3 levels, at least in terms of net photosynthesis, and that this tolerance overlapped with the constitutive adaptability to water stress, i.e. P. latifolia > A. unedo > L. nobilis (Nali et al., 2004).

Transpiration changed similarly to g_s, suggesting T varied because of partial stomatal closure or opening (Kolb and Matyssek, 2001). Transpiration showed a species-specific behaviour as it did not significantly vary among treatments in A. unedo, decreased and increased in L. nobilis and P. latifolia, respectively, at 110 ppb compared to 0 ppb. Stomatal responses to air pollutants are known to be complex, varying among species, leaf and tree age, and in conjunction with other environmental stressors (Mansfield, 1998; Robinson et al., 1998). As a result of the coupling of T vs. g_s, WUE showed similar results when expressed both as A/T and as A/g_s, i.e. no variation in L. nobilis and a progressive decline with increasing O_3 doses in P. latifolia (on average –23% and –48% at 55 and 110 ppb, respectively, compared to 0 ppb) and A. unedo (-14% and –40%). In the latter species, the reduction was significant only when WUE was expressed as A/T, suggesting that O_3 could increase loss of water by cuticular transpiration. In Mediterranean summers, when stomata are closed for a long dry period, cuticular transpiration is likely to become more and more important (Petropoulou et al., 1995). An O_3–induced decrease in WUE has been frequently reported (Mansfield, 1998). Here it was detected in the species that are supposed to be more conservative in terms of water, i.e. the sclerophyllous A. unedo and especially P. latifolia. Detoxification of absorbed O_3 was likely to protect the photosynthetic machinery in these species, not their water control ability. Therefore, elevated O_3 concentrations may adversely affect Mediterranean sclerophyll seedlings, by reducing their constitutional ability to maximixe photosynthesis and minimize water loss. In the long-term, these species-specific responses to O_3 in co-occurring plant species may lead to altered patterns of competition.

The relationship between gas exchange and O_3 visible injury was studied in leaves from both the O_3 treatments. To avoid interferences due to previsual O_3 effects on gas exchange, leaves free of symptoms were not included in the analysis. Transpiration increased with increasing visible injury. As a result, instantaneous water use efficiency A/T declined with increasing symptoms. Also A/g_s declined with increasing symptoms. Therefore, the appearance of O_3 visible injury in leaves of these species may adversely affect their WUE. Conversely, visible injury was not related to photosynthesis in our species, while a negative correlation had been found in Nicotiana tabacum cultivars (Saitanis et al., 2001) and in needles of mature Pinus jeffreyi trees (Patterson and Rundel, 1995).

On the basis of our results, visible injury is representative of O_3 damage to the leaf-level WUE in these species, even if less than 80% of variability is expressed by the investigated variables. Moreover, O_3 symptoms should be interpreted with great caution until the underlying causes of that variation are fully understood (Davison et al., 2003). Generalizations of sensitivity of forest trees to O_3 are complicated by tree development stage, microclimate, leaf phenology, compensatory processes, within-species variation, edaphic and climatic factors, and other interacting stresses. In cultivars of Citrullus lanatus and Cucumis melo, no correlation between a variety of methods used to assess O_3 sensitivity and visible injury was found (Fernandez-Bayon et al., 1993). This observation draws clear attention to the dangers in ranking plants for O_3 sensitivity purely on the basis of visible symptoms. Investigations with more sensitive species should address the critical point of O_3 visible injury representativeness of leaf functionality.

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AIR POLLUTANT EFFECTS TO LEAF, BARK AND WOOD PHOTOSYNTHESISOF TREMBLING ASPEN (POPULUS TREMULA L.)

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Summary

Gaseous air pollutants penetrate stomata in leaves and lenticels in stems, thus reaching the mesophyllic or parenchymal interior of the organs. As inner bark tissues in young twigs and branches are able to perform photosynthesis, the effects of different air pollutants to chlorenchymal photosynthesis were examined. Short time exposure to selected air pollutants was studied in the aqueous phase to mimic the apoplastic cellwall environment and the inhibitory concentrations were compared between leaf, stem, bark and proper wood photosynthesis of trembling aspen (Populus tremula L.). Peridermal tissues proved to be effective barriers for pollutant diffusion. Photosynthetic cells of the proper wood seemed to be most tolerant, a fact that needs further studies.

Introduction

Besides leaves and needles also twigs and branches (and sometimes even old stems) of woody plants participate in C acquisition. Below the dead periderms and rhytidomes of most of the trees and shrubs a green cortical tissue the chlorenchyme is located which is able to photosynthesise and thus reduce the respirational carbon loss (Foote and Schaedle1978; Nilsen, 1995; Pfanz andAschan, 2001). Also green cells of the woody parenchymal ray cells and in young twigs of distinct species even the pith may be photosynthetically active (Pfanz et al., 2002).

Much is known on the uptake of pollutants into leaves and their action within the leaf (Zeevart, 1976; Weinstein, 1977; Pfanz et al., 1987; Van Hove and Van Kooten, 1989; Führer et al., 1993, Yin et al, 1996). Quite in contrast the effects of air pollutants on the limb level have been commonly neglected. The parameters studied so far are mostly tree ring width, basal area increment, stem density and chemical composition and/or isotopic composition (Weber and Grulke, 1995 and references therein). Yet, gaseous pollutants penetrate the twigs and branches of woody plants via the lenticels and partially through the dead peridermal and rhytidomal cells and may thus be damaging. Actual fluxes are not known as there are no data on resistances available (but see Groh et al., 2002). As inner bark tissues are able to photosynthesise, the pollutants are supposed to react in a similar manner as within the chloroplast containing mesophyll cells of leaves.

To compare short time effects of air pollutants on the photosynthetic action of twig organs and tissues, leaves, isolated chlorenchymes, wood portions as well as pith segments were studied. Trembling aspen (Populus tremula) was chosen and the action of ammonia (NH_3), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), and hydrogen fluoride (HF) on the photosynthetic action of different tree organs was studied using short time exposures. The experiments were carried out with isolated organ parts in a liquid medium in an oxygen electrode. The aqueous phase was used to mimic the situation when pollutants that have diffused via stomata and lenticels are dissolved in the aqueous phase of the respective cell walls (Pfanz, 1994).

Material and methods

Representative trembling aspen trees (Populus tremula L.) grown on the southern edge of a urban forest site (230m asl) in Remscheid (Germany) were selected for sampling. Leaves, twigs, bark and wood were freshly harvested from one-year-old organs directly before the measurements. Measurements were performed from June to October to allow easy peeling of periderms because of the highly active cambium. Isolated inner cortex tissues (chlorenchyma) could thus be obtained without cell ruptures. The proper wood portions of the twigs were cut out with razor blades and immediately immersed with incubation buffer. The medium consisted of 50 mM MES, 4 mM KCl, 1 mM $MgSO_4$, 1 mM $Ca(NO_3)_2$, buffered at pH

6. The different air pollutants were added to the measuring solution in form of their inorganic salts so as to mimic the real situation in the apoplastic cell wall solution (Pfanz, 1994).

Results and Discussion

Freshly harvested trembling aspen leaf sections showed photosynthetic control rates of around 2.2 µmol O_2 m^{-2} s^{-1} when no pollutant was added to the incubation medium. When sulphite was added in time steps of 15 mins to yield 0.5 to 15 mM sulphite in solution, photosynthetic rates slightly increased under very low sulphite concentrations. Subsequently, photosynthetic rates dropped continuously and at 5 mM sulphite no photosynthesis was measurable and the leaves respired in the light. Respiration increased with increasing sulphite to reach values of up to -4.5 µmol O_2 m^{-2} s^{-1}. Using isolated bark chlorenchyme a rather similar reaction was held. Even though apparent net photosynthesis was not detected, bark respiration increased with rising sulphite concentrations almost linearly (Fig. 1 left panel). Yet maximum respiration rates (-2.9 µmol O_2 m^{-2} s^{-1}) were also smaller than those of the respective leaves. Although no net photosynthesis was to be measured with isolated wood fractions, increasing sulphite clearly increased the mitochondrial respiration (Fig. 1 right panel). Wood photosynthesis, which is the photosynthetic activity of the parenchymal ray cells in the wood, was shown to normally have a very low activity due to the extremely shade adapted chloroplasts (PFANZ & AL. 2002). A similar reaction was found using a non-peeled twig segment. Mentionably, also in all stem organs resp. tissues a slight decrease in respiration rates was observed under low sulphite concentrations (0.5 to 1.25 mM). In all poplar organs examined, a 50% inhibtion of O_2 exchange is reached under the same sulphite concentrations of about 6-7 mM.

Using the same method as in the experiment of Fig. 1 the effects of other pollutants on the photosynthetic reaction of the selected tree organs and tissues were studied. Besides sulphur dioxide, ammonia, nitrogen dioxide and fluoride were used. Table 1 summarises the concentrations of the respective pollutant to yield 10 to 90% inhibition of photosynthesis. Using ammonia (or ammonium ions in the cell wall solution) the isolated bark proofed to be less sensitive than intact twig segments or leaf tissues.

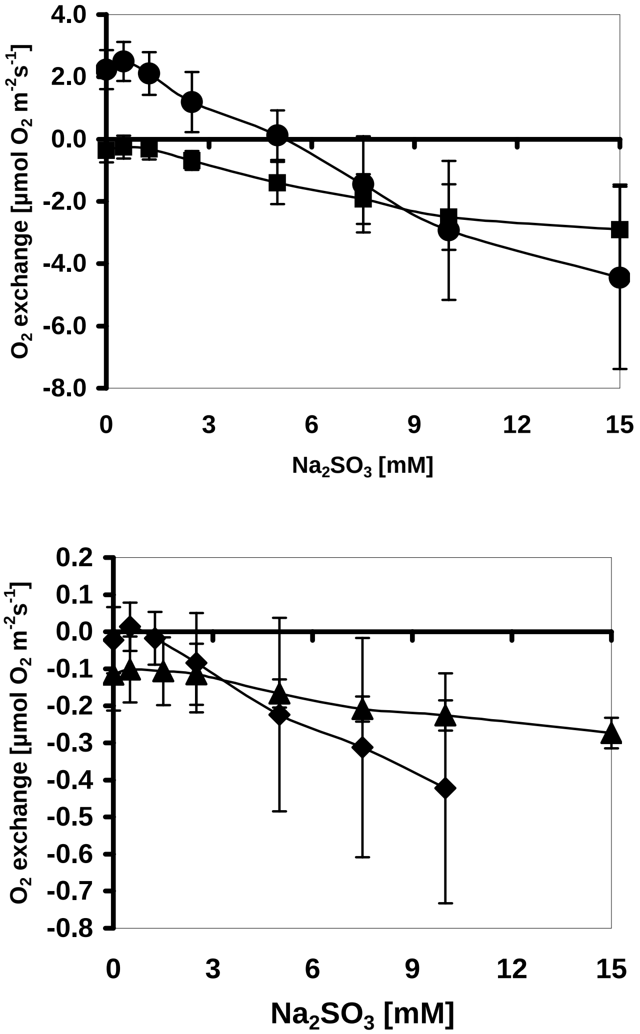

Fig. 1. Influence of Na_2SO_3 on the oxygen gas exchange of leaves (circles), peeled bark (squares) (left panel), isolated wood (triangles) and intact twigs (diamonds) (right panel) from P. tremula as measured with a Clark-type oxygen electrode. The different organs were incubated in aqueous solutions (buffered at pH 6) with the respective inorganic salts of the pollutant simulating the situation in the cell apoplast. Light intensity used was 1000 µmol photons $m^{-2}s^{-1}$ (means ±SD, n = 10-12).

Table 1. Inhibition of organ photosynthesis of leaves, one-year old twig segments, isolated cortical chlorenchyme and isolated wood fractions of Populus tremula as measured by O_2-gas exchange in a Clark-type oxygen electrode. The different organs were incubated in aqueous solutions (buffered at pH 6) with the respective inorganic salts of the pollutant simulating the situation in the cell apoplast. Light intensity used was 1000 µmol photons $m^{-2}s^{-1}$ (means ±SD, n = 10-12). „Not reached“ means that no inhibition was to be measured with the pollutant concentrations used.

		Populus tremula		
		Inhibition [pollutant mM]		
	pollutant	10%	50%	90%
Leaf	NH_4Cl	60	180	260
	Na_2SO_3	1,5	6,5	13
	NO_2	0,6	9	20
	NaF	9	23	400
peeled bark	NH_4Cl	100	225	450
	Na_2SO_3	2	6	14
	NO_2	2,5	14	22
	NaF	4	25	75
isolated wood	NH_4Cl	not reached	not reached	not reached
	Na_2SO_3	3	6	14
	NO_2	not reached	not reached	not reached
	NaF	not reached	not reached	not reached
intact twig	NH_4Cl	3	8	15
	Na_2SO_3	not reached	not reached	not reached
	NO_2	not reached	not reached	not reached
	NaF	not reached	not reached	not reached

In contrast to the isolated chlorenchyme the intact twig segments were still shielded by peridermal layers which reduced the pollutant flux into the twig interior. The difference between the inhibitory concentrations between intact twigs and the isolated inner chlorenchymal tissue is thus an indirect measure for peridermal resistances to the diffusion of the special pollutant. Quite interestingly, isolated wood could not be inhibited in the presence of ammonia, nitrogen oxides or fluorides, although sulphur dioxide was inhibitory. Even with concentrations of 200mM ammonia no inhibition was to be recorded. The reason for this behaviour is still unclear, but it may due to an incomplete photosynthetic apparatus of these extremely shade adapted tissues (Pfanz et al., 2002; Larcher et al., 1988; Höll, 1974).

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ENHANCED UV-B RADIATION RESPONSES IN BIRCHES

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Introduction

Outdoor UV-B supplementation studies with higher plants have revealed some significant responses, but plant responses to UV-B generally seem to be more subtle than expected (Seartles et al., 2001). Northern birches have been responsive in climate change and enhanced air pollution experiments (Vanhatalo et al., 2001). Leaf surface and epidermis is the first cell layer to intercept UV-B radiation, and it is hence an important protective barrier for plants. The purpose of this study was to identify the protective and growth-related UV-B responses and signs of acclimation in three different birch types: red-leaved pubescent birch Betula pubescens f. rubra with anthocyanins in leaves and buds, mountain birch Betula pubescens ssp czerepanovii adapted to mountain and timberline ecosystems and local pubescent birch Betula pubescens. The hypothesis was that enhanced UV-B treatment would increase leaf hairs and protective wax structures in the leaves of the different birches and decrease birch growth.

Material and methods

Four-year-old micropropagated birches were grown under enhanced UV-B radiation (simulation of 20 % ozone depletion) for the growing seasons 2002 and 2003 on the experimental field in Oulu (65º40'N, 25º28'E). There were 14 lamp banks on the field (7 ambient and 7 ambient + supplemental UV-B). The lamps were covered with filters (cellulose acetate transmission down to 290 nm; polyester filter absorption below 315nm). The effects of enhanced radiation on birch growth and leaf anatomy were studied at the end of each growing season. Newly and fully developed undamaged leaves were collected from the upper third main branches of the red, pubescent and mountain birch plantlets at the end of

August. From randomly selected leaves, two leaf discs (Ø=10 mm) per leaf were cut between veins and attached to brass stubs with alternately the abaxial and adaxial sides facing upwards (n=7). The leaf discs were sputtered with gold-palladium (80:20, 45 nm) at 11, 2 nm min-1, under 2, 5 kW and 20 mA (Polaron E5100). Two fields per leaf disc were randomly selected and micrographed at 100 × and 500 × magnifications with FESEM (JEOL JSM 6300F) operated at 6 kV with 6 mm distance and an exposure of 40 s. The amounts of leaf wax surface deposits were estimated from two 100 × magnification micrographs per leaf and classified according to the following criteria by Vanhatalo et al. (2001): Class I = 0-10% (no or few wax sculptures), Class II = 10-30% (some wax sculptures), Class III = 30-50% (moderate wax sculptures), Class IV = 50-70% (high wax sculptures), Class V = 70-100% (abundant wax sculptures). The amount of guard cell surface wax deposits was estimated from 500 × magnification micrographs and classified according to the following criteria by Vanhatalo et al. (2001): Class I = 0% (no wax sculptures), Class II = 1-10% (few wax sculptures), Class III = 10-50% (moderate wax sculptures), Class IV = 50-100% (abundant wax sculptures). The amounts of non-glandular and glandular trichomes on leaf surfaces were evaluated from the 100 × magnification micrographs according to the following criteria: Class I = none, Class II = single/few and Class III = abundant. Height of the birches was measured with a measuring reel and the radial diameter of the base with a caliper rule at the beginning of June and at the end of August. Statistical analyses were performed with SPSS version 11.5. The median values of the wax and trichome occurrence classes were analyzed by non-parametric Kruskal-Wallis test and the average values of the growth parameters were analyzed by one-way ANOVA

Results and discussion

Newly developed leaves of Betula pubescens f. rubra, red birch, were densely covered with non-glandular, long leaf hairs in control treatment, and moderately covered in the UV-B treatment. Fully grown leaves had only a few or no non-glandular leaf hairs (Table 1, Fig 1 A-D). The difference between newly and fully developed leaves was statistically significant in both the control ($p<0, 01^{**}$) and UV-B treatments ($p<0, 01^{**}$). The occurrence of glandular leaf trichomes was higher in newly developed leaves in both the control and UV-B-treatments (UV-B treatment p<0, 05*) (Table 1, Fig 1 A-D). When comparisons were made between the treatments, separately for newly and fully developed leaves, there was a statistically significant difference in the amount of non-glandular leaf trichomes both in newly developed (p<0, 01**) and in fully grown (p<0, 05*) leaves. The leaves of red birch were more densely pubescent in the control treatments than in UV-B treatments. No or a few wax sculptures were observed on the surface of the newly and fully grown leaves of red birch, and there was no difference in the amount of leaf surface or guard cell wax deposits in the control or UV-B treatments (Table 1, Fig 1 A-D, Fig 2 A-B).

In Betula pubescens, pubescent birch, newly developed leaves were more densely pubescent than fully developed with non-glandular leaf hairs in both the control and UV-B treatments (Table 1, Fig 1 E-H). The difference was statistically significant in the control treatment (p<0, 01**). As far as glandular trichomes were concerned, the difference between the studied leaves of different ages was significant in the UV-B treatment (p<0.05*), where newly developed leaves were more densely covered with glandular trichomes than fully developed ones (Table 1, Fig 1 E-H). When comparisons were made between the treatments with pubescent birch, there was a significant difference in the amount of glandular trichomes in new leaves (p<0, 01**) between the control and UV-B treatments, the amount of glandular trichomes being higher in the UV-B treatment. No or a few wax sculptures were observed on the surfaces of new and fully grown leaves of pubescent birch, and there was no difference in the amount of leaf surface or guard cell wax deposits in either treatment (Table 1, Fig 1E-H). There were more wax deposits in the stomatal areas of fully grown leaves in the UV-B treatment compared to the controls, but the difference was not statistically significant (Fig 2 C-D).

With Betula pubescens subsp. czerepanovii, i.e. mountain birch, there were only a few non-glandular leaf hairs on the surface of newly developed leaves and none on the surface of fully developed leaves, with no difference between the UV-B and control treatments. The amount of glandular trichomes on the surface of the leaf epidermis was small, and there was no statistical difference between newly and fully developed leaves in either treatment (Table 1, Fig 1 I-L). When the waxes on the surface of mountain birch leaves were studied, the amounts of wax deposits in the leaf area and in the stomatal area were small in both treatments with leaves of different ages (Table1, Fig 1 I-L). In the UV-B treatment, the difference in the amount of leaf surface wax deposits was statistically significant between new and fully developed leaves ($p<0{,}01$**); fully developed leaves had more waxes on their leaf surface (Fig 2 E-F). Between the treatments, there was a statistically significant difference ($p<0, 05$*) in the amount of wax deposits on the leaf surface of newly developed leaves. There were more leaf surface waxes in the control treatment compared to the UV-B treatment (Table 1).

When newly developed leaves in both treatments were compared between species, red birch had significantly more non-glandular trichomes in the control treatment than other birches ($p<0.01$**). When fully developed leaves were compared in both treatments, there were no significant differences between the species in non-glandular or glandular trichome density or wax deposits (Table 1).

Table 1. Occurrence of non-glandular and glandular trichomes and wax deposits on the surface of new and fully grown leaves of the birch plantlets in the control and UV-B treatments. (Distribution class of wax deposits on leaf area: Class I = 0-10% (no or few wax sculptures), Class II = 10-30% (some wax sculptures), Class III = 30-50% (moderate wax sculptures), Class IV = 50-70% (high wax sculptures), Class V = 70-100% (abundant wax sculptures). Distribution class of wax deposits on stomata area: Class I = 0% (no wax sculptures), Class II = 1-10% (few wax sculptures), Class III = 10-50% (moderate wax sculptures), Class IV = 50-100% (abundant wax sculptures). Non-glandular and glandular trichome occurrence: Class I = none, Class II = single/few and Class III = abundant.)

	Leaf developmental stage	Non-glandular trichome occurrence		Glandular trichome occurrence		Distribution class of wax deposits on leaf area		Distribution class of wax deposits on stomata area	
		Control	UV-B	Control	UV-B	Control	UV-B	Control	UV-B
Betula pubescens f. rubra	Newly developed	III	II	I-II	II	I	I	I	I
	Fully grown	I-II	I	I	I-II	I	I	I	I
Betula pubescens	Newly developed	II	II	I	II	I	I	I	I
	Fully grown	I	I-II	II	I-II	I	I	I	II
Betula pubescens subsp. czerepanovii	Newly developed	I-II	I-II	I	II	I-II	I	I	I
	Fully grown	I	I	II	II	II	I	I	II

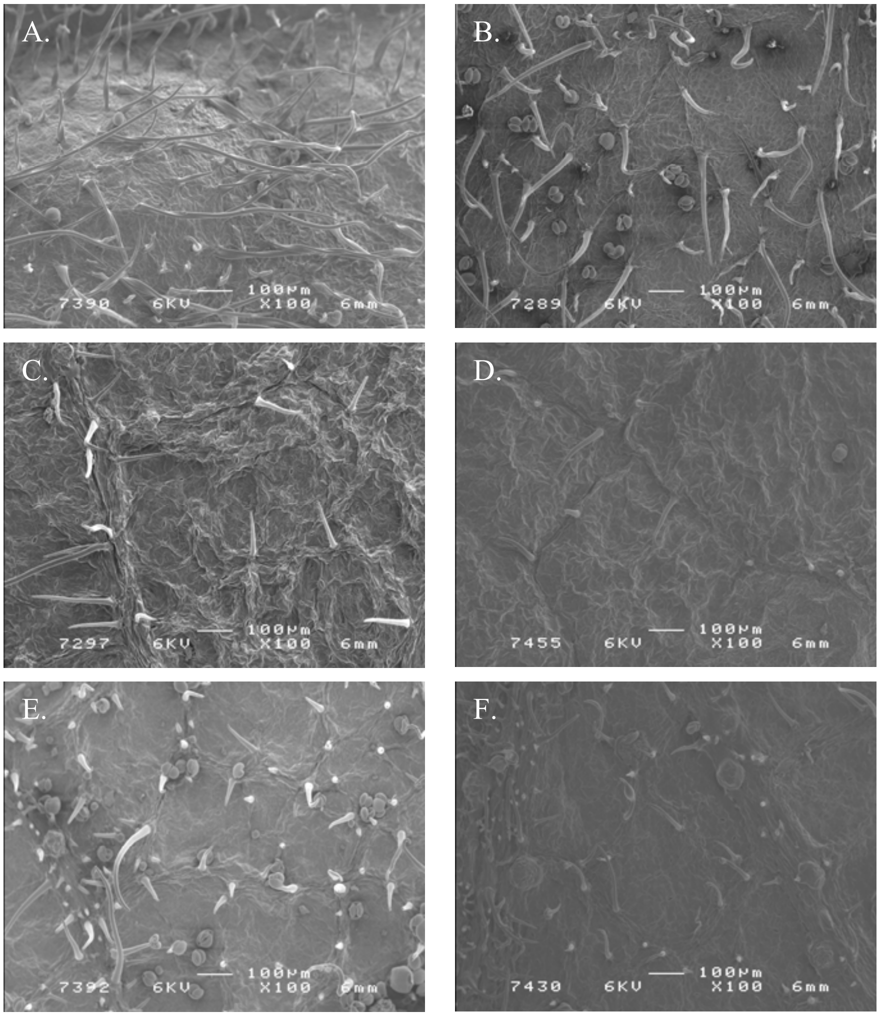

Fig 1 A-F. Field emission scanning electron microscope micrographs (adaxial surfaces) of the studied birches (100 ×). (A) Pubescent leaf surface of a newly developed red birch leaf in the UV-B treatment. (B) A newly developed red birch leaf showing non-glandular trichomes in the control treatment. Sparsely pubescent, fully grown leaves of red birch from the UV-B (C) and control (D) treatments. (E) Pubescent surface of newly developed leaves of pubescent birch from the UV-B (E) and control (F) treatments, showing non-glandular and glandular trichomes.

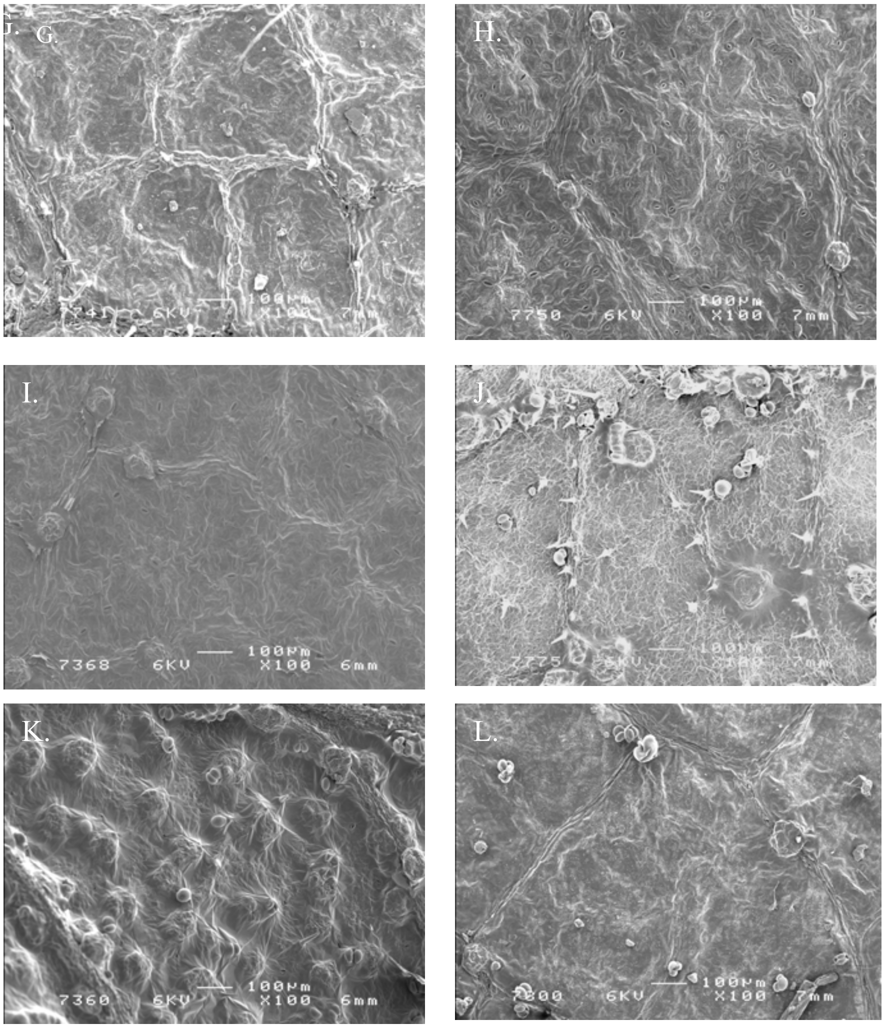

Fig 1 G-L. Field emission scanning electron microscope micrographs (adaxial surfaces) of the studied birches (100 ×). Surface of a fully developed leaf of pubescent birch in the UV-B (G) and control (H) treatments. Surfaces of newly developed mountain birch leaves with few glandular trichomes in the UV-B treatment (I) and in the controls (J) with few non-glandular and glandular leaf hairs. Fully grown leaves of mountain birch in the UV-B (K) and control (L) treatments.

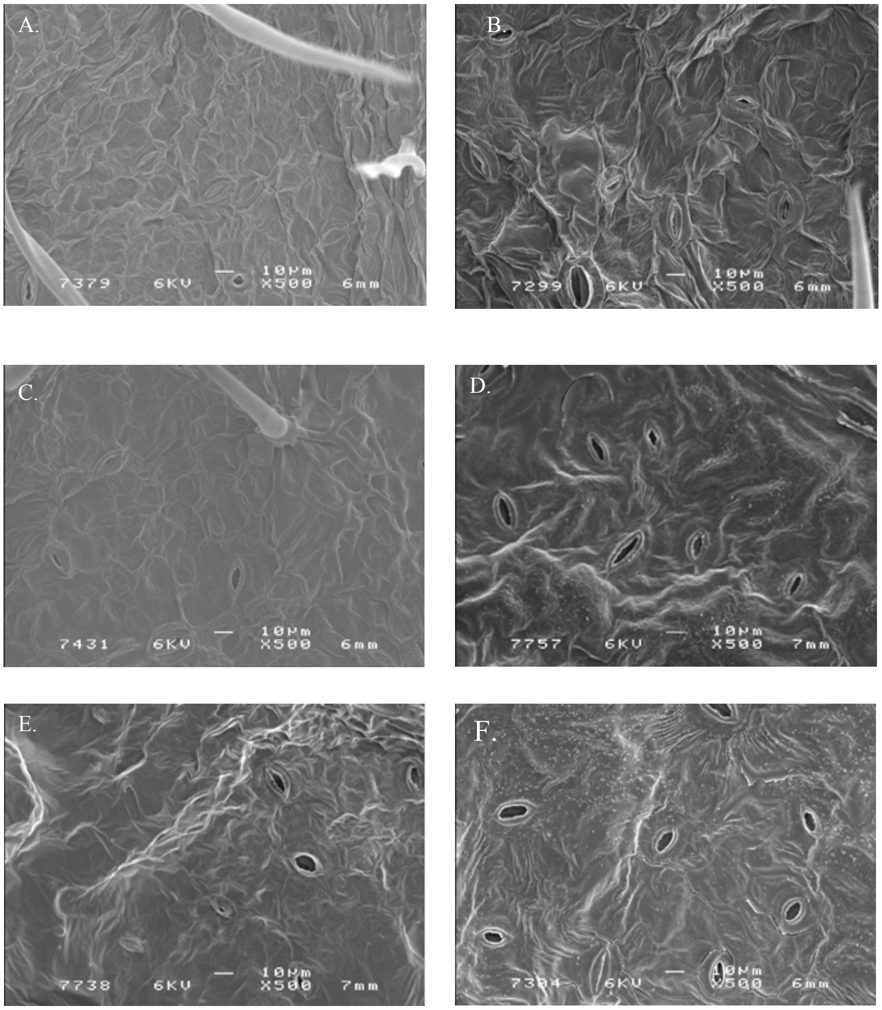

Fig 2 A-F. Field emission scanning electron microscope micrographs (abaxial surfaces) of the studied birches in the UV-B treatment (500 ×). (A) Surface of a newly and (B) fully developed red birch leaves with absent wax deposits on the leaf surface and around stomata. (C) Surface of newly developed leaf with absent wax deposits and (D) fully developed leaf of pubescent birch, with some wax deposits on the leaf surface. (E) Surface of newly developed leaf of mountain birch with absent wax deposits and (F) fully grown leaf with wax sculptures on the leaf area.

Enhanced UV-B radiation had no significant effect on the growth of the studied birches (Fig 3 A-D). In Kostina et al.'s (2001) study enhanced

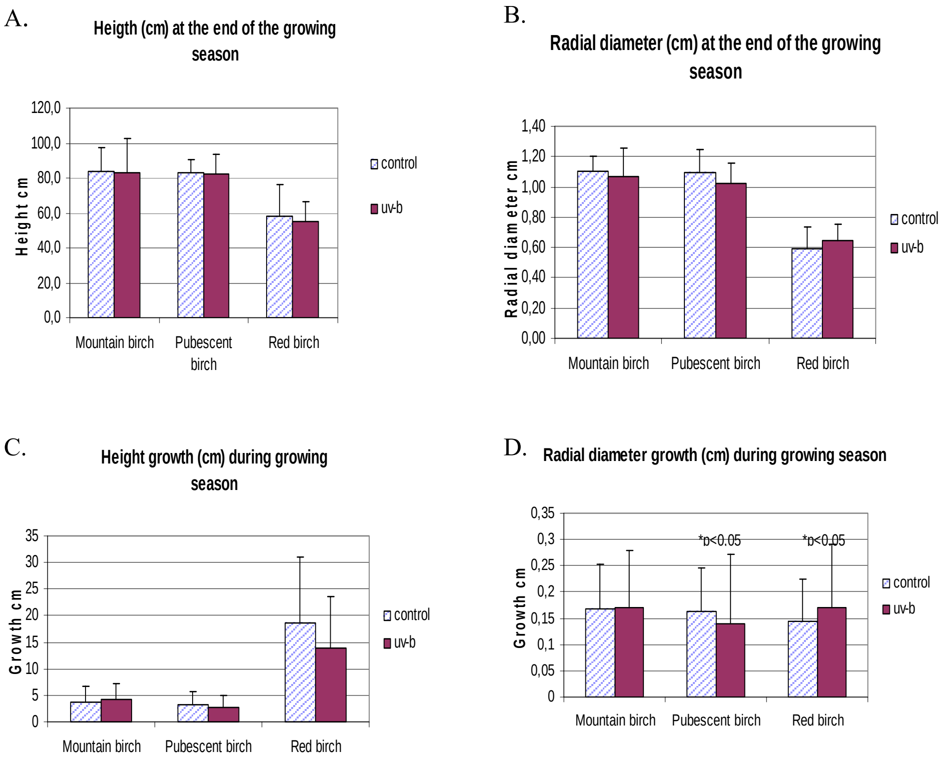

Fig 3 A-D. Growth of the birch plantlets. Height (A) and radial diameter (B) of the birches at the end of the growing season 2003. (C) and (D) average annual growth rates of the birches.

UV-B radiation had no significant effect on the growth of Betula pendula Roth seedlings and it is likely that two year exposure period is too short to induce changes in birch growth (Kostina et al., 2001). Red birch plantlets had a significantly ($p<0,001$***) faster growth rate compared to the other two birch types both under enhanced UV-B radiation and in the control treatments.

The newly developed leaves of all the studied birches were hairy (non-glandular trichomes) on both leaf surfaces, and as they developed, especially adaxial non-glandular trichomes decreased. Also, the number of glandular trichomes was, in most cases, higher in the newly developed leaves. The number of glandular trichomes on newly developed leaves was higher in the UV-B treatment in all birches, but no trend in non-glandular trichomes between the treatments was seen. Glandular trichomes have been reported to resemble structurally the resin glands of Betula pendula, and cells of young birch glandular trichomes have high metabolic activity, especially producing terpenoids (Valkama et al., 2003). The functional role of trichomes is probably the most important in the early stages of birch leaf development (Valkama et al., 2004). New leaves of studied birch types are well protected against environmental factors during development.

Epicuticular waxes may enhance the epidermal reflectance of UV radiation. No trend between treatments in the amount of wax deposits was, however, seen in red or mountain birch. In red birch anthocyanins, located optimally for UV-B screening in the epidermis, may have an additional protective effect (Gould et al., 2000; Pihlajaniemi et al., 2002). In pubescent birch, there were more wax deposits in the stomatal area of fully grown leaves in the UV-B –treatment, but the difference was not statistically significant.

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HIGH UV-B AND HERBIVORY - A DETRIMENTAL COMBINATION FOR MOUNTAIN BIRCH?

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Northern environment sets multiple limitations to the resources required for plant growth and success. Short summer with low temperature sum followed by harsh and long winter limit the function of photosynthetic machinery and hence the success of plants. UV-B radiation has proposed to pose threat to plant functions, especially in the polar regions (and in the areas nearby) where stratospheric ozone depletion is causing enhanced UV-B levels. In addition to abiotic limitations, herbivores can set serious limits for plant success. We studied these effects by exposing mountain birches (Betula pubescens ssp. czerepanovii) originating from northern Finland to both increased UV-B radiation and to simulated herbivory. We exposed two months old mountain birch seedlings to elevated UV-B levels (simulating 25% O^3-layer depletion) and to simulated herbivory (all leaves removed either in the beginning of the experiment, two weeks or four weeks after placing seedlings under elevated UV-B). Seedlings were kept under high UV-B levels for eight weeks after which the experiment was finished (Fig. 1).

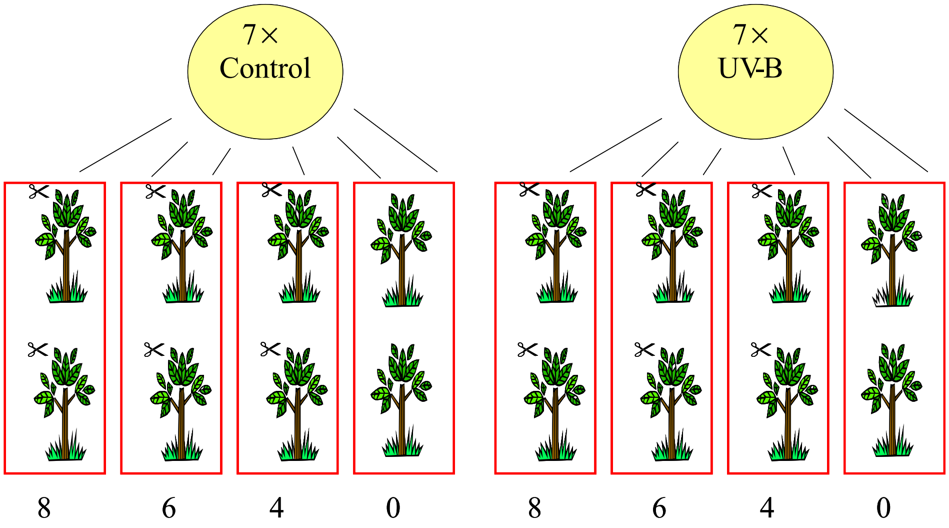

Fig. 1. Experimental set-up. Seedlings were grown on 14 frames half of which were placed under UV-B lamps and the other half under control lamps. Two seedlings of the eight on each frame were selected to one of the four defoliation treatments. Seedlings were defoliated so that they grew 8, 6, 4 or 0 (intact) weeks under the lights after the defoliation. A mean of the two seedlings were computed before analysis (i.e. N = 7 in both treatments).

We found that the elevated UV-B level as such did not have a significant effect on birch growth (measured in terms of growth in length and shoot dry weight, Figs. 2 and 3). Herbivory in the early phase of growth decreased the birch growth when compared to the seedlings defoliated either in later phase of their growth season or left intact. Herbivory and UV-B seemed not to have any significant combined effect (no significant interaction) on birch growth. However, since we observed trend towards a decreased growth in height in seedlings defoliated in very early phase of their growth and grown under increased UV-B radiation (line marked with 8 in Fig. 2) we cannot rule out the possibility that enhanced UV-B radiation poses an additional stress for mountain birches that are stressed also by herbivores.

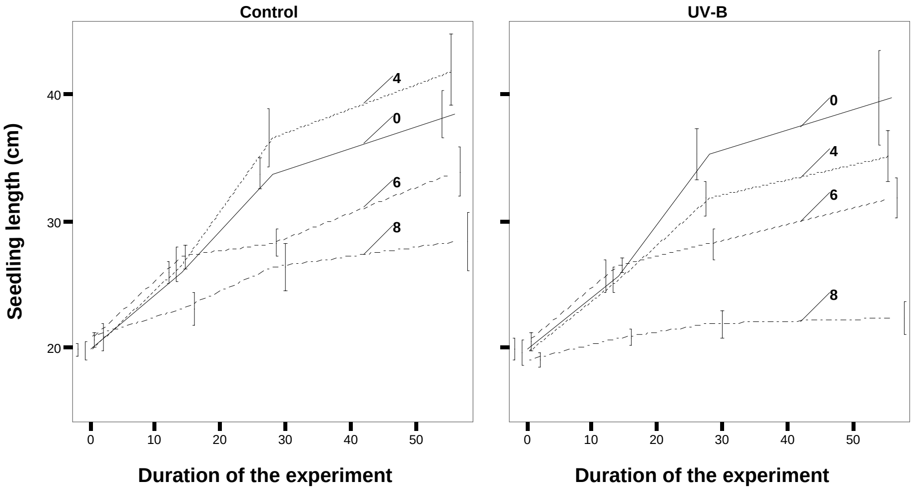

Fig. 2. Seedling length measured during the experiment (mean ± 1 S.E.). Numbers connected to the lines indicate the time (0 = intact seedlings, 4, 6 or 8 weeks) that seedlings were exposed to UV or control light after defoliation. Experiment lasted 56 days (8 weeks).

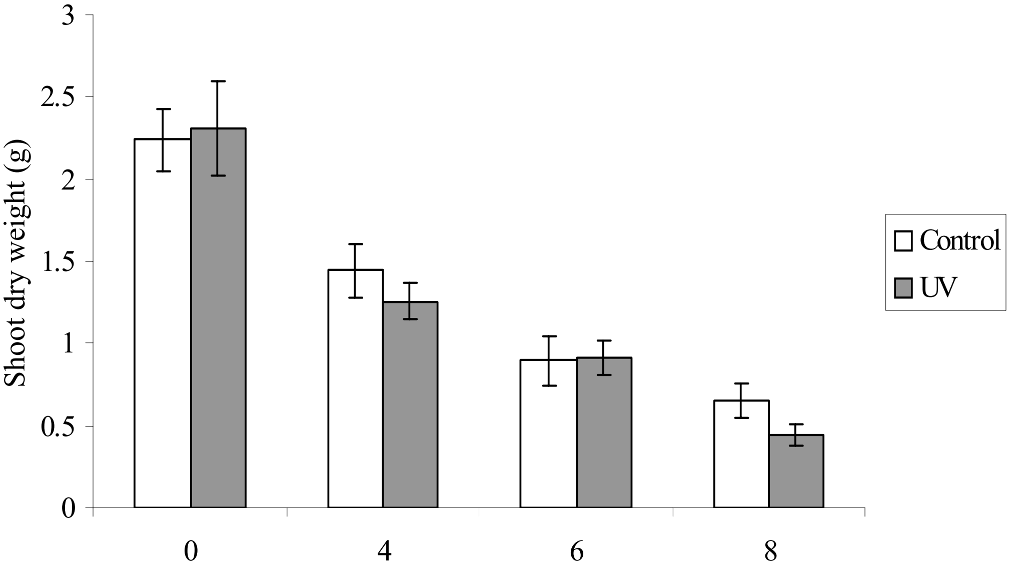

Fig. 3. Shoot dry weight (grams, mean ± 1 S.E.) measured after the experiment. X-axis indicates the time (weeks) that seedlings were exposed to control vs. UV light after the defoliation.

PRELIMINARY ESTIMATES OF OZONE UPTAKE FOR FAGUS SYLVATICA L. CANOPY STANDS ON SELECTED EU/UN-ECE INTENSIVE MONITORING PLOTS IN SOUTH-WESTERN EUROPE

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Current levels of tropospheric ozone have been shown to cause damage to forest trees, agricultural crops and semi-natural vegetation (Kärenlampi and Skärby, 1996). A rise in ozone concentrations has occurred in a large-scale over the past decades, and as a result of the continuing rise in the emission of precursor substances further increases must be expected in many parts of the world (Reich, 1987; Runeckles and Krupa, 1994; Fuhrer, 2000). It is widely accepted that the general receptor-specific maximum leaf conductance is modified by environmental and phenological parameters: soil factors, such as soil moisture deficit and irrigation, plant development factors such as phenological stages, and factors influencing the instantaneous ozone uptake by plants, including temperature, leaf-to-air vapour pressure deficit, global radiation, wind speed. In order to improve our ability to identify areas potentially at risk for ozone impacts an extension of the existing database and a more detailed understanding of the flux-response relationships is needed. Based on earlier models by Jarvis (1976) and Körner et al., (1995), a model has been developed by Emberson et al., (2000a, b) to estimate stomatal ozone flux across Europe for a number of important species. An initial application of this model is illustrated for three species, wheat, beech (Emberson et al., 2000a) and Norway spruce (Emberson et al. 2000b). Within the framework of the EU project 'Ozone in South-Western European Forests' (Ferretti et al., 2004), this preliminary study aims to apply and test the ozone flux model by Emberson et al., (2000a) for canopy beech (Fagus sylvatica L.) stands on selected EU/UN-ECE intensive monitoring plots. Furthermore, we aim to produce ozone flux estimates for canopy beech stands in Switzerland and Italy and compare those with the AOT40 values calculated within the working package of ‘Ozone in South-Western European Forests’ project. The gained experience and the output should give us a better understanding of the data requirements, the advantages and difficulties of such flux based models for future attempts to estimate the ozone risk on European forest ecosystems. The output will enable further investigations of flux – effect relationships for the estimate of a critical flux to protect European beech stands from adverse ozone effects.

The ozone flux model by Emberson et al., (2000a) was parameterized for beech canopy stands and the environmental conditions of Switzerland. The input parameters for 2000 – 2002 were gathered from the EU/UN-ECE intensive monitoring plots from Switzerland, Italy and Spain. The test for completeness and plausibility resulted in 6 plots (5 within Switzerland, 1 within Italy) out of total 20 possible beech plots. Ozone concentrations were calculated from weekly and bi-weekly passive sampling data. The results of this preliminary study are comparable with the ones produced by Emberson et al., (2000a) for beech canopy stands. The results suggest that the output of the biological more meaningful flux approach may not be comparable with the values from the ozone concentration based AOT40 approach due to a strong influence of environmental factors such as vapour pressure deficit and soil moisture deficit on the stomatal ozone flux (Fig. 1). For a future extension of this project over more European countries and different species, the needed time for quality assurance of the input data and the validation of the model output should not be underestimated. In order to define ozone risk on forest ecosystems by the means of a critical flux the estimated model output needs to be compared with possible adverse plant responses such as visible injury or reduced growth.

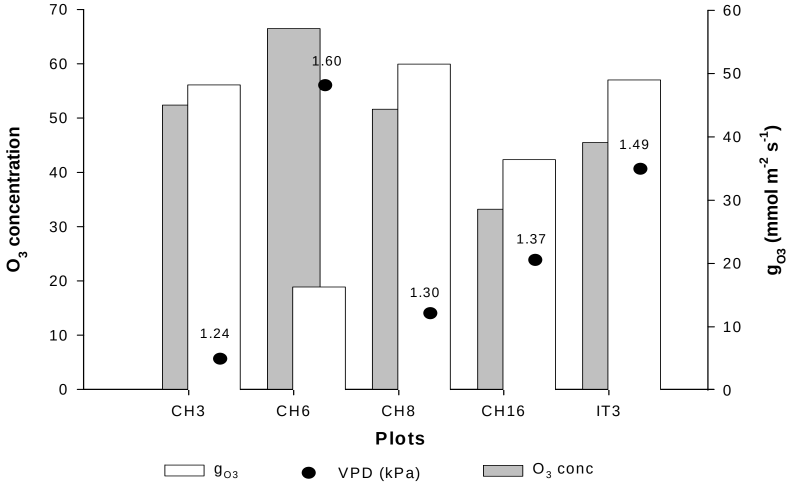

Fig. 1. Monthly means for stomatal conductance for ozone (gO_3), vapour pressure deficit (VPD) (kPa) and ozone concentration at four Swiss and one Italian intensive monitoring plot for June 2002.

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SUSTAINABLE USE OF MOUNTAIN BIRCH FORESTS IN A CHANGED CLIMATE

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Introduction

The mountain birch is a major constituent of the Fennoscandian subarctic forest ecotone with a large capacity for biomass production and CO_2 binding. It has been investigated in many different ways, i.e. experimentally to find its temperature and light responses and its distribution and ecology has been thoroughly described. However, little is known about how much and in what ways human populations have used and intend to use the birch ecosystem culturally and economically. Large areas in northern Europe have been deforested by extensive cutting and grazing for centuries. Local populations, the aboriginal Sàmi and immigrant Scandinavian and Finnish settlers have over long periods utilised this northern forests for hunting, fishing and herding as well as for agriculture, forestry, tourism and recreation. There is a strong need for more basic knowledge of the system to predict the consequences of long-term climatic change and anthropogenic influence for the interaction between insect outbreaks and mountain birch populations.

Summary of results

In order to investigate the objectives mentioned above, a 3-year (2000-03) EU-funded project ”Human Interactions with the Mountain Birch Forest ECOsystem: Implications for Sustainable Development” (HIBECO) was initiated. The main outcome of the project was as follows:

1. Productivity of the birch forests.

Birch performance. Soil and climate were found to be the most important factors determining the performance of northern Betula pubescens trees. Winter temperatures were also found to be the most important limiting factor for survival of the geometrid moth, Epirrita autumnata. Early leafing and monocormic birch provenances seemed to be preferred. Remote sensing studies and earlier investigations have shown substantial overgrazing by reindeer in northern Fennoscandia and by sheep in Scotland and Iceland.

Effects of climate change. In addition to the human impact due to overgrazing, logging and tourism, there has been a significant climatic shift during the last decades, resulting in warmer winters and a more humid climate. The removal of lichen cover by reindeer grazing has accelerated these vegetation changes. At Finnmarksvidda in northern Norway a change in vegetation over the last 40 years has been registered; blueberry (Vaccinium myrtillus) and the dwarf cornell (Cornus suecica) are currently more common than 40 years ago. In contrast, lichens preferred by reindeers have decreased in abundance. The reason for this change is believed to be a combination of a changed climate and changes in human use of these areas. Similarly, there has been an increase in the extent of the birch forests in some areas, for instance at Màze at Finnmarksvidda, Norway the coverage of mountain birch forests has increased by about 90% during the last 45 years, probably due to increased precipitation combined with higher temperatures and formation of ice crusts. Removal of lichen cover may cause more open soil, which in turn may result in better germination of many higher plants, for instance mountain birch.

Currently damages by extreme (winter and spring) temperatures are of relatively small and local importance only, but potentially this type of threat may be more important in the future with a changed climate. On the other hand, mountain birch shows a large phenotypic plasticity regarding phenology and other characteristics. This may improve its ability to cope with a changed climate. In a transplant study the ability to adapt to different photoperiods and temperature changes was found to be higher in northern coastal birch provenances than in birch from more continental origin. These ecotypes seem to be more subjected to spring frost damages than before, because they lose their hardiness too early, leading to reduced growth during the following season.

2. Herbivory

Reindeer and sheep. A review of existing literature on experimental studies of reindeer grazing on different pasture components shows consistently reduction of lichen coverage in grazed areas However, the other pasture components exhibited highly variable responses to reindeer grazing. Birch tended to have lower coverage or biomass, lower height and lower seedling density in grazed areas. Grasses tended to have a higher coverage and biomass in grazed areas. In Iceland, heavy grazing by sheep has even resulted in widespread erosion, not just vegetation damage, which can be very difficult to re-establish.

Experiments with simulated browsing as well as field studies during the HIBECO project showed that sheep grazing influenced all growth parameters, e.g. height, numbers of branches, leaves and shoots (long and short), canopy area and basal stem diameter. Strong browsing at late season was more detrimental to growth than winter browsing, but on the other hand induced growth of compensatory shoots.

Insects. Insects such as autumnal and winter moths rarely cause defoliation in southern latitudes. However, in some areas along the Scandinavian mountain chain they show rather regular cycles or outbreaks of varying amplitude. In the northernmost Finland E. autumnata defoliated birches in hundreds of square kilometres in 1965-66, The recovery of the birch forest has been very slow, partly due to overgrazing by reindeer, large areas remaining virtually treeless even about four decades after the damage. This has clearly decreased the possibilities for traditional livelihoods (reindeer herding, grouse hunting) in the area. Cold winter temperatures (below –35°C) kill the eggs of autumnal moths and restrict outbreaks both geographically and locally. Higher winter temperatures would therefore increase the frequency of insect outbreaks in the continental areas but may not affect the defoliation risks in the oceanic areas. On the other hand, increasing summer temperatures may restrict the area and intensity of outbreaks due to increased pressure from the natural enemies of defoliating insects. Warmer summers would also enhance the capacity of birches to recover from damage.

3. Human interactions

Past and contemporary use of mountain birch resources. The research team completed more than 140 interviews with individual and institutional users of the mountain birch in northernmost Finland, Norway and Sweden in communities with Finnish, Norwegian, Sami and Swedish populations. The interviews covered different aspects of mountain birch use, i.e. traditional use of birch for construction, firewood and handicraft, management practises and perception of birch forests. In areas with recreational activities or heavy overgrazing by reindeer trampling damages may be a serious problem, in addition to the grazing impact. In a separate study long-term mechanical trampling, in combination with reindeer grazing was found to influence the structure and function of vegetation and soils.

The HIBECO project has revealed extensive overgrazing by reindeer, at the same time there has been an increase in the area and biomass of birch forests due to removal of lichen cover and climate change, therefore a less intensive grazing and a lower reindeer population is recommended. However, more intensive cutting of birch for fuel should be possible, and this would tend to compensate for the loss of income on longer terms.

REINDEER SUMMER PASTURES AND ULTRAVIOLET (UV) RADIATION

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Introduction

Most reindeer in Finland graze year around in the coniferous forest zone. The most important summer habitats of reindeer are peatlands, open mountains and river banks. Reindeer choose their forage plants carefully, preferring plants that have high nitrogen content and avoiding those containing much phenolic compounds (Danell et al., 1994).

An increase of UV radiation is a particular ecological concern in the Arctic areas. Ozone depletion increases penetration of solar radiation in those wavelengths (UV-B 280-320 nm) where ozone is the principal absorber. The increase in UV radiation is highest in late winter, early spring and midsummer. It has been predicted that subarctic regions will be subjected to 14% maximum increases in the annual UV dose in 2010-20 relative to 1979-92 (Taalas et al., 2002).

In northern ecosystems, changes in plant productivity or in their production of chemical defence compounds due to increased UV-B radiation (Gwynn-Jones, 1999) may have indirect impacts on the preconditions of northern sources of livelihoods such as reindeer pastures. For reindeer, annual growth occurs primarily on summer pastures, and changes in the quality of summer pastures may affect feed selection or nutrition of reindeer. In this study, we investigated the effects of UV-radiation on the chemical composition and digestibility of a few of the most important summer pasture plants of reindeer.

Objectives

We focused on the following key questions:

1. Does the chemical composition of summer pasture plants of reindeer change due to UV radiation? - Do the plants, for example, produce higher concentrations of defence compounds, such as soluble phenolics?

2. Does the digestibility of those pasture plants that are important in the diet of reindeer change due to the effects of UV on their chemical composition?

This research was a subproject of the RENMAN project (The Challenges of Modernity for Reindeer Management: Integration and Sustainable Development in Europe's Subarctic and Boreal Regions; Forbes 2004) funded by EU fifth framework program. The final results of the work are published in Soppela et al. 2004.

Material and methods

The studies were conducted in a natural peatland ecosystem with UV-B filtration experiment in reindeer pastures of the Lappi Reindeer Herding District in Vuotso (67ºN, 27ºE), in the Eastern Lapland, Finland during 2002-2003 (Fig. 1).

A pilot study was conducted in 2001 with the selection of the field site and plant species. The two dominant vascular plant species in the field site were Menyanthes trifoliata and Eriophorum russoleum. They both are important grazing plants of reindeer. Plant species studied included also Betula nana, E. angustifolium, Rubus chamaemorus and Carex spp. The UV-filtration experiment was conducted with three treatments during summers 2002 and 2003. The treatments consisted of plots fenced with wooden frames and covered with plastic filters adjusted over a natural peatland ecosystem: 1) UV-B exclusion treatment (a clear polyester plastic), 2) control treatment (a clear cellulose acetate) and 3) ambient plots (plots with frames, but no plastic filters). The plant cover was determined yearly by the ITEX method (Walker). Total content of soluble phenolics was determined from the plant samples (Martin and Martin, 1982). In addition, nitrogen and fiber fractions were determined. In vitro digestibility was analysed by using cattle rumen fluid method modified to reindeer forages (Tilley and Terry, 1963).

Fig. 1. View to the UV-filtration experiment in Vuotso, Finland. The filters were placed to the plots in early June and checked throughout summer.

Results

Total concentration of soluble phenolics varied considerably between plant species (Fig. 2). Soluble phenolics were lowest in both M. trifoliata and E. russoleum under UV-B exclusion treatment in both 2002 and 2003. Morevover, E. russoleum responded more sensitively in the UV-B exclusion treatment than M. trifoliata. Total concentration of soluble phenolics was significantly higher in E. russoleum in ambient plots and in the treatment controlling the effects of plastic filter that in the UV-B exclusion treatment ($P<0.01$) in 2003 (Fig. 3).

The concentration of nitrogen and fibers and *in vitro* digestibility (data not shown) varied significantly between the plant species. There were no statistical differences in nitrogen concentration, fiber fractions or digestibility between the treatments, either in M. trifoliata or in E. russoleum. In vitro digestibility of leaves and root of M. trifoliata was very high, and significantly higher (2 times) than digestibility of leaves of R.

chamaemorus and B. nana. In the UV-filtration experiment, there were no statistical differences in digestibility between the UV-treatments either in M. trifoliata or in E. russoleum.

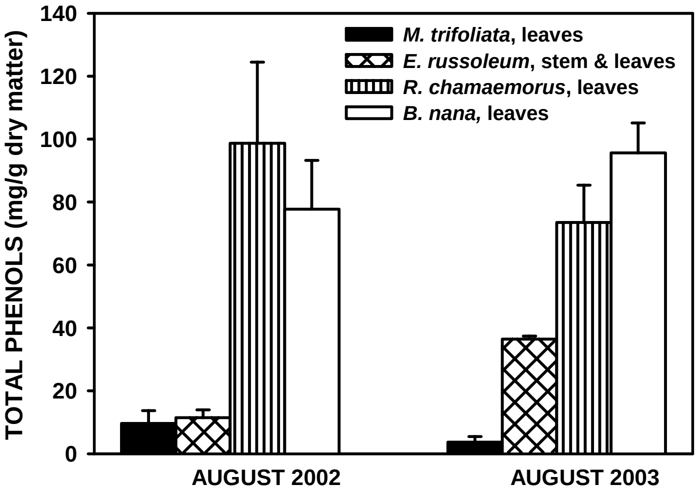

Fig. 2. Total concentration of soluble phenolics (mean ± SD) in different plant species collected from unmanipulated peatland areas (Soppela et al. 2004).

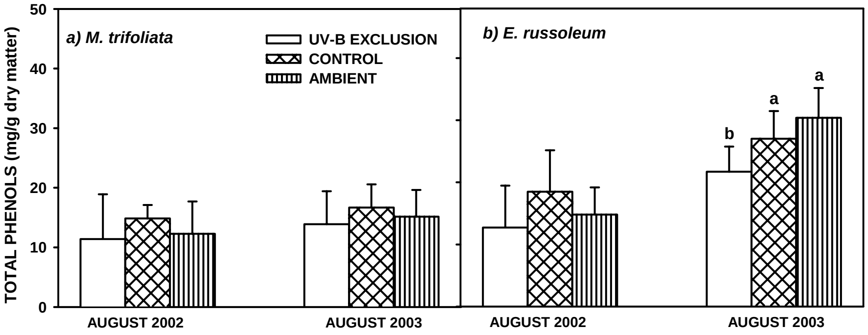

Fig. 3. Total concentration of soluble phenolics (mean ± SD) (a) in leaves of M. trifoliata and (b) in stem and leaves of E. russoleum in different treatments of the UV-filtration experiment in 2002 and 2003. Significant differences between treatments are indicated by different superscript letter (one-way ANOVA P<0.01, followed by LSD test) (Soppela et al., 2004).

Conclusions

The results show that UV radiation induced production of UV-B absorbing phenolic compounds in E. russoleum during second summer of research. There were no significant differences between treatments in the UV experiment in nitrogen, fibers or digestibility. The effects of UV radiation were, however, rather small and not the same in all forage plants. The excess of phenolic defence compounds may reduce the quality of reindeer forage and affect the feeding behaviour of reindeer. Further and longer-term studies are necessary to confirm the preliminary results.

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POSITIVE GROWTH RESPONSES OF YOUNG SCOTS PINE SEEDLINGS TO URBAN FOREST SOILS

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Introduction

The productivity of many boreal ecosystems is limited by the supply of biologically available nitrogen. This leads to a high vulnerability of boreal forests to anthropogenic nitrogen emissions. In many urban areas these emissions exceed natural N inputs. Elevated N availability increases productivity and biomass accumulation (Tilman, 1996), but generally reduces the biological diversity of ecosystems (Tilman, 1987; Vitousek et al., 1997). The increase of limiting nutrients can drastically change species dominance in ecosystems (e.g. Lilleskov et al., 2001; Vitousek et al., 1997; Wallenda and Kottke, 1998). The impacts vary between different types of organisms (Gehring et al., 1998) depending on the sensitivity of the species for example to atmospheric ammonia (Fangmeier et al., 1994) and their capacity to compete with other species under changed nutrient conditions (Kaye and Hart, 1997).

The biological processes of the humus layer have been studied since 1987 in Scots pine forests along an urban pollution (mainly nitrogen and sulphur) gradient around the city of Oulu in northern Finland (e.g. Markkola et al., 1990, 2002; Ohtonen, 1994; Ohtonen et al., 1990, 1992, 1993; Tarvainen et al., 2003, 2004). Our study area provides an interesting forum for restoration experiments, since both the N and S emissions have been cut down during the last decade, but the humus layer still contains high N and S concentrations affecting the vegetation and fungal communities (Tarvainen et al., 2003). Using sown-origin and nursery seedlings of Scots pine as bait plants we were able to study the nutrient availability for the growth.

Materials and Methods

The study area is located in the middle boreal vegetation zone (Ahti et al., 1968) around the city of Oulu in northern Finland (65º00´N, 25º30´E). The area is characterized by nutrient-poor mineral soil. The study sites were chosen in dry-dryish pine stands in different pollution zones determined by reference to the sulfur concentration in the pine needles - zone I: not measured, zone II: <1100, zone III: 1100-1300, and in the zone IV: >1300 mg g^{-1} (Karhu, 1986). The principal emissions in the area have decreased during the last decade (Anonymous, 2001). In our earlier studies we found increasing nitrogen and sulphur concentration in forest humus layer along the pollution gradient (Markkola et al., 1995; Tarvainen et al., 2003). In order to reduce the nitrogen load in forest soil, a restoration experiment was established in June 2001 in urban forests of Oulu (Zone IV) and natural forests of Muhos. The effects of humus disturbance and removal on the early development of sown-origin Scots pine seedlings were studied also in a greenhouse experiment, established in June 2001. The plant growth parameters and peroxidase (POD) activity were measured from above and/or below ground part of the seedlings.

Results and Discussion

The effects of soil quality on performance of Scots pine bait seedlings were significant. The differences were observed when seedlings were grown urban forest soils either in field or greenhouse experiments. On the contrary, the pollution effects via air were not significant in our study (Tarvainen et al., 2004). The seedling growth was not affected by the exposure to any combinations of low level of gaseous O_3, SO_2 and NO_x.

Our field observations (Markkola et al., 1990) and greenhouse experiments (Markkola et al., 2002; Tarvainen et al., 2004) show that certain factors in urban polluted forest soils elicit high POD activity in the roots of both mature trees and seedlings of Scots pine. Only a slight positive relation between root POD activity and low levels of ammonium sulphate added to the soil was found in our study (Markkola et al., 2002). The possible role of elevated heavy metal concentrations detected in the urban polluted forest soils cannot be excluded in the elicitation of POD activity. The effect of Cu in eliciting POD activity in Scots pine roots may be minor according to previous studies (Kukkola et al., 2000; Roitto et al., 1998), but it is still possible that other heavy metals such as Pb, Cr and V in our urban polluted soils (Ohtonen et al., 1990) could act as POD elicitors. As an alternative hypothesis we also suggest, that indirect biotic factors in the soil like changes in fungal community at polluted sites (Markkola et al., 1995; Tarvainen et al., 2003) or shift in ECM community in the fine roots of nursery seedlings after planting into the nutrient poor forest humus (Tarvainen et al., 2004) may be important. According to Münzenberger et al. (1997), ECM fungi have the ability to specifically suppress the POD activity, as found in Picea abies and Larix decidua seedlings. It has been suggested that auxins produced by the ECM fungi (Charvet-Candela et al., 2002; Salzer and Hager, 1993) reduce peroxidase-catalysed cross-linking of cell-wall constituents and the roots with less rigid cell walls would allow colonization of the intercellular space by symbiotic fungi (Tarkka et al., 2001). On the contrary, certain mycorrhizal fungi may inrease POD activity in the roots (Günther et al., 1998; Salzer and Hager, 1993), but the activity decreases when the mycorrhiza formation is established (Spanu and Bonfante-Fasolo, 1988).

As a conclusion, we can say that long-term accumulation of nitrogen compounds to urban forest humus promotes positive growth responses to urban nitrogen rich environment. However, we found increased stress expressed as POD enzyme activity in the roots, and the possible changes in winter hardiness of the seedlings were not studied.

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DIAGNOSTICS IN BEECH EXPOSED TO CHRONIC FREE AIR O_3 FUMIGATION: 2. COMPARISON BETWEEN YOUNG AND ADULT TREES AT THE BRANCH AND TREE LEVEL

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Introduction

Most research on O_3 effects has been carried out so far under controlled laboratory conditions or in open-top chambers with seedlings (Reich, 1987; Sandermann et al., 1997; Matyssek and Innes, 1999). However, these results cannot be transferred to predict O_3 responses of adult trees in the field (Kelly et al., 1995; Kolb et al., 1997; Kolb and Matyssek, 2001) because microclimatic conditions in exposure chambers exposure differ from field conditions (Koch and Lautenschlager, 1988) and because ozone sensitivity of young and old trees differs due to ontogenic differences in morphology and physiology thus strongly influencing the responses to O_3 (Ryan et al., 1997; Bond, 2000). Furthermore, it is still an open question if findings from former branch-bag experiments are relevant for whole-tree risk assessment. Therefore, it was the aim of this study to compare the O_3 sensitivity, of juvenile and adult beech trees when both ages were assessed under the same climatic conditions and to clarify the still open question whether beside carbon autonomy (Sprugel et al., 1991) twigs also possess autonomy in their defense metabolism (Sandermann et al., 1997).

Materials and Methods

The study was carried out in an about 60-year-old beech/spruce stand at Kranzberg Forest (Freising/Germany, 48° N, 11°) throughout the growing season of 2003. Scaffolding and a research crane provided access to sun and shade crowns. A free-air O_3 fumigation system (Nunn et al., 2002; Werner and Fabian, 2002; Häberle et al., 2003) was installed within the forest canopy, allowing entire crowns of neighbouring five beech trees to be exposed to an experimentally enhanced, twice-ambient O_3 regime ($2xO_3$). Comparisons were made with trees under ambient air ($1xO_3$ = control). Maximum O_3 levels at $2xO_3$ were restricted to 150 nl O_3 l^{-1} to prevent risk of acute O_3 injury.

In order to exclude microclimatic differences between seedlings and adult trees, container grown seedlings (9 containers per treatment with 6 plants each) were positioned on a special scaffolding within non-fumigated and O_3-fumigated areas in the sun crown of 60-year old beech trees. Containers were watered regularly in order to avoid severe soil drought. In addition, transparent fumigation cuvettes tracking ambient climatic conditions and O_3 levels (Havranek and Wieser, 1994) were mounted around terminal sections of branches in the sun crown of beech under both O_3 regimes. Six cuvettes received $1xO_3$ air in trees under $2xO_3$ and vice versa (Fig. 1).

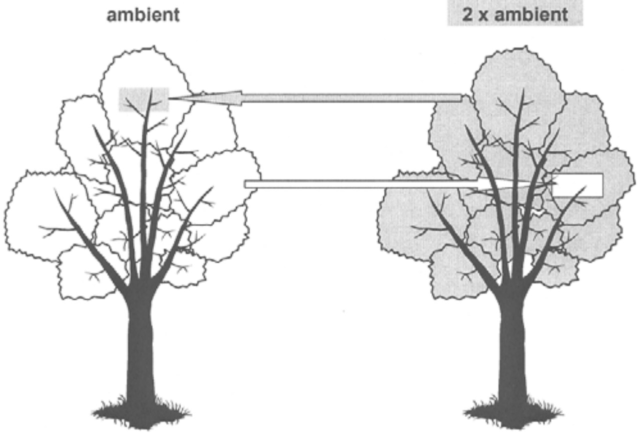

Fig. 1. Experimental design for validating branch chamber O_3 fumigations for their applicability for whole tree risk assessment

At the end of the fumigation period leaves were examined for differences in gas exchange under controlled conditions and antioxidant contents. Gas exchange measurements were made in situ with a CIRAS1 (PP-Systems; Herts, U.K.) under standardised conditions (20°C leaf temperature, 10

PakPa^{-1} leaf to air water vapour pressure difference, 360 µmol mol^{-1} CO_2, light saturation). Total ascorbate, total glutathione, and α – tocopherol were detemined as described previously by Tausz et al. (2003) and Wildi and Lütz (1996), respectively.

Results and Discussion

Mean O_3 concentrations, SUM0, AOT40 values and cumulative O_3 uptake (CU) throughout the fumigation period are summarised in Table 1. As a consequence of the dry summer 2003 stomatal conductance for O_3 (gO_3) was low and shows no significant differences with respect to O_3 exposure. Consequently, CU into the leaves was mainly dependent on external O_3 concentration.

Table 1. Exposure indices for the ambient ($1xO_3$) and twice ambient ($2xO_3$) ozone treatment during the experimental period April 15 to September 5, 2003. SUM0 = total external O_3 exposure, AOT40 = accumulated exposure over a threshold of 40 ppb, CU = cumulative O_3 uptake, g_{O3} = stomatal conductance for O_3. CU and g_{O3} values are daily mean values over the experimental period per cuvette ozone treatment (n = 6).

Treatment	Mean O_3 [ppb]	SUM0 [ppm-h]	AOT40 [ppm-h]	CU [µmol m^{-2} d^{-1}]	gO3 [mmol m^{-2} s^{-1}]
$1xO_3$	49	169	31	74 ± 32	17 ± 7
$2xO_3$	63	219	80	92 ± 19	16 ± 3

Independent from O_3 exposure tree age had a significant effect on photosynthetic capacity (Pn), stomatal conductances (gH_2O), and CO_2 concentration in the leaf internal air spaces (c_i) with adult trees having higher Pn and lower gH_2O and c_i values than seedlings (Fig. 2). $2xO_3$ caused a significant decline in Pn of seedlings and no effects were observed in adult trees (Fig. 2). These findings clearly emphasize that seedlings are uncertain surrogates for estimating the susceptibility to O_3 in adult trees, even under comparable site conditions. However, $2xO_3$ did not affect Pn of seedlings grown in the shade of the forest floor (Then et al. unpublished), the natural habitat of beech seedlings.

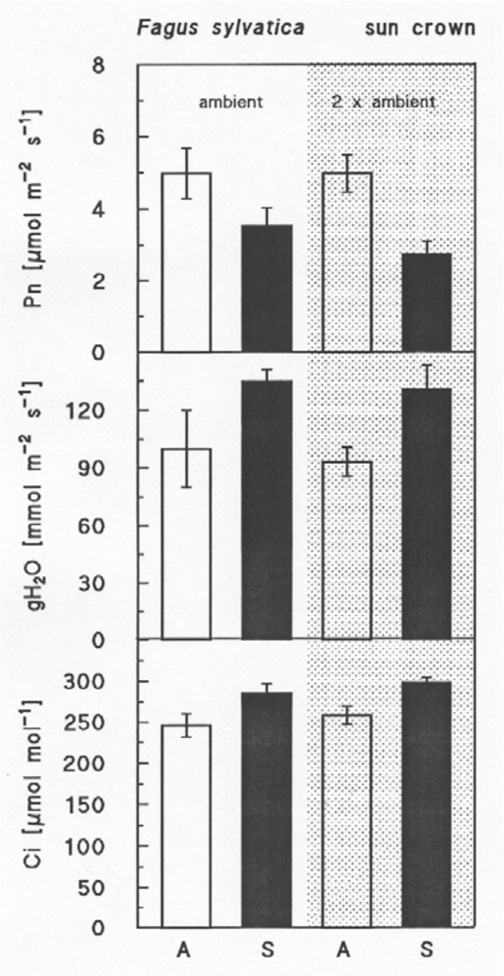

Fig. 2. Net photosynthesis (Pn), stomatal conductance to water vapour (gH_2O), and CO_2 concentration in the mesophyll internal air spaces (c_i) in the sun crown of Fagus sylvativa leaves of adult trees (A) and seedlings (S) after exposure to $1xO_3$ (open area) and $2xO_3$ (hatched area) throughout the growing season of 2003. Data are means of 5 trees ± SE.

After exposure to both O_3 regimes employed in branch chambers, a comparison of leaves of enclosed twigs with leaves of twigs outside chambers fails to find significant differences in foliar gas exchange and antioxidant levels (Fig. 3).

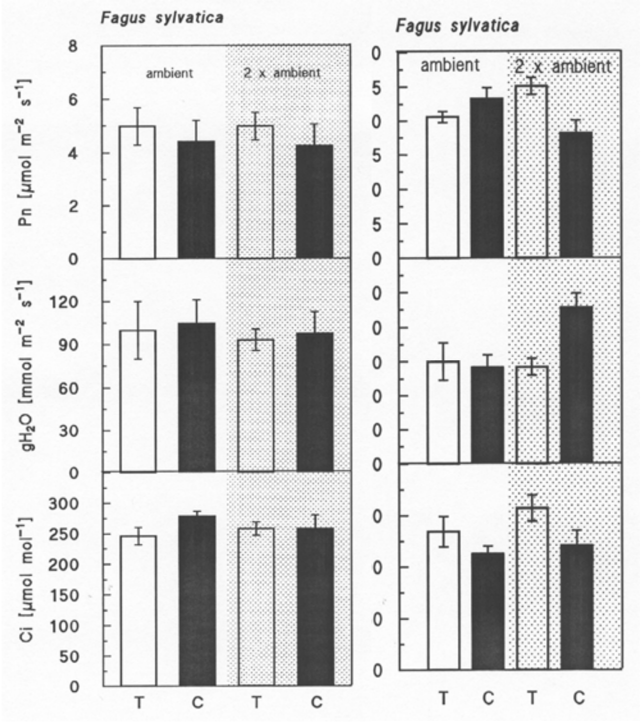

Fig. 3. Net photosynthesis (Pn), stomatal conductance to water vapour (gH_2O), and CO_2 concentration in the mesophyll internal air spaces (c_i) (left); as well as total ascorbate, total glutathione, and α – tocopherol contents (right) in leaves of adult beech (Fagus sylvatica) trees on twigs outside (T) and inside (C) the cuvettes after exposure to $1xO_3$ (open area) and $2xO_3$ (hatched area) throughout the growing season 2003. Data are means of 6 cuvettes ± SE.

Observed differences within treatments were within the natural variation observed at the study site (Wieser et al., 2003). These findings clearly demonstrate that in analogy to the postulated carbon autonomy (Sprugel et al., 1991) twigs possess autonomy in their defense metabolism. This is also emphazised by the fact that ascorbate regeneration is driven by light processes of photosynthesis (Smirnoff, 1996; Noctor and Foyer, 1998) and that the ascorbat pool correlates with the photosynthetic capacity and supply of soluble carbohydrates (Smirnoff and Pallanca, 1996).

Conclusions

In conclusion, even when comparing two different ontogenetic stages in the field under comparable micro-climatic conditions of sun crowns, beech seedlings responded more sensitively to O_3. Branch cuvettes by contrast, were found to be useful surrogates for examining the response of entire tree crowns to elevated O_3.

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HIGH STOMATA DENSITY CONTRIBUTE TO OZONE SENSITIVITY

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Abstract

The differences in leaf anatomy between O_3-sensitive (Potentilla palustris, Trifolium repens and Phleum alpinum) and tolerant (Alchemilla alpina, Gnaphalium norwegicum, Anthoxanthum odoratum and Anthoxanthum odoratum ssp. alpinum) species were studied in situ, in open field and in open-top chambers (OTCs). The tolerant forb species were characterized by amorphous smooth epicuticular wax surfaces. The sensitive species had moderate or low trichome density and dense structural epicuticular waxes characterized by platelets and plates. The forb species grown in situ for at least 3 years showed a relationship between the previously observed O_3 sensitivity, high stomatal density and small size of mesophyll cells. In the plants growing for the first year in open-field plots or OTCs high stomatal density was related to sensitivity both in grasses and forb species. It was concluded that high stomatal density contributes to O_3 sensitivity. OTC treatment did not affect the leaf anatomical characteristics.

Introduction

The sensitivity of plants to tropospheric O_3 is governed by avoidance, compensation, defence and repair mechanisms (Kolb et al., 1997). Low O_3 uptake is the fundamental avoidance mechanism. Stomata constitute the primary route for the passage of O_3 into the leaf. Hairs, trichomes, cuticles and glandular structures influence the passage of air over the epidermis and into the stomata (Kerstiens and Ledzian, 1989; Runeckles, 1992; Bolhar-Nordenkampf and Draxler, 1993; Evans et al., 1996, Dickison, 2000). Intercellular space constitutes a continuous system from the substomatal chambers to the cell walls in the mesophyll. Internal resistance to the movement of O_3 and its harmful reaction products is possibly dependent on the volume of this intercellular space, which generally accounts for 10-40 % of total leaf volume in the palisade mesophyll and 50-80 % in the spongy mesophyll. Increased mesophyll intercellular space will allow a greater volume of O_3 and may increase the probability on cell injury. The intercellular volume increases during ontogeny and shows daily changes according to the water content of the leaves (Nobel 1983; Bennett et al., 1992; Bolhar-Nordenkampf and Draxler, 1993). Stomatal density is known to be influenced by such external factors as wind speed, light intensity and atmospheric CO_2 concentration (Packham and Willis, 1982; Woodward and Bazzaz, 1988; Retuerto and Woodward, 1993; Woodward and Kelly, 1995; Beerling and Kelly, 1997), which may differ between OTCs and open-field plots.

Small mesophyll cells have a higher surface- to volume- ratio than large mesophyll cells, which means that they may be affected by a larger amount of O_3. Although there is a greater fraction of air in the spongy mesophyll region, palisade cells usually have a greater total cell wall area exposed to the intercellular air spaces than spongy mesophyll cells due to their higher cell density (Turrell, 1936; Nobel, 1983). Ozone sensitivity may also be related to the relative growth rate (RGR). Several studies suggest that fast growing species are more O_3- sensitive than slowly growing ones. This is propably due to the higher stomatal conductance of fast growing species, which results in larger amounts of O_3 entering the intercellular space (Reiling and Davison, 1992; Danielsson et al., 1999; Bungener et al., 1999).

The aim of this study was to determine if the O_3 sensitivity of forbs and grasses observed in experimental studies (Timonen et al., 2004 and references therein) is related to the leaf anatomy of the species. The hypothesis was that O_3 sensitivity is associated with O_3 diffusion on and into leaves, and that it is explained by surface structure, stomatal density, leaf thickness, mesophyll density and the size of the intercellular air space.

Material and methods

The plant species for the anatomical study were chosen on the basis of a review article on O_3 studies of wild field layer plant species in northern Europe (Timonen et al., 2004). Three species that readily show growth reduction upon exposure to elevated O_3 concentrations: Potentilla palustris, Trifolium repens and Phleum alpinum were chosen to represent sensitive species (visible symptoms or growth reduction). Gnaphalium norwegicum, Alchemilla alpina and Anthoxanthum odoratum, which seem to tolerate elevated concentrations, represented tolerant species (Table 1.).

Leaf samples were taken in August and/or September 2001 and 2003. In the year 2001, in situ samples were collected from plants in the collections of the Botanical Garden of the University of Oulu. The species had been growing in the garden for 2 months to 9 years. P. palustris was growing wild by the pond in the Botanical Garden (year 2001) and on shore of nearby Lake Kuivasjärvi (year 2003). The other species studied in the year 2003 were grown from seeds sown in mid-May in the Botanical Garden of the University of Oulu. They were potted in a medium fine sand rich in organic matter, limed and fertilized light Sphagnum B2 peat (Kekkilä Finnpeat) and gravel (2:2:1) and transferred outdoors in their growing pots. On the 9th of June 2003, all plants were transferred to the experimental field (65°03´N, 25°29´E) in the Botanical Garden. They were divided between 12 open-top chambers (OTC) and 6 open-field plots. The plants were watered with lake water. No fertilizers were added. For studies both OTC and open-field plot conditions were used.

Table 1. Studied species and populations and transplanting and sampling dates. S: sensitive species, T: Tolerant species.

Year	Species	Origin	Date of transplanting outdoors	Sampling date
2001 in situ				
	T Alchemilla alpina L.	Nordnes, Storfjord, Norway (69°22´N, 20°13´E)	17th of September 1998	16th of September 2001
	T Anthoxanthum odoratum L.	Purola, Kajaani, Finland (64°13´N, 27°41´E)	10th of August 1992	16th of September 2001
	S Phleum alpinum L.	Saana, Enontekiö, Finland (69°02´N, 20°52´E, 500 m),	9th of July 2001	16th of September 2001
	S Potentilla palustris (L.) Scop.	Botanical gardens, Oulu, Finland (65°03´N, 25°28´E)	-	16th of September 2001
	S Trifolium repens L.	Commercial seed mixture, Vihertalo ky., Finland	5th of June 1997	16th of September 2001
2003 OTC + open field				
	T Anthoxanthum odoratum L.	Tervik, Pernaja, Finland (60°27´N, 25°57´E)	9th of June 2003	11th of August 2003
	T Anthoxanthum odoratum ssp. alpinum	Øverbygd, Rostadalen, Norway (68°49´N,21°52´E, 550m)	28th of May 2003	11th of August 2003
	T Gnaphalium norwegicum Gunnerus	Gåldajärvi, Kiruna, Sweden (69°05’N,20°05’E, 500m)	8th of July 2003	10th of September 2003
	T Gnaphalium norwegicum Gunnerus	Storfjord, Rostadalen, Norway (68°49’N,21°52’E, 250m)	8th of July 2003	10th of September 2003
	S Phleum alpinum L.	Rovijok, Storfjord, Norway (69°13’N,20°42’E, 325m)	9th of June 2003	11th of August 2003
	S Potentilla palustris (L.) Scop	Kuivasjärvi, Oulu, Finland (65°03´N, 25°29´E)	-	11th of August 2003

Sun-exposed leaves from the middle part of the shoot on the eastern side of the plant from five fully grown individuals were sampled for microscopy in 2001, and 3rd fully grown leaf from the apex was sampled from six fully grown individuals in the year 2003. Gnaphalium populations germinated so late that the plants were not big enough for sampling before the 10th of September. No O_3 was added to the OTCs but the chambers were ventilated to avoid excessive temperatures. The plants were watered before sampling. For each plant, two replicates were used in both years. The samples were cut between the midrib and the leaf margin halfway between the base and the apex of the lamina. Sample pieces (4 x 7 mm) for light- microscopic studies were fixed in FAA and embedded in paraffin. Transverse sections (5 µm) were cut and stained with Delafield´s hematoxylin. Samples were prepared from the adaxial (upper) and abaxial (lower) sides of each sample leaf, and stomatal densities (number of stomata per square millimetre) were counted and the structure of epicuticular wax examined under a JEOL JSM-6300F FESEM (Field Emission Scanning Electron Microscope). Magnification was 500x for stomatal and trichome density and 10 000x for wax structure. In A. alpina and G. norwegicum, trichomes had to be peeled off with tweezers to expose the stomata and cuticle of the leaf's abaxial surface.

From the cross-sections, the thickness of the leaf, the adaxial and abaxial epidermis and the palisade and spongy parenchyma, the areas of palisade and spongy mesophyll cells and the intercellular spaces of both palisade and spongy parenchyma were measured with Nikon Optiphot-2 microscope, Hitachi CCD KP-C571 video camera, Nikon FX-35DX camera and the image analysis software applications Scion IMAGE/NIH IMAGE and ImageJ1.27Z. The numbers of palisade and spongy parenchyma cell layers were counted. Five views of each leaf were analysed. In grasses, the leaves were longitudinally undulated. Measurements were conducted on both thick and thin areas 5 from each.

The data were analysed by using the GLM Univariate procedure (i.e. analysis of variance) and, in cases of inhomogeneous variance or non-normal distribution by non-parametric Kruskall-Wallis H test (Mann-Whitney U as a post hoc test) of SPSS for Windows Release 10.1.3. The numbers of sample plants were used as the number of replicates.

Results

Stomatal density and wax structure

In this study OTC treatment did not affect stomatal density even though it is known to be influenced by external factors as wind speed, light intensity, atmospheric CO_2 concentration, which differ in OTC and open field. Since there was no OTC effect, nor OTC x species interactions, the data of OTC and open-field plots were pooled together in the analysis. The species differed significantly in most parameters. In sensitive forb species the number of stomata per mm^2 was between 252-325, in tolerant ones 148-220 mm^{-2} and in grasses 92-183 and 56-111, respectively (Tables 2 and 3). Grass species had lower stomatal densities than forb species. P. palustris and A. alpina were hypostomatous (stomata restricted to the abaxial leaf surface) and the other species amphistomatous (stomata located on both the adaxial and the abaxial leaf surface). Explanation for the grasses having higher stomatal density on the adaxial compared to the abaxial leaf surface is probably that they fold longitudinally so it is the adaxial side that gets to the shelter reducing water loss.

The stomatal density of G. norwegicum abaxial side was 4-fold to the adaxial side, and density was low compared to the other forb species. O_3-sensitive P. palustris had higher stomatal densities than the other forbs. In grasses O_3-sensitive P. alpinum had higher stomatal densities than the O_3-tolerant A. odoratum, but the difference was statistically significant only in the year 2003.

Table 2. Leaf anatomy characteristics of the forb species, mean±s.d. Year 2001 represents in situ (n=5) and year 2003 OTC + open field plots (n=12).

	Sensitive species		Tolerant species		
	Potentilla palustris	Trifolium repens	Alchemilla alpina	Gnaphalium norwegicum Gåldajärvi	Gnaphalium norwegicum Rostadalen
2001 in situ					
Leaf thickness (µm)	109±29.2 a	132±17.0 a	148±34.3 a		
Adaxial epidermis thickness (µm)	16±3.5 a	18±2.8 a	22±5.7 a		
Abaxial epidermis thickness (µm)	11±2.5 a*	16±2.2 b *	13±0.9 a*		
Palisade parenchyma thickness (µm)	34±13.3 a*	42±9.3 a, b*	60±13.2 b*		
Spongy parenchyma thickness µm)	47±11.2 a	59±7.8 a	58±15.7 a		
Spongy thickness/ palisade thickness	1.46±0.4 a*	1.45±0.2 a*	0.98±0.2 b**		
Proportion of intercellular space in palisade layer (%)	9±3.4 a	4±1.5 a	16±8.9 a		
Proportion of intercellular space in spongy layer (%)	67±8.2 a**	5±1.5 b**	46±12.2 c**		
Proportion of intercellular space in total mesophyll (%)	44±8.1 a*/**	5±1.1 b**	28±9.5 c*/**		
Palisade cell area (µm^2)	210±49.4 a**	429±165.7 b*/**	661±177.7 c*/**		
Spongy cell area (µm^2)	157±29.8 a*/**	307±93.6 b**	252±54.8 b*		
Stomatal density adaxial (mm^{-2})	0±0.0 a**	157±85.0 b**	0±0.0 a**		
Stomatal density abaxial (mm^{-2})	252±51.0 a	169±102 a	220±49.0 a		

Stomatal density total (mm^{-2})	252±51.0 a	325±99.0 a	220±49.0 a		
2003 OTC + open field					
Leaf thickness (µm)	144±21.9 a***			177±18.1b ***	159±16.4 a.b***
Adaxial epidermis thickness (µm)	16±2.3 a**			21±4.4 b**	19±3.5 a.b**
Abaxial epidermis thickness (µm)	13±2.1 a***			17±2.4 b***	13±2.1 a***
Palisade parenchyma thickness (µm)	70±10.3 a***			54±3.1 b**/***	45±7.4 c**/***
Spongy parenchyma thickness (µm)	57±11.9 a ***			101±12.6 b***	94±11.0 b***
Spongy thickness/ palisade thickness	0.8± 0.2 a***			1.9±0.3 b***	2.1±0.3 b***
Proportion of intercellular space in palisade layer (%)	23±6.5 a*/***			14±4.1 b***	17±3.8 b*
Proportion of intercellular space in spongy layer (%)	59±10.3 a***			26±10.1 b***	20±7.7 b***
Proportion of intercellular space in total mesophyll (%)	41±7.5 a***			20±5.8 b***	19±4.1 b***
Palisade cell area (µm^2)	359±122.7 a***			749±92.7 b***	649±121.7 b***
Spongy cell area (µm^2)	167±75.6 a***			632±116.5 b***	489±55.7 c***
Stomatal density adaxial (mm^{-2})	0±0.0 a***			28±19.4 b***	38±33.9 b***
Stomatal density abaxial (mm^{-2})	287±85.3 a***			120±38.7 b***	126±47.5 b***
Stomatal density total (mm^{-2})	287±85.3 a**/***			148±33.8 b***	164±56.3 b**

Means within the same row followed by different letters are significantly different by analysis of variance or Mann-Whitney U. ***p<0.001, **0.001<p<0.01, *0.01<p<0.05. */** means that 0.01<p<0.05 to one species and 0.001<p<0.01 to another.

Table 3. Leaf anatomy characteristics of grass species, mean±s.d. Year 2001 represent in situ (n=5) and year 2003 OTC + open field plots (n=12).

	Sensitive species	Tolerant species	
	Phleum alpinum	Anthoxanthum odoratum	Anthoxanthum odoratum ssp. alpinum
2001 in situ			
Leaf thickness (µm)	116±24.6 a	102±8.2 a	
Adaxial epidermis thickness (µm)	22±8.2 a	19±1.9 a	
Abaxial epidermis thickness (µm)	14±2.0 a	15±0.8 a	
Proportion of intercellular space (%)	38±5.9 a	35±15.8 a	
Mesophyll cell area (µm^2)	307±69.3 a*	439±197.0 b*	
Stomatal density adaxial (mm^{-2})	77±33.8 a	45±14.4 a	
Stomatal density abaxial (mm^{-2})	16±10.6 a	11±19.5 a	
Stomatal density total (mm^{-2})	92±35.6 a	56±9.1 a	
2003 OTC + open field			
Leaf thickness (µm)	178±27.5 a***	163±14.4 a**	130±15.6 b**/***
Adaxial epidermis thickness (µm)	20±3.2 a**	21±3.6 a***	15±2.6 b**/***
Abaxial epidermis thickness (µm)	23±3.7 a***	23±2.8 a***	15±3.6 b***
Proportion of intercellular space (%)	18±7.2 a	16±6.1 a	17±5.5 a
Mesophyll cell area (µm^2)	519±80.5 a***	531±135.3 a***	304±60.2 b***
Stomatal density adaxial (mm^{-2})	115±41.5 a***	54±15.3 b***	79±51.2 ab***
Stomatal density abaxial (mm^{-2})	68±30.7 a**	45±16.7 ab**	32±33.7 b**
Stomatal density total (mm^{-2})	183±45.7 a***	100±26.5 b***	111±50.3 b***

Means within the same row followed by different letter are significantly different by analysis of variance or Mann-Whitney U. ***$p<0.001$, **$0.001<p<0.01$, *$0.01<p<0.05$. **/*** means that $0.001<p<0.01$ to one species and $p<0.001$ to another.

The O_3-tolerant species A. alpina and G. norwegicum had a high density of abaxial trichomes, while in O_3-sensitive species P. palustris their density was moderate. In the other species, abaxial surfaces were totally (P. alpinum and T. repens) or nearly (A. odoratum and A. odoratum ssp. alpinum) glabrous. Adaxial leaf surfaces were moderately pubescent in A. odoratum ssp. alpinum and G. norwegicum. They were sparsely pubescent In P. palustris and A. odoratum, while the other species were glabrous.

In most species, epicuticular wax was arranged in irregular platelets or plates (nomenclature follows Barthlott et al. (1998)) on both leaf surfaces (Figs. 1 and 2). The adaxial surfaces of A. alpina were sparsely covered with membraneous platelets, and the abaxial surface had net-like coverage of membraneous platelets (Fig. 1a and 1b). Tolerant G. norwegicum had smooth amorphous adaxial and abaxial leaf surfaces (Fig. 1c and 1d). Sensitive species exhibited more microstructural variability. P. palustris had dense adaxial wax with membraneous platelets and coiled rodlets and abaxial wax resembling coiled rodlets (Fig. 1e and 1f). T. repens had a waxy surface with particularly dense small platelets on both sides (Fig. 1g and 1h).

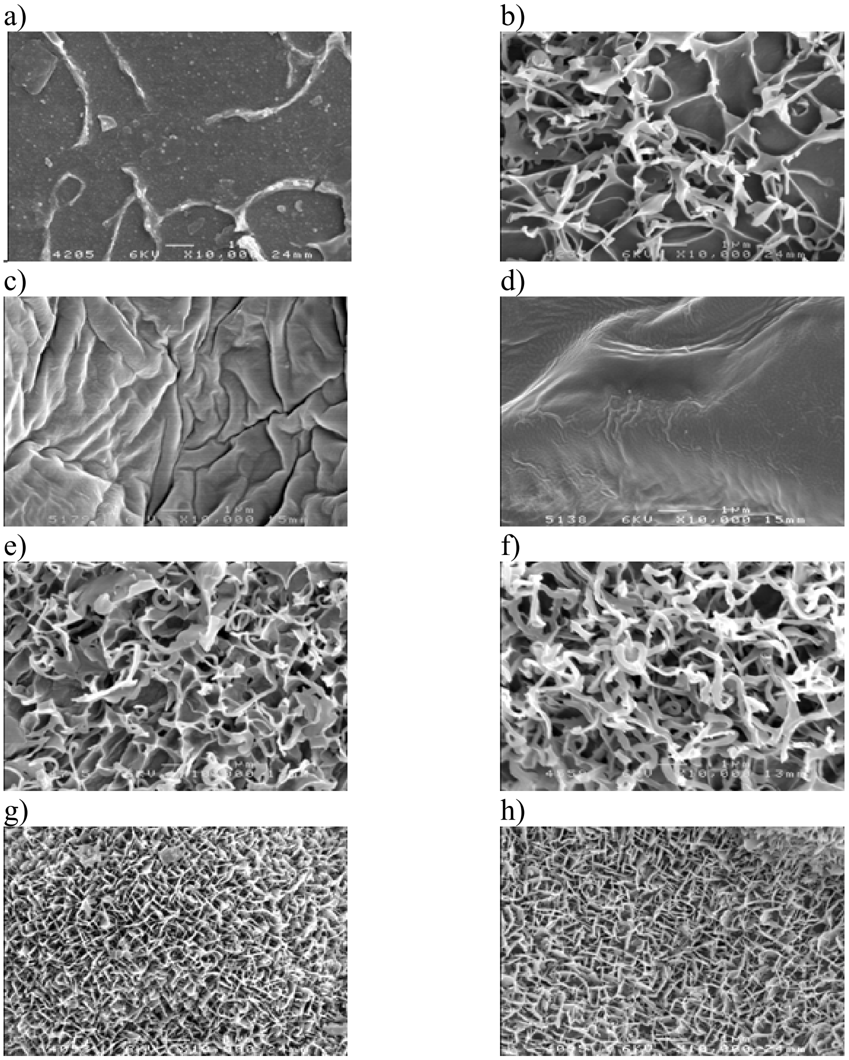

Fig.1. **F**ESEM views of epicuticular waxes of forb species collected in the summers 2001 and 2003, magnification 10 000x. a) Alchemilla alpina adaxial in situ, b) Alchemilla alpina abaxial in situ,c) Gnaphalium norwegicum adaxial open field, d) Gnaphalium norwegicum abaxial open field, e) Potentilla palustris adaxial open field, f) Potentilla palustris abaxial open field, g) Trifolium repens adaxial in situ, h*)* Trifolium repens abaxial in situ.

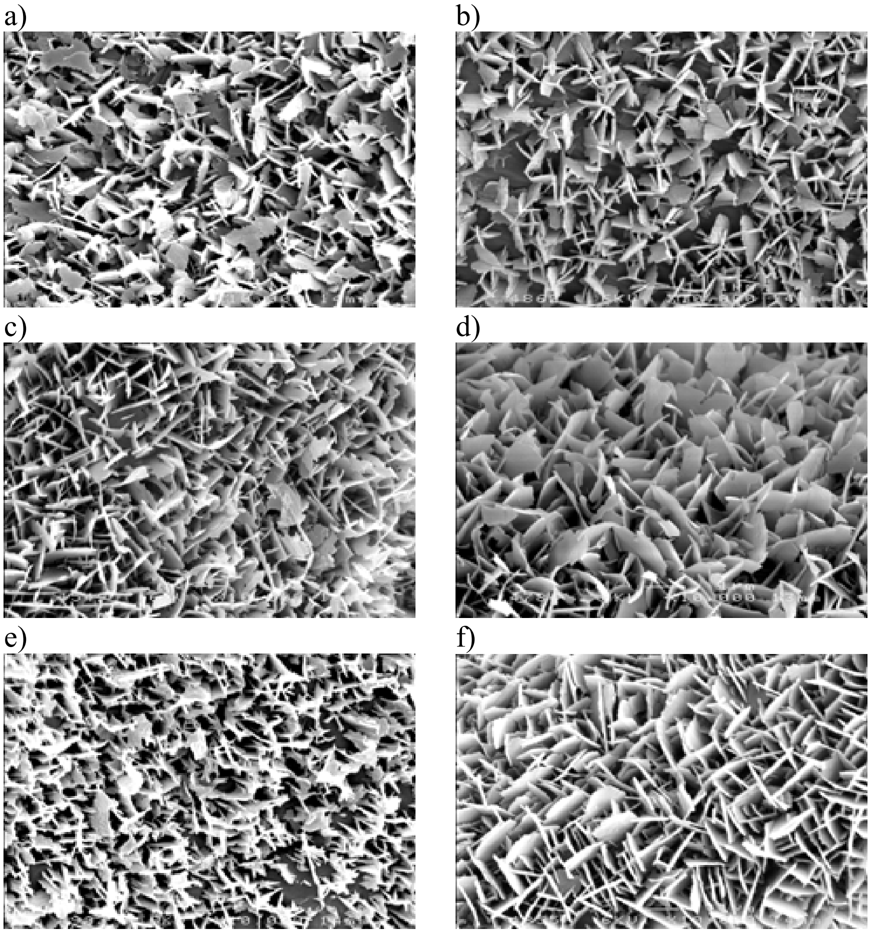

Fig. 2. FESEM views of epicuticular waxes of grass species collected in the summers 2003, magnification 10 000x. a) Anthoxanthum odoratum adaxial open field, b) Anthoxanthum odoratum abaxial open field, c) Anthoxanthum odoratum ssp. alpinum adaxial open field, d) Anthoxhantum odoratum ssp. alpinum abaxial open field, e) Phleum alpinum adaxial open field, f) Phleum alpinum abaxial open field.

The adaxial surface of A. odoratum was covered by platelets with some polymorphism and the abaxial surface was covered by small platelets (Fig. 2a and 2b). A. odoratum ssp. alpinum had similar structure on the adaxial surface, but the abaxial surface was characterized by plates. The adaxial surface of sensitive P. alpinum had small platelets, and the abaxial surface was covered by dense plates (Fig. 2e and 2f).

Leaf anatomy

The in situ leaf anatomy of O_3-sensitive T. repens revealed thick abaxial epidermis (Table 2). The palisade parenchyma layer was thin in the wet site species P. palustris. The thicknesses of the spongy parenchyma did not differ between species. The ratio of spongy to palisade parenchyma was higher in O_3-sensitive P. palustris and T. repens than in tolerant A. alpina. The total mesophyll intercellular space was smallest in T. repens and largest in the other sensitive species, P. palustris, the difference being mostly caused by differences in the spongy layers. The palisade parenchyma cell size was smaller in O_3- sensitive P. palustris than in tolerant A. alpina.

The O_3-sensitive wet site species, P. palustris, had a large intercellular space and the smallest parenchyma cells of the forb species (Table 2 and Fig. 3a-d.). Among the grasses, the in situ O_3-sensitive P. alpinum had smaller mesophyll cells than the O_3-tolerant A. odoratum (Table 3). No such difference was seen in the OTCs and open field, but A. odoratum ssp. alpinum had thinner leaves and adaxial and abaxial epidermis and smaller mesophyll cells than the other two grasses (Table 3, Figs. 3e-g). In sensitive forb species (T. repens and P. palustris) palisade parenchyma cells were in 2 layers and in the tolerant ones (A. alpina and G. norwegicum) in a single layer. The cells were typically rod-shaped. In T. repens they became shorter and more spherical towards the spongy parenchyma. The grass species had homogenous mesophylls.

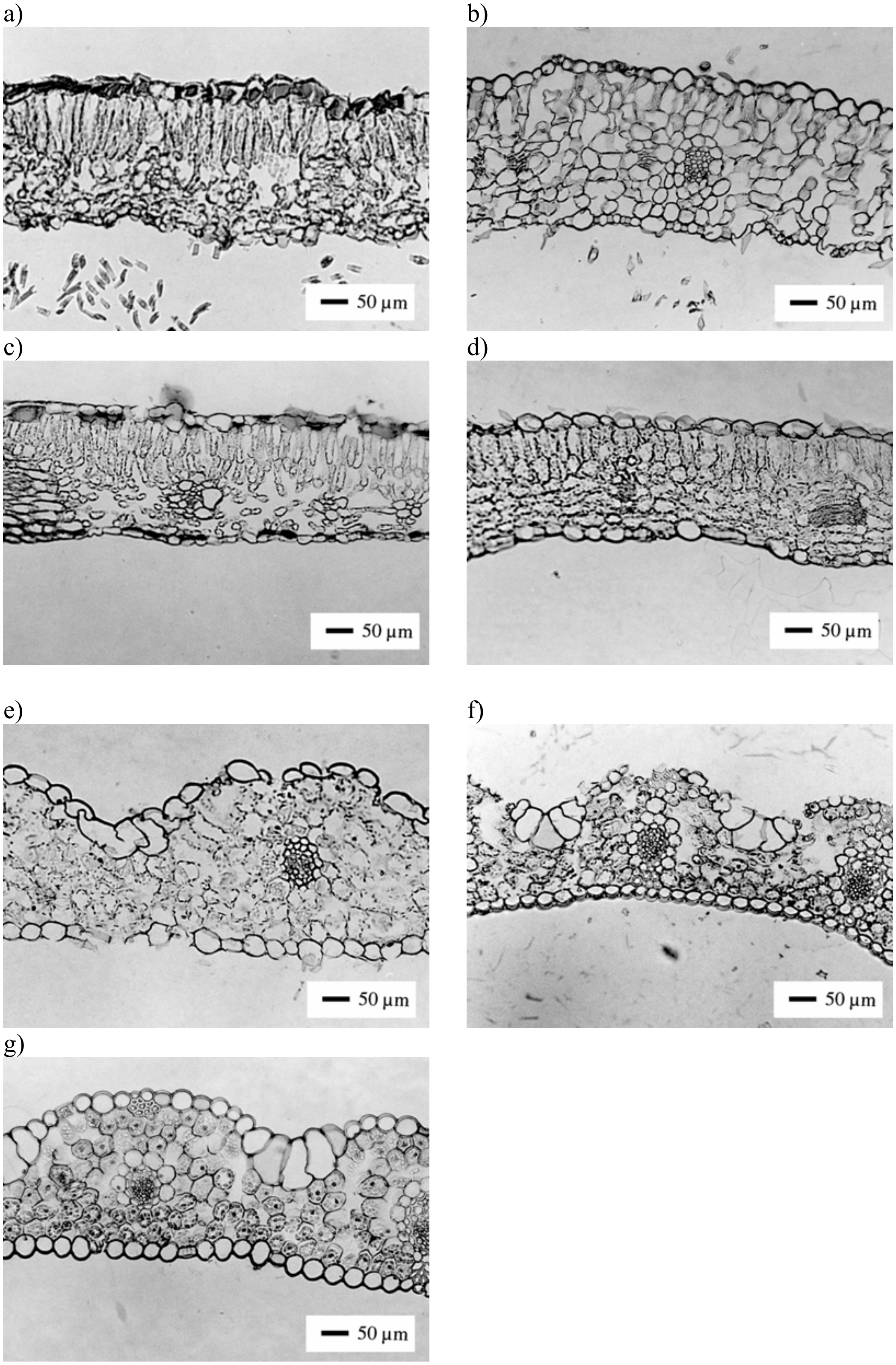

Fig. 3. Cross- sectional views of leaves collected in the summers 2001 and 2003, magnification 140 x. a) Alchemilla alpina in situ, b) Gnaphalium norwegicum, Gåldajärvi open field, c) Potentilla palustris open field, d) Trifolium repens in situ, e) Anthoxanthum odoratum open field, f) Anthoxanthum odoratum ssp. alpinum open field, g) Phleum alpinum open field.

Discussion

In this study the O_3-sensitive species had higher stomatal density than tolerant ones. Also Evans and Miller (1972), Evans et al. (1996) and Ferdinand et al. (2000) pointed out that sensitive species/genotypes have higher stomatal density, but sensitivity has also been found to correlate with low stomatal density in some species, such as Betula pendula Roth. clones (Pääkkönen et al., 1997). Bennett et al. (1992), on the other hand, did not find any correlation between sensitivity and stomatal density.

There was no anatomical acclimation to the OTC circumstances. The O_3-sensitive forb species were characterized by dense epicuticular structural wax. The wax structure of O_3-sensitive, wet site favouring P. palustris with its coiled rodlets differed from the other sensitive species, which were characterized by wax platelets or plates. Rough surfaces cause turbulence in air streams (Pleijel et al., 1995). This may increase the O_3 flux to the leaf interior and thus explain the observed O_3 responses of the species that have been ranked as sensitive ones. The O_3-tolerant forb species had smooth amorphous surfaces. The high abaxial trichome density of A. alpina and G. norwegicum is probably due to their being alpine plants which often have dense hair coverage to reduce gas diffusion (Ripley et al., 1999; Dickison, 2000). This also diminishes the amount of O_3 entering the leaf interior and possibly decreases the risk of harmful O_3 effects on alpine and other hairy plants. Respectively, the O_3 sensitivity of P. alpinum may be related to the lack of trichomes which would reduce the O_3 uptake.

The present study revealed no relationship between the thickness of the leaf or epidermis and the O_3 sensitivity of the species. Nor did Evans et al. (1996) find thick epidermis to contribute to O_3 tolerance on Rudbeckia laciniata, Rubus canadensis, Aster divaricatus, Sassafras albidum, Liguidamber styraciflua and Magnolia tripetala. On the other hand, sensitive genotypes of Prunus serotina Ehrh. and Betula pendula Roth. have been documented to have 6 % and Trifolium repens L. 15 % smaller total leaf thickness compared to tolerant genotypes (Bennett et al., 1992; Postiglione, 2000).

The tolerant species studied here, A. alpina and G. norwegicum, had large palisade parenchyma cells, and G. norwegicum also spongy parenchyma cells. Thus, there is a discrepancy compared to the result of Evans et al.

(1996), who found the sensitive species to have larger palisade parenchyma cells and no difference in spongy parenchyma cell size. In this study, the results of grass species did not support the hypothesis that O_3 sensitivity would be related to small cell size. Sensitive P. alpinum had smaller mesophyll cells than tolerant A. odoratum in situ, but not in OTCs or open-field plots. Bennett et al. (1992) and Evans and Miller (1972) did not find any relationship between sensitivity and the cross-sectional area of mesophyll cells.

P. palustris had aerenchymatous parenchyma with a lot of intercellular space, especially in the spongy parenchyma. The other sensitive forb, T. repens, had clearly the densest parenchyma of all species, and especially its spongy parenchyma had very small intercellular spaces. Bennett et al. (1992) also failed to find any difference in the volume of intercellular space in the palisade mesophyll layer between sensitive and tolerant genotypes. Still according to Barth and Conklin (2003), the O_3 sensitivity of Arabidopsis thaliana mutant lcd1-1 may be, at least partly, explained by it having lower palisade cell density than the non-sensitive wild type. This results in more oxygen radicals attacking a single cell. As, in this study, results of grass species did not support the hypothesis of a large intercellular space explaining the O_3 sensitivity, either, it is concluded that there is no relationship between large intercellular space and O_3 sensitivity.

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MASS BALANCE OF NATURAL AND ANTHROPOGENIC CARBON DIOXIDE FLOWS

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Introduction

Climate warming is named to be one of the major problems in the world at present. The main component contributing to the green house effect, causing atmospheric temperature increase is indicated to be carbon dioxide gas (CO_2, (g)) (IPCC 2001 a, b). Resolution of this huge problem need several actions heading to the same target: decrease the CO_2 concentration in the atmosphere. In this study authors have taken an engineering approach involving applied methods and practices used in process and chemical engineering to describe the problem of CO_2 accumulation into the atmosphere. The study includes a schematic mass flow diagram with a mass balance, continuing with a discussion on indirect effects of pollutants, named acidic pollutants, on the carbon flows.

The mass balance of the global carbon dioxide flows

Since the CO_2 accumulation into the atmosphere is indicated to be a mass balance problem, then by applying the practice used in chemical and process engineering, a mass balance over the problem area is created. In this special case, the focus of the problem is atmosphere, which could be described as an intermediate vessel, into where CO_2 flows from the various sources and from where it is consumed by several anthropogenic and natural processes (IPCC 2001a; Turunen et al., 2003). A schematic presentation of the flows, which are directly interfaced with atmosphere, is presented in Fig. 1. So, for example photosynthesis of terrestrial plants is shown, but not that of marine plants, while they use dissolved CO_2 from water (Sieglerhaler and Sarmineto, 1993).

From the basis of thermodynamics it is known, that the gas-liquid equilibrium exists for dissolved volatile components. The components are transferred between the liquid and gas phases towards equilibrium composition at each temperature and pressure. Analogically, equilibrium relations can be derived for gas-solid systems (Sandler, 1989). These kinds of CO_2-flows can be identified between atmosphere and hydrosphere. The flows between atmosphere and cryosphere are not discussed so much (IPCC 2001a, b), however, this phenomenon is also shown in Fig. 1. Since there is a continuous CO_2-exhange between atmosphere and hydrosphere, in this illustration the aqueous system, together with atmosphere is considered as an intermediate storage, although oceans are generally known to be large sinks of CO_2 as well (IPCC 2001a, b; Sieglerhaler and Sarmineto, 1993; FAO, 2001).

The numeric annual mass balance, based on the structure in Fig. 1, is presented in Table 1. All the values for the distinguished flows could not be identified, however since lumped values were available, the overall picture could be figured out. Some values are still missing or intentionally ignored, like forest fires, volcanic eruptions or fixation by the mineral carbonation. Chemical fixation of CO_2 in the form of carbonate minerals is known in nature, but the rate of this reaction is so slow, that it occurs on the geological time scale (Lackner et al., 1995). It is also worth mentioning, that the value of emissions from anthropogenic processes, 6.5

GtC/a, includes burning of fossil fuels and emissions from cement industry, but excludes burning of biofuels or biomaterial by human activity. Although, the table is not complete, and there is variation in some of the figures e.g. in terrestrial activity (IPCC 2001b; Sieglerhaler and Sarmineto, 1993), this method however, provides a simple and perspicuous method to compare different elements of system with each other.

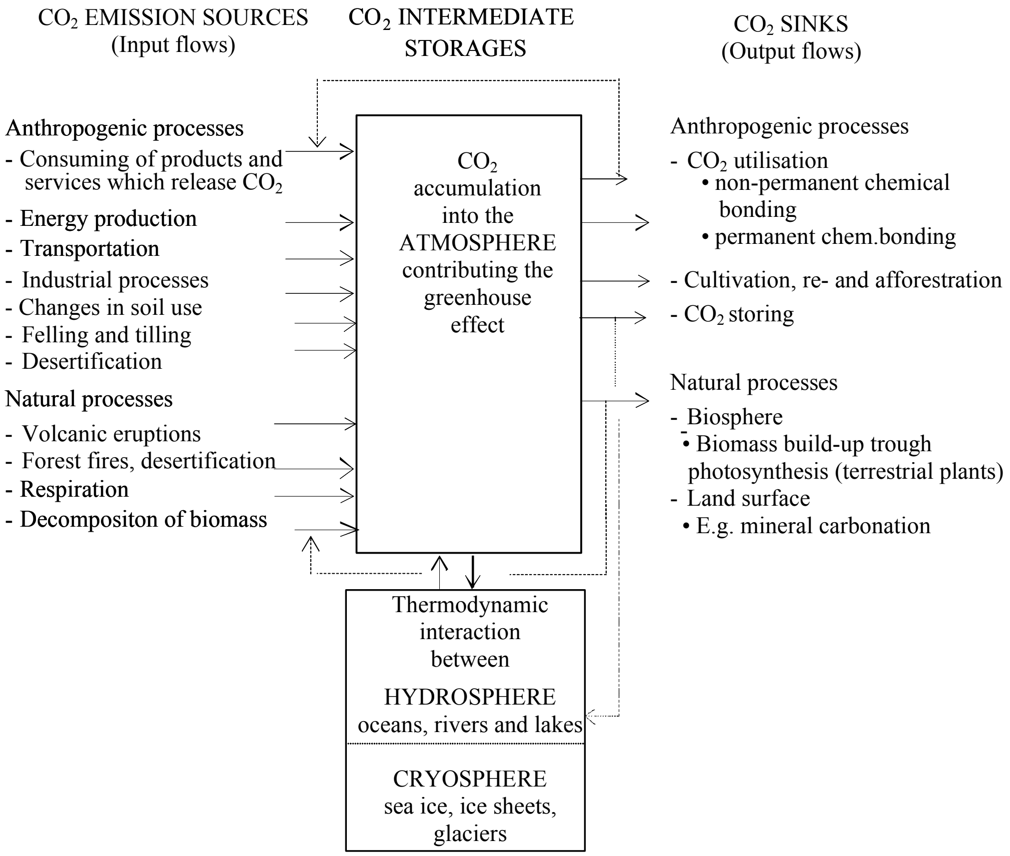


Fig. 1. Schematic mass flow diagram for CO_2 over the atmosphere. The solid arrows show the CO_2 flows and dashed arrow lines represent products, natural or artificial, in which CO_2 has been fixed.

According to the practice used in process engineering input and output flows are listed and counted in their own columns in Table 1. If the figures in both columns are equal, the system is in balance. If the input flows are smaller than the output flows, then the particular process vessel is draining empty. If the input flows are greater than output flows, then there is an accumulation problem. In terms of CO_2-flows, this is the ongoing process in the atmosphere. Accumulation rate is about 3.5 GtC/a (equal to PgC/yr), which is the same magnitude as reported in literature (IPCC 2001 a, b; Sieglerhaler and Sarmineto, 1993; FAO, 2001). When comparing that with the total flow rates, which are 158.5 and -155.0 GtC/a (cf. Table1), it is noticed that the accumulation represents about 2.2% of these flows.

Additionally it is obvious, that natural flows are clearly the largest flows in this system. Although the natural flows cannot be controlled by human consciousness in the same way as anthropogenic flows, it is worth having a closer look at them.

Some phenomena and their characteristics are as follows:

1. Interaction flows between atmosphere and hydrosphere described with thermodynamic equations

On a global scale this is the largest flow pair (90/-92 GtC). On the basis of the mass balance, only 1% change (~0.9 GtC/a) in this flow may change the accumulation amount by a quarter (0.9/3.5*100%=26%). This would be a significant change.

Carbon in natural aquatic systems can be in the form of dissolved CO_2 (aq), which further reacts to inorganic components. These reactions involve the formation of calcium carbonate, which can be dissolved or precipitated. Also organic carbon is found from natural aquatic systems (Baes, 1983). This mixture of different components and reactions in aquatic system is creating interaction flows between hydrosphere and atmosphere. On the other hand, in natural processes, equilibrium is a fundamental property of physical and chemical interactions.

Table 1. The mass balance of CO$_2$, numerical presentation.

CO$_2$ GLOBAL MASS BALANCE			
INPUT FLOWS		**OUTPUT FLOWS**	
	GtC/a	GtC/a	
Anthropogenic processes			***Anthropogenic processes***
- Consumption of products and services which release CO$_2$ 6)	6.5		- Cultivation, re- and afforestation
		-0.03	- CO$_2$ utilisation (long and short term fixation) 8)
- Energy production			
- Transportation			- CO$_2$ storing (enhanced oil recovery)
- Industrial processes			
- Felling and tilling			
- Changes in soil use (deforestation) 6)	2		
- Desertification			
Natural processes			***Natural processes***
Biosphere and land surface			*Biosphere*
- Decomposition of biomass 6)	60	-63	6) - Photosynthesis (terrestrial plants)
- Respiration			*Land surface*
- Desertification	-	-	- Mineral carbonation
- Forest fires	-		
- Volcanic eruptions	-		
Hydrosphere			*Hydrosphere*
- Flow towards thermodynamic equilibrium 3), 6)	90	-92	3), 6) - Flow towards thermodynamic equilibrium
Cryosphere			*Cryosphere*
- Flow towards thermodynamic equil.	-	-	- Flow towards thermodyn.equil.
Total input flow	158.5	-155.03	Total output flow
=> accumulation into the atmosphere	**3.47**	**GtC/a**	
Accumulation represents	2.19	% of the total global input flows	
	2.24	% of the total global output flows	

In thermodynamics an equilibrium state plays a central role. The equilibrium state will always occur in a closed system, if sufficient time has been given. In addition, a system open to flows of mass and energy, depending on the nature of these interactions between the system and its surroundings, may also evolve to an equilibrium state (Sandler, 1989).

The following sections 3 and 4, describe further the thermodynamic equilibrium in multiphase aqueous systems. The equilibrium state at constant temperature and pressure is the state for which the so-called Gibbs free energy (G) achieves a minimum value from among all states consistent with the reaction stoichiometry. The identification of the equilibrium state is then a problem of minimising the Gibbs free energy (Sandler, 1989).

2. Photosynthesis

This is the second largest flow pair of the system (60/-63 GtC/a). The 1 % change (~0.6 GtC/a) of this flow may change the accumulation also significantly (0.6/3.5*100%=17%).

Photosynthesis is the process, which converts CO_2 and water into oxygen and organic compounds using energy of light in the presence of chlorophyll. This is a very important process, occurring in many types of bacteria, algae and vascular plants, providing energy for the whole living world (Lawlor, 2001). This is a natural process, which uses CO_2 as a raw material. If carbon sequestration is considered through natural photosynthesis by afforestation and reforestation, the amount of sequestrated carbon is depending e.g. on the forest type and latitude (FAO, 2001), nutrients, hydrological situation and so on (FAO, 2001; Malhi et al., 2002).

Thermodynamic equilibrium of natural aqueous system

Chemical and energetic changes in macroscopic systems can be treated simultaneously using multicomponent equilibrium routines including minimization of the Gibbs energy. The chemical equilibrium can then be reached by modern computer aided calculation methods. By modelling both the phase and chemical equilibrium one is provided with a practical tool for studying aqueous chemical and biological processes involving sources and sinks of CO_2. The non-equilibrium decomposition of biological matter to CO_2 can be taken into account using dynamic constraints in the thermodynamic model and shows potentially new possibilities in modelling industrial and environmental systems. The dynamic Gibbs energy model was used to describe the chemical states of biological production of CO_2. The observed CO_2 emissions into the air from a lake ecosystem are related to the decomposition process of organic matter that produces carbon dioxide. The reaction processes between the solid, liquid and gas phases are affected by chemical equilibrium of gas solubility phenomena. The thermochemical model allows for the calculation of measurable quantities as solubilities of solids and gases as well as the ionic concentrations and activities in terms of temperature, pressure and pH. It gives the speciation and solubilities of chemical species and the direction of the change at given boundary conditions that can be theoretical or empirical.

Environmental modelling

The total Gibbs energy of a system is constructed by writing the chemical potential μ_i for each species. The chemical potentials of the species in a solution can be expressed in general as a sum of ideal and non-ideal parts. The Gibbs energy G of a mixture in different phases (α) is made up of contributions of the pure components or of the solutions taken as ideal or non-ideal in the form,

$$G = \sum_{\alpha} \sum_{i} n_i^{\alpha} \mu_i^{\alpha} \tag{1}$$

In the eqn(1) n_i^{α} is the amount of species i and μ_i^{α} its chemical potential in a phase α. The chemical equilibrium in a closed system at constant temperature and pressure is achieved at the minimum of the total Gibbs energy, *min*(*G*), satisfying the material balance conditions, which means that the system is in equilibrium.

The chemistry in the model system is described by means of a system species matrix. It includes all chemical constituents, atoms and charges that are in the system. The calculations are carried out by means of the total Gibbs energy of the system at given temperature and pressure. A comparison of the results obtained with the multiphase model with the stoichiometric equilibrium constant data is straightforward. The results of the *min*(*G*) matrix can then be verified with the literature data where the equilibrium constants have traditionally been used. Reactions for the system studied are given through the reaction equations.

$$CO_2\ (g) \Leftrightarrow CO_2\ (aq) \tag{2}$$

$$CO_2\ (aq) + H_2O \Leftrightarrow H^+ + HCO_3^- \tag{3}$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \tag{4}$$

$$CaCO_3(s) \Leftrightarrow Ca^{2+} + CO_3^{2-} \tag{5}$$

$$Na_2CO_3(s) \Leftrightarrow 2Na^+ + CO_3^{2-} \tag{6}$$

$$H_2SO_4(l) \Leftrightarrow HSO_4^- + H^+ \tag{7}$$

$$HSO_4^- \Leftrightarrow H^+ + SO_4^{2-} \tag{8}$$

$$C(organic) + O_2(aq) \Rightarrow CO_2(aq) \tag{9}$$

The reaction equation (9) shows the irreversible decomposition of the organic matter through the natural oxidation process. The decomposition of the organic matter is a complex phenomenon but eventually carbon dioxide is produced and remains at higher concentrations than equilibrium solubility of atmospheric CO_2 alone would suggest.

The pH and CO_2 equilibrium values in of freshwater systems are affected by temperature, partial pressure and acid concentrations. Also the amount of dissolved neutral $CO_2(aq)$ is temperature dependent. That is related to the temperature dependence of Henry's law constant and chemical potential in the dissolution process (Henry's law describes gas solubility into liquid) (Salminen et al., 2004). The organic carbon oxidising into the form of CO_2 and then dissolving into the water shows as increased alkalinity. That is a measurable quantity and defined as:

$$[\mathrm{Alk}] = [\mathrm{HCO_3^-}] + 2[\mathrm{CO_3^{2-}}] + [\mathrm{OH^-}] - [\mathrm{H^+}] \tag{10}$$

This is a dynamic behaviour and has to be taken into account in the thermodynamic model by including dynamic constraints. Eventually the decay of organic material shows as an increase in the CO_2 emissions into the air (Kobylin et al., 2003; Salminen, 2004), see Fig. 2.

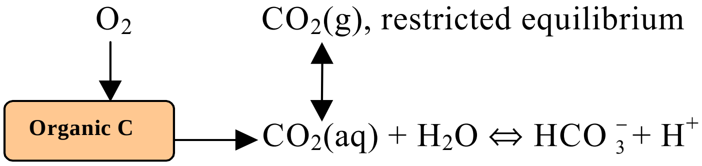
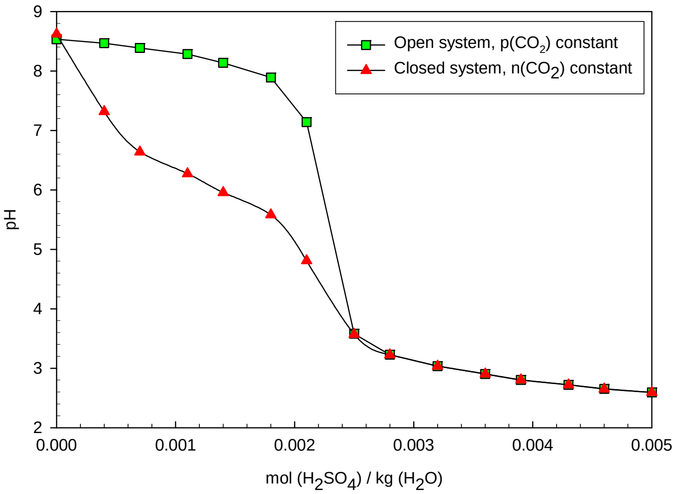


Fig. 2. The oxidation of organic matter produces eventually carbon dioxide that remains in the water at higher quantities than partial pressure of air would indicate. Due to the pressure gradient the excess CO_2 will eventually be emitted into the atmosphere.

A slight acidity of aqueous system, wherein CO_2 is dissolved, can be noticed. This is due to proton ions, which are formed according to the reactions shown in Equations (2)-(4) as well as in Fig. 2. This will rise a question on CO_2 dissolution into water in a case, that aqueous system has increased H^+ concentration, i.e. low pH value, due to some other acidic substance, like trough reactions (7) and (8). Fig. 3 shows the results of pH change as a function of sulphuric acid addition in open and closed systems (different thermodynamic state) using artificial seawater, the composition of which is also displayed in Fig. 3. In an open system the CO_2 partial pressure has been kept constant and in a closed system the amount of CO_2 is constant. In a closed system, even a small increase in sulphuric acid decreases the pH. In an open system, the effect is not so dramatic. The points in Fig. 3 are calculation results using the method of minimisation of the total Gibbs energy. At higher acid concentrations calculations in open and closed systems give similar pH results. The measured range of H_2SO_4 concentration in natural waters is <0.0002 mol/kgH$_2$O.

As shown by a number of studies the CO_2 partial pressure in the atmosphere has increased from a pre-industrial level of 280 ppm to the present 360 ppm in just over 100 years. The present rate of increase is estimated at 0.5% per year (Sigman and Boyle, 2000; Falkowski et al., 2000). The equilibrium relations of the dissolved carbon dioxide in fresh waters and oceans are complex functions of chemical and biological composition, temperature and pH. Approximately 98% of the carbon in the oceans is in the form of dissolved carbon (reactions 3 and 4) and calcite. The pH is effectively buffered by the vast reservoirs of calcite on the ocean floor.

a)



b)

Compound	m/g
NaCl	26.518
$MgSO_4$	3.305
$MgCl_2$	2.447
H_2O	965.579
$NaHCO_3$	0.202
$CaCl_2$	1.141
NaBr	0.083
KCl	0.725

Fig. 3. a) The effect of addition of sulphuric acid on pH in constant carbon dioxide partial pressure (amount of CO_2 molecules are varied, open system) and constant amount of all chemical components (closed system). The results confirm those presented by Pilson for artificial seawater (Pilson, 1998).
b) The composition of 1 kg of artificial seawater.

In global scale, annual anthropogenic SO_x emissions are estimated at 65-90 MtS per year (IPCC, 2004). That converted into acid rain has an effect on the chemical speciation of the water systems. Calcite plays a major role in the CO_2 balance in oceans, acting as an effective buffer. Consequently the change in pH is small as CO_2 or sulphuric acid dissolves in the ocean. Since the chemical reactions take place in the surface waters of the ocean the mixing depth is an important factor in the solid-liquid-gas interactions. Carbon dioxide has an important chemical effect on the carbonate balance in natural waters through the dissolution process of atmospheric CO_2. The addition of sulphuric acid (reactions (7) and (8)) in calcite solution decreases the pH and increases the calcite solubility (reaction(5)). Finally the reactions produce more soluble calcium sulphate (11) and carbon dioxide (reaction 3).

$$Ca^{2+}\,(aq) + SO_4^{2-}\,(aq) \Leftrightarrow CaSO_4\,(s) \qquad (11)$$

According to calculations in saturated calcite solution circa 0.2 units' decrease in pH is reached by doubling the CO_2 partial pressure from the present level $p(CO_2) = 3.63 \cdot 10^{-4}$ bar (equal to 363 ppm). The addition of sulphuric acid increases the concentration of proton H^+ and the bisulphate ions HSO_4^- in the solution through reactions (7) and (8). As the calcium carbonate is dissolved (reaction (5)) the formation of bicarbonate ions HCO_3^- gives the pH buffer capacity. The carbon dioxide reaction product dissolves in its saturation level against with the global partial pressure $p(CO_2)$ and the non equilibrium CO_2 is eventually released into the gas phase. The Gibbsian models provide an efficient and practical tool for evaluating the effects of changes in different temperatures, pressures and chemistries.

In terms of aquatic ecosystems the CO_2 partial pressure is relatively constant, although during last 100 years, it has been increased. From this point of view the aquatic ecosystem is similar to the open system presented in Fig. 3. On the other hand, if system boundaries are set large enough, thus including the whole atmosphere and hydrosphere, also the amount of carbon stays relatively constant. Carbon content in atmosphere is 730 GtC and in ocean 38 000 GtC (IPCC 2001a; FAO, 2001). The total annual change in these systems are: accumulation into atmosphere 3.5 GtC/a, dissolution of CO_2 from atmosphere into ocean 2 GtC/a and carbon flow from rivers into oceans 0.8 GtC/a (IPCC 2001a), annual change is then $(3.5+2+0.8)/(730+38000)*100\% = 4*10^{-5}\%$, i.e. the system is approaching the closed system in terms of amount of carbon.

If only the physical phenomenon, gas diffusion into the liquid and dissolved CO_2 desorption from the liquid is studied, the area of this kind of an examination is the very thin layer on both sides of the phase boundary, and changes in this thin layer are affecting the thermodynamic equilibrium state. This state will change if new components are introduced into the system. It seems that the addition of sulphuric acid removes aqueous system equilibrium towards the state, where liquid has a lower ability to store CO_2 in a dissolved form.

Acidic air pollutants, forests and carbon sequestration

One of the most important acidifying substances that are deposited from the atmosphere are weak solutions of sulphuric and nitric acids, which arrive in the form of acidic precipitation. In addition, the dry deposition of gaseous sulphur dioxide and oxides of nitrogen, and of certain particulates such as ammonium sulphate and ammonium nitrate, can contribute further acidifying potential to ecosystems (Freedman, 1989). Gaseous emissions, such as sulphur dioxide (SO_2) and nitrogen oxides (NO_x), can travel over very long distances, transported by the winds over hundreds of kilometres and then deposited by wet deposition in rain, occult (in cloud) deposition and dry deposition to surfaces and may cause impacts far from the source of pollution (Kuylenstierna et al., 2002).

The direct impacts of increased SO_2 concentrations are largely confirmed in urban areas, pre-urban areas and close to industrial sources of pollution, although dry and wet deposition of resulting acidifying substances occurs on a continental scale. Gaseous air pollutants may affect vegetation through visible injury and/or effects on growth and yield (invisible injury) and through subtle physiological, chemical or anatomical changes. A large body of knowledge has shown that significant yield losses can occur in the absence of visible symptoms (Kuylenstierna et al., 2002).

Carbon accumulates in forest ecosystems through the absorption of atmospheric CO_2, and its assimilation into biomass. Carbon is stored in living biomass, including standing timber, branches, foliage and roots; and in dead biomass, including litter, woody debris, soil organic matter and forest products. Overall, forests contain just half of the carbon residing in terrestrial vegetation and soil, amounting to some 1200 GtC. Boreal forests account for more carbon than any other terrestrial ecosystem (26% of total terrestrial stocks), while tropical and temperate forests account for 20% and 7%, respectively. /6/ It is estimated that 8% of global forests cover received an annual sulphate deposition above an estimated threshold for effects on acid sensitive soils, and that this will increase to 17% in 2050 (IPCC, 2001a).

As from the previous can be noticed, the role of the forests in carbon sequestration is meaningful. Human activity should be pointed the way that the natural rate of carbon sequestration trough photosynthesis shall stay on the highest possible level, also the activities should be the kind, that carbon stored into forests shall be conserved in there, and if stored carbon is used, that shall be done on a sustainable way.

Dynamic states of natural processes

Climate system is a complicated combination of physical, chemical, biological and geological processes. Each of these processes may respond differently (in time scale) to the imposed changes. However, even without changes in external forcing, the climate may vary naturally, because in a system where components have very different response times and non-linear interactions, the components are never in equilibrium and are constantly varying. This means that there is no simple proportional relation between the cause and effect. A complex, non-linear system may display what is technically called chaotic behaviour. Thus, the behaviour of the system is critically dependent on very small changes in the initial conditions (IPCC, 2001b).

In the controlled industrial systems it is known, that even a slight increase in the amount of impurities, e.g. in raw materials, may change significantly the process chemistry, cause side reactions or in some other way disturb process operations (Kletz, 1986, 2003). Especially sulphur components are named to be catalyst poisons, causing decrease in reaction yield (Carberry, 1976). Sulphur components may also leach other materials and cause corrosion, which may further be the cause of harmful reactions (Kuylenstierna et al., 2002; Kletz, 2003).

It might be worth to study further the effects of impurities, like acid rain, to the processes and flows in the ecosystem. Special attention should be paid to those physical, chemical and biological processes, which are storing or using atmospheric CO_2.

Conclusions

In this study the problem of global warming is approached as a problem of CO_2 accumulation into the atmosphere, which is shown to be the main greenhouse gas. A schematic mass flow diagram over the atmosphere divided into input and output flows, classified into anthropogenic and natural processes, including living, chemical and physical phenomena, may help to formulate the CO_2 accumulation problem.

From the CO_2 global mass balance, which was created following the structure of flow diagram, it is noticed that the natural flows of CO_2 are playing the major role. By studying the interaction fluxes between hydrosphere and atmosphere using the thermodynamic calculation method of minimisation of the total Gibbs energy, it is found, that in a closed system, in spite of buffering effect of calcite, even the addition of small amounts of acid, like sulphuric acid, decreases significantly the aquatic system pH. Low pH decreases system ability to maintain CO_2 in dissolved form in aquatic solution. Setting the system boundaries for the ecosystem is not a simply task, however, the study shows, that in certain conditions, dramatic changes can take place.

Forests play a key role in carbon sequestration, especially boreal forests are important. There are strong evidences, that acid rain may locally inhibit the normal growth of biomass. However, it is estimated that the area, imposed to the acid rain, will still increase in the future.

A complex, non-linear system, such as climate system, may respond critically to very small changes in the initial conditions. Dramatic changes in process behaviour of industrial processes due to small amounts of impurities are also reported. The global mass balance of CO_2 flows show, that increasing existing output flows, even only by a couple of percents, in comparing to the input flows, could be sufficient to move the mass balance into the equilibrium at the annual level. Thermodynamics is the discipline, which study equilibrium as well as dynamic states of system. Methods used in thermodynamics are applicable also to natural processes. While searching possible mitigation strategies for green house gases, it would be worth to study further the possible inhibiting effect of air pollutants, for example acid rain, caused by anthropogenic processes, to the processes related to the climate system. Special attention should be paid to the processes, which store or use CO_2 from the atmosphere. These are the interaction flows between atmosphere and hydrosphere and photosynthesis. By increasing the understanding the dynamic related into these processes we are able to define and ensure the circumstances, when the highest possible CO_2 storing capacity could be reached in natural processes.

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UV RADIATION AND DEFENCE MECHANISMS OF SCOTS PINE (PINUS SYLVESTRIS L.) AT THE SUBARCTIC: PHOTOPROTECTIVE PIGMENTS

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Abstract

Photoprotective pigments, particularly xanthophyll cycle pigments and soluble phenolics were studied in needles of Scots pine (Pinus sylvestris L.) saplings in a UV exclusion open-top chamber experiment in Sodankylä, Finland. The experiment used following plastic filters in exclosure treatments to manipulate the spectral balance of natural irradiance: (1) “control” (a ployethene plastic filter); (2) “UV-B exclusion” (a clear polyester filter); and (3) “UV-B/UV-A exclusion” (a clear acryl plate). The research also included (4) “Ambient” Scots pine saplings that were not subjected to any treatment exclosures. Significant variation among the sampling times and/or the treatments was detected for both xanthophyll cycle pigments (e.g. violaxanthin, antheraxanthin and zeaxanthin) and soluble phenolics (e.g. dicoumaroyl-astragalin) of Scots pine needles during the spring 2002.

Introduction

In the subarctic and Arctic ecosystems the plants may experience strong, but often short radiation stress at the start of their growing season due to ambient and reflected irradiance from the surrounding snow cover and can therefore be exposed to the direct effects of UV-B radiation (Ottander et al., 1995; Gröbner et al., 2000). Many pigments play a significant photoprotective role in the plants. Carotenoids are long-chained compounds that include carotenes and xantophylls. They perform an essential photoprotective role in quenching triplet-state chlorophyll and scavenging singlet oxygen and other toxic oxygen species formed within the chloroplast. Xanthophyll cycle is a photoprotective mechanism involving two reversible reactions: light-dependent de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin as an intermediate, and light-independent epoxidation of zeaxanthin to antheraxanthin and violaxanthin (Ottander et al., 1995). A unique characteristic of UV protecting pigments, flavonoids and related phenolic compounds, is that they absorb UV radiation, while simultaneously transmitting the visible PAR (photosynthetically active radiation) to the chloroplast-containing mesophyll cells within the leaf interior (Schnitzler et al., 1996; Jordan, 2002). The aim of this work was to study the effect of UV radiation on the photoprotective pigment metabolism in needles of Scots pine saplings at the subarctic.

Material and Methods

UV exclusion experiment was arranged with Scots pine (Pinus sylvestris L.) saplings during 2001-2002 on a randomized block design with four treatments, each replicated ten times, altogether 40 plots. The individual treatment exclosures (chambers) consisted of wooden frames with plastic covers adjusted over the whole sapling: (1) Control (a polyethene plastic filter) (2) UV-B-exclusion (a clear polyester filter) and (3) UV-B/UV-A exclusion (a clear acryl plate). The chamber structure, the daylight transmissions of all plastic filters and temperatures within the chambers were tested. The research also included (4) Ambient control plants that did not have plastic filter (Fig. 1) (Turunen et al., 2002). Soluble phenolics (Kallo et al., 2003) and xanthophyll cycle pigments were analysed by HPLC. The epoxidation stage (EPS) of xanthophyll cycle pigments was calculated as follows: EPS=0.5 antheraxanthin+violaxanthin/violaxanthin

+ antheraxanthin + zeaxanthin (Wingsle and Hällgren, 1993; Ottander et al., 1995).

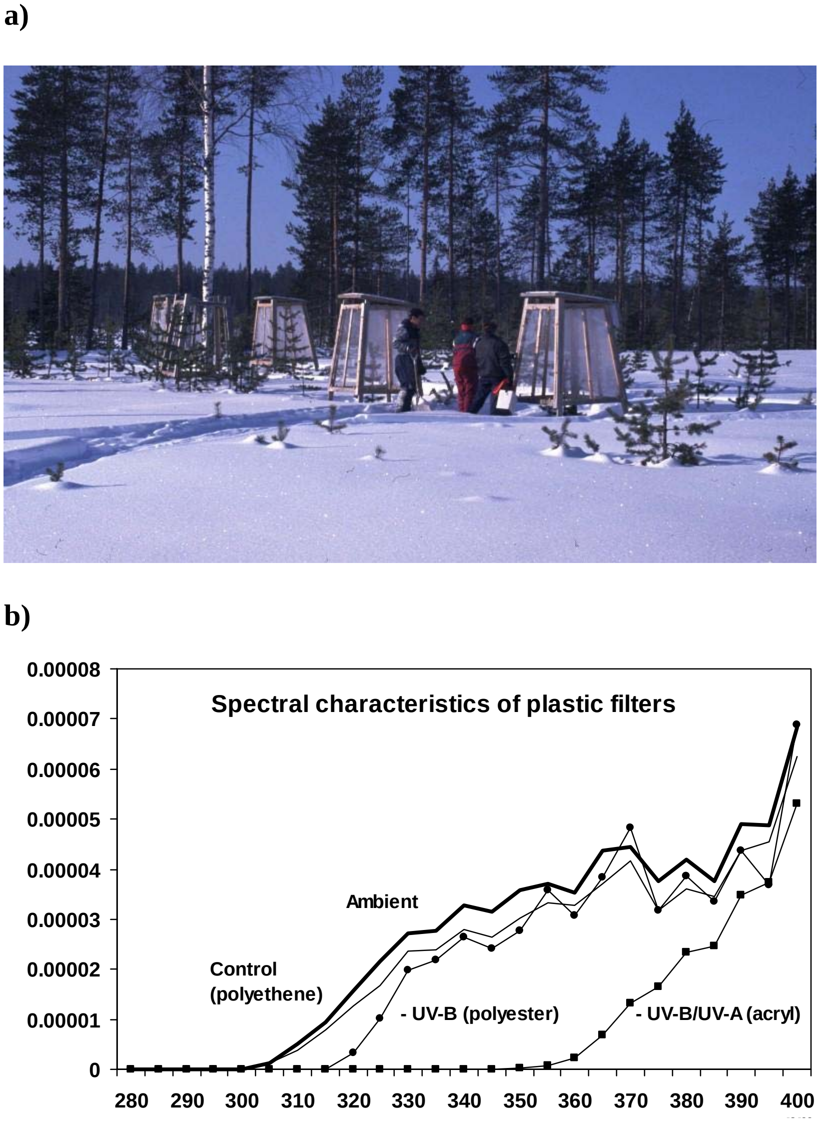

Fig. 1. UV exclusion open-top chambers with Scots pine saplings in Sodankylä, Finland **(a)**, and spectral characteristics of the plastic filters used in the experiment **(b).**

Results

Xanthophyll cycle pigments

There was a rapid increase in the concentration of violaxanthin and a decrease in zeaxanthin of Scots pine needles in April. The EPS (epoxidation stage) of xanthophylls (0.5A+V / V+A+Z) increased in April reflecting a conversion of zeaxanthin into violaxanthin. Significant, but transient changes in xanthophyll cycle pigments could be seen among the treatments (Fig. 2).

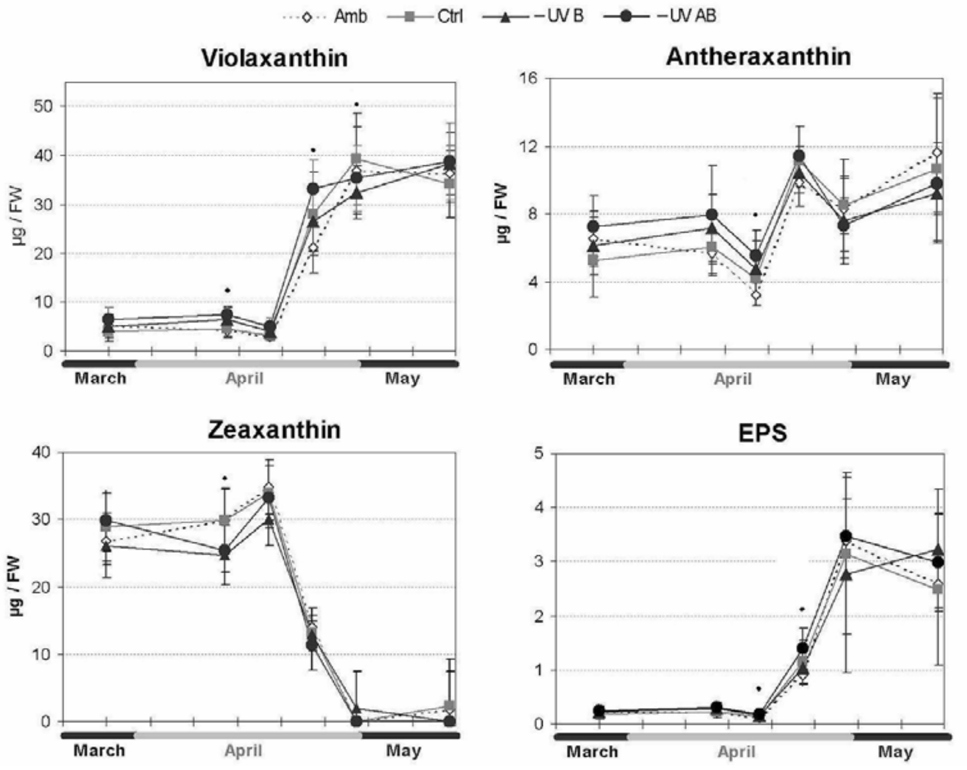

Fig. 2. Concentration of violaxanthin, antheraxanthin, zeaxanthin and the epoxidation status (EPS) of the xanthophylls of Scots pine needles in different UV exclusion treatments during spring 2002. Anova results shown among the treatments ($p<0.05$) are marked with black dot.

Soluble phenolics

Significant changes in soluble phenolics of Scots pine needles between the treatments could be observed. For example, the peak area of dicoumaroyl-astragalin is higher under UVB/UVA exclusion compared to the other treatments, but for unknown U2 compound it is smaller than in other treatments. Also, there is a trend for higher PMS (pinosylvin monomethylether) in UVB/UVA exclusion compared to the other treatments (Fig. 3).

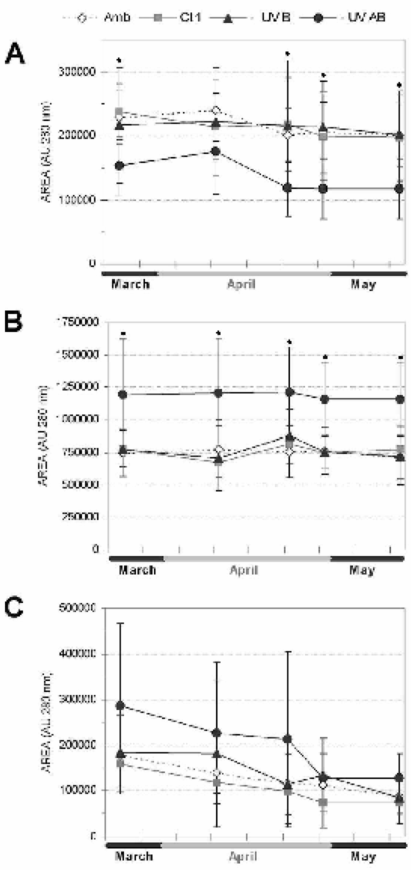

Fig. 3. Peak areas of unknown U2 (a), dicoumaroyl-astragalin (b) and pinosylvin monomethylether (PSM) (c) from the HPLC chromatogram of soluble phenolics of Scots pine needles in different UV exclusion treatments during spring 2002. Anova results shown among the treatments (p<0.05) are marked with black dot.

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PALJAKKA ENVIRONMENTAL SPECIMEN BANK

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Increasing demand for competent long-term storage of environmental samples has emerged due to a growing shortage of proper storage facilities, improvements in environmental monitoring programs and development of more accurate analysing methods. One solution for the need of adequate specimen storage are Environmental Specimen Banks (ESB), which primarily concerns on long-term preservation of environmental specimens for deferred or retrospective analyses of the condition of environment (Olsson and Bignert, 1997). There are two ESBs in Finland presently, one for the storage of frozen animal specimen in the Finnish Environment Institute in Helsinki and another for the storage of dried plant specimen in the Finnish Forest Research Institute in Paljakka research area.

Paljakka ESB was established in 1994 and extended in 1999. Total floor area of 770 m^2 consist specimen storage space 296 m^2 divided in nine fireproof storage rooms in the sizes of 19-62 m^2. Total storage shelve length is presently 3 960 m. Sample pre-treatment and office rooms as well as auditorium and accommodation services are also offered in the Paljakka ESB. Pre-treated and dried plant samples are stored in mobile steel shelves opening to a single aisle between rows at a time, which increase the number of shelving that can be accommodated within a finite space. Real-time monitoring of air temperature and humidity is established inside the storage rooms to ensure accurate conditions, since stabilized storage conditions can increase specimen “lifetime” for decades or even hundreds of years. Both the ambient air inside storage rooms and air in storage bags and boxes are monitored.

Total amount of individual samples (mainly mosses, epiphytic lichen, pine bark, humus and forest litter) stored in the Paljakka ESB is estimated to exceed 200 000. Sample material include specimen from several different studies, e.g. from forest health and heavy metal deposit monitoring surveys (Kubin et al., 1997; Poikolainen et al., 2004) as a part of National Forest Inventory (NFI) and ICP Forests survey. All of Metla's dried plant samples that require long-term storage are lodged in the Paljakka ESB. Forest litter material collected since 1958 can be sorted into several sub-components, such as seeds, flowers, cones, needles, leaves, bark and insect remains (Kouki and Hokkanen, 1992; Poikolainen and Kuusinen, 2000). In addition to sample material from different studies and experiments, reference moss material is prepared and stored in the Paljakka ESB for checking the analytical standard of laboratories participating in heavy-metal deposition surveys in Europe (Steinnes et al., 1997). Additional information about the Paljakka ESB is available in http://www.metla.fi/mu/index-en.htm.

Increased interest for the long-term storage of environmental samples created a need to develop and unify specimen and database availability in Finnish environmental institutes. For that purpose, The Ministry of Education and Metla conduct research and development project “Environmental specimen banking and co-operation between different institutes” between 2003 and 2006. This project focuses on long-term storage of environmental samples and co-operation between the institutes involved in specimen banking in Finland. Main target of the current project is to develop a model for the storage and use of environmental samples in Finland. Practical work consist development of Paljakka ESB due to improvements in sample storage and information services of the specimen database. All the advancements in Paljakka ESB can be utilized in final model, and existing specimen banks could be set as central units in Finnish specimen storage net. Information about the stored specimen can be connected to different international data processing systems more effectively by centralized up-to-date databases. In addition, all sample information could be centralized by establishing libraries for manuals, standard operating procedures, publications etc. from the samples stored in existing ESBs.

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Air Pollution Impacts on Forest Ecosystems: Key-Results from the 2004 IUFRO RG 7.04.00 Meeting

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Introduction

The 21st meeting for specialists in air pollution effects on forest ecosystems "Forests under changing climate, enhanced UV and air pollution" was held on August 25-30, 2004, in Oulu, Finland, hosted by Department of Biology, Thule Institute and University of Oulu, on behalf of the IUFRO Research Group 7.04.00 "Air Pollution Impacts on Forest Ecosystems". A satellite microscopy workshop was also held as part of the IUFRO Working Party 7.04.02 "Mechanisms of action and indicator development".

When studying environmental problems in forest ecosystems, particularly in trees, the key question is: which changes or injury do we observe and what is their origin? Field studies and experiments are carried out to document exposure and uptake of pollutants, effects at tree and ecosystem level, to prove dose-response relationship, and to clarify the multiple relationships existing between site factors, climate, pollutants, competition for resources, successional dynamics and tree/ecosystem responses. In the past 20 years, experimental conditions to test air pollution effects have constantly improved using increasingly sophisticated technical installations. These approaches have included greenhouses, climate chambers, open-top chambers, branch and single tree chambers, and recently various free-air release fumigations systems in tree plantations or forest canopies - all with the purpose of measuring the effects in near-natural systems. Research has also focused on the 'mechanisms of action' of different stress factors, such as gas exchange, anatomical and biochemical responses. Introductory work has been done using molecular biology methods. Applications using bioindication in the field (initially with exposed sensitive plants and recently with natural vegetation) have been developed. Results from O_3 injury assessments were reported in different sessions of this meeting during which much new work was dedicated to understand the effects of increased O_3, elevated CO_2, nitrogen, and UV-B on ecosystems. Both field studies and experimental works were presented, providing evidence documenting the air pollution impact on forest trees and/or ecosystems in Europe and North America. Here we select and resume some key-results from the oral presentations, whose degree of innovation may contribute to address future research.

DIAGNOSIS, MONITORING AND EVALUATION

Two main topics were addressed: (i) importance of the design in field studies aimed at assessing air pollution effects on forests, and (ii) statistical relationships existing between crown condition (defoliation and needle longevity) and environmental parameters. The importance of both topics in forest monitoring is well known since some time and – as such – the topics cannot be considered entirely new. Yet, when examining the structure and results of forest monitoring programmes in Europe, it is clear that design issues as well as data processing issues could be improved. In this respect, some of the presentations given during the meeting added new information. The role of monitoring design was examined also in relation to acute, source-related air pollution impacts on forests and to biogeochemical studies (Kozlov, Bastrup-Birk). In many cases, studies carried out over the last 10 years still suffer poor design (ambiguous sampling design, lack of replication), and this confirms that little progress has been made in this field (see Hurlbert, 1984; Eberhardt and Thomas, 1991). It is important that this point was taken up once again: presentations emphasized the clear demand for a design-based, statistically-oriented monitoring programme. Future efforts in forest monitoring and assessment have to be based on rigorous statistical designs. This is also true in relation to data analysis, which cannot be properly undertaken without an unbiased sampling design.

The relationships between forest health and air pollution were investigated according to different approaches. Correlative studies between defoliation and environmental parameters were undertaken in Lithuania on different statistical populations (Serafinaviciute, Augustaitis). In both cases a number of relationships were found with meteorological, site and air pollution parameters (up to 78% variance explained). In particular, the role of temperature and summer drought was found to be significant, together with O_3 levels on broadleaves and sulphate deposition on conifers. A less formal approach was adopted in the Carpathians, where low pollution levels (with the possible exception of NO_2) and relatively good forest health were detected (Bytnerowicz). Biogeochemical cycling studies and associated modeling were also reported for Poland (Malek). The new information coming out from these presentations is related more to the geographical area considered. To date, most of the correlative studies undertaken were related to North and central Europe with relatively limited information available on Southern and Eastern European countries. New information about this area is valuable, even if issues such as multi co-linearity of the variables were not addressed and this is a point were substantial effort is needed.

Ozone

Characteristics of tropospheric O_3 pollution have substantially changed during recent years in Europe and North America because of stringent regulations and technological progress (Martin). Main results are reduced peaks and increased annual averages (Manning). However, in parts of South-east Asia and Central and South America, emissions are on the rise. Intercontinental transport of O_3 precursors (from North America and Asia to Europe) has been demonstrated to be an efficient process (Derwent et al., 2004).

OZONE HAS BEEN SHOWN TO BE A SIGNIFICANT PREDICTOR OF CROWN DEFOLIATION IN FORESTS IN SOUTH WESTERN EUROPE, BUT STILL LESS IMPORTANT THAN FACTORS LIKE THE GEOGRAPHICAL AND TOPOGRAPHICAL LOCATION OF THE TREES AND THE SOIL

PROPERTIES (FERRETTI). PASSIVE SAMPLERS FOR AMBIENT O_3 MONITORING COMBINED WITH SELECTION OF NATIVE BIOINDICATORS HAVE PROVEN TO BE VERY USEFUL TOOLS IN EVALUATION OF O_3 EFFECTS ON FORESTS (SANZ). THERE IS A CLEAR NEED FOR BETTER INDICES EXPLAINING O_3 PHYTOTOXIC EFFECTS IN THE FIELD. IN THIS REGARD, PROGRESS HAS BEEN MADE IN USE OF PASSIVE SAMPLERS DATA FOR RECONSTRUCTION OF REAL-TIME O_3 CONCENTRATIONS (FERRETTI). COMPUTER-BASED CHARTS OF O_3-CAUSED INJURY OF NATIVE PLANTS MAY GREATLY HELP SCIENTISTS AND LAND MANAGERS IN THE EVALUATION OF O_3 EFFECTS ON NATIVE FLORA (SANZ). PROGRESS IS BEING MADE IN EUROPE IN DEVELOPING AN O_3-EFFECTIVE FLUX APPROACH COMBINED WITH INFORMATION ON PHYSIOLOGICAL/BIOCHEMICAL DEFENCE MECHANISMS OF PLANTS THAT COULD EVENTUALLY REPLACE THE STRONGLY CRITICIZED AOT40 APPROACH. IN FREE-AIR O_3 EXPOSURE, THE AOT40 EXCEEDANCE DOES NOT WORK FOR ADULT TREES, ESPECIALLY NOT IN DRY YEARS, AND THE ONLY ALTERNATIVE APPEARS THE FLUX CONCEPT (LÖW). AFTER VALIDATION WITH DATA FROM FREE-AIR O_3 FUMIGATION IN GERMANY, THE EMBERSON ET AL. (2000) O_3 FLUX MODEL WAS USED ON LEVEL II EUROPEAN BEECH PLOTS IN SWITZERLAND (SCHAUB), DEMONSTRATING THAT IT IS VALUABLE TO MODEL O_3 UPTAKE IN LEAVES.

Short-term O_3 exposure stimulates stomatal closure as a reaction to an increased internal CO_2 concentration resulting from mesophyllic limitations to carbon assimilation (Paoletti). Prompt stomatal closure accounts for plant resistance to O_3 peaks. Long-term exposure causes stomata to become sluggish in response to changes in environmental conditions. As a consequence, plant water control may be impaired. Further investigations should lead to a better understanding of the physiological basis of sluggish stomatal responses across a broader spectrum of species. More information is also needed to understand the relationship between O_3 sensitivity and stomatal density.

The free-air O_3 canopy fumigation experiment at Kranzberger Forst, Germany, showed that O_3 effects that have been reported in young trees – for example, extensive foliar injury, accelerated leaf loss, reduced assimilation rate, decreased growth rate - are found only to a minor degree in adult beech trees in the field. Young beech in phytotrons were more sensitive to O_3 injury, because of slightly higher O_3 uptake when exposed to the same external O_3 regimes, lower photosynthetic rates in relation to O_3 uptake, and lower levels of antioxidants related to leaf surface area (Nunn). High O_3 concentrations per se do not cause injuries under dry conditions, as stomata close (Löw). Irrespective of O_3 exposure, cuvette enclosure did not affect gas exchange behavior and antioxidant contents in leaves of adult trees (Then). However, branch bag O_3 experiments are still relevant for whole-tree risk assessment in adult field grown trees. Biochemical parameters (antioxidant contents) additionally demonstrated that twigs possess autonomy in their defense metabolism (Then). Chamber experiments suggest that the effect of O_3 on carbohydrates results in carbon gain/allocation to below ground parts. The enzyme activity is also affected, in that Rubisco activity decreases and PEPc activity increases. Analysis within adult beech canopies showed high heterogeneity and no clear effect of O_3 on enzyme activity of Rubisco and PEPc (Blumenröther). The question was whether the carbohydrate and enzyme activity pattern is a biochemical leaf response to O_3 uptake or to the drought in 2003. Parallel assessment of photosynthesis and carbohydrate content showed no differences in photosynthesis between sun and shade trees, suggesting that drought cannot be the only reason for the observed symptoms. When investigating biochemical defense in the chloroplast pigments, for example tocopherols and the ascorbate and glutathione systems, significant differences among tree age and canopy positions were found (Herbinger). Sun leaves exhibited lower pigment content and total glutathione, and higher deepoxidation state of xanthophyll and α-tocopherol, mainly in seedlings. No O_3 effects were found on the lipophilic thylakoid compounds, but the aqueous glutathione system responded significantly to $2xO_3$.

UV-B

Reduced emission of the relevant pollutants has been slowing the depletion of stratospheric ozone. However, progress was slow because of the inherent inertia of atmospheric processes. Consequently, UV-B enhancement continues and a return to background levels is decades away (Martin).

Mixed results were obtained for more than three-dozen tree species studied with some species showing deleterious effects of UV-B (Sullivan). More negative effects were observed in indoor conditions compared with field studies. Out of 160 species of the high Arctic tundra, only a few showed sensitivity to the increased UV-B (Rozema). Taken as a whole it is concluded that minor reduction in stratospheric ozone and subsequent increase of UV-B radiation are not likely to result in losses in tree or forest productivity. However, the assessment of the role of ambient UV-B in controlling forest health and ecosystem processes or possible indirect consequences of UV-B radiation are unknown and difficult to investigate. To better understand the potential for UV-B induced changes, long-term experiments should be continued.

Acidifying Depositions and Nitrogen

Powerful scientific programmes developed the knowledge to guide an effective policy to reduce acidifying deposition in North America, Europe, and the former Soviet Union (Martin). However, other parts of the world are now experiencing rising emissions of sulphur and nitrogen oxides. For example, in Thailand, the chemical analysis of deposition in mixed deciduous forests showed increased acidity (Luangjame). Actual levels of acidity were low, however the results may be used as a baseline in a region where increased acid rain may be expected.

Stopping acid treatments increased spruce root growth, while stopping N fertilization had no detectable changes (Sheppard). Positive seedling growth responses to the more favorable N status in soils subjected to prolonged pollutant inputs was shown in urban soils (Tarvainen). A clearly negative effect of soil acidification on fine root growth was reported for a field study on beech (Braun). The issue of soil acidification and plant response was raised in several of the presentations, and clearly remains an important theme in pollutant research in Europe (Malek, Braun, Sheppard, Potocic). This does not necessarily imply that soil acidity is always a factor in response to pollutants, however, especially in Mediterranean climates where base saturation is usually high (Grulke).

Forest fire is very relevant to nitrogen budgets, especially in Mediterranean-type ecosystems, and has a significant impact on nutrient budgets even for infrequent fires. Fire N loss equals more than 10,000 times the annual N leaching rate and post-fire N fixation is much larger than atmospheric deposition in a forest in the Sierra Nevada Mountains, USA (Johnson). Nearly all N in burned organic matter is gasified and lost to the system. Fire causes immediate losses in soil C and N but no significant changes in Ca^{2+} or Mg^{2+}. Forest fire may be more important than water for N cycling even for infrequent and restricted fire.

The role of needle position on the crown of Scots pine and of various environmental factors was investigated in Finland along a N deposition gradient (Lamppu). Crown defoliation, i.e the "traditional" forest health indicator in Europe, is determined by many factors, including needle longevity. Needle longevity decreases in the upper crown and in relation to N content (probably caused by N deposition). This information may help explain the crown defoliation patterns in N polluted areas. Nevertheless, California black oak in N-amended sites showed slower leaf loss as compared to unfertilized sites (Grulke). At a highly polluted site, N deposition appeared to deleteriously interact with drought stress, and exacerbated O_3 injury expression on the oak leaves.

Heavy metals

In both spruce and maple seedlings exposed to combinations of heavy metals and O_3, soil-applied heavy metals resulted in the greatest growth reductions, while O_3 effects were minor and variable (Günthardt-Goerg). Multiple stress interactions are by their nature complex, and this study showed how combined stresses can alter growth and tissue nutrient concentrations.

Organic chemicals

The signing of the Stockholm Convention in 2001 focused attention on POPs (persistent toxic chemicals), even if implications for forests are not clear: some POPs are beneficial to control forest pathogens; some POPs, mainly those including metals, may have direct harmful impacts on forests (Martin).

Accumulation of polycyclic aromatic hydrocarbons (PAHs) in the needles may be successfully used to characterize emission rates of PAHs from different sources as well as to describe the hazard posed to the environment from these compounds on a large-scale (Staszewski). The spruce needle method of biological monitoring seems to be a convenient tool for a preliminary recognition of the hazard level from PAHs.

Climate Change

Climate change is still a topic of considerable debate in spite of evidence linking increasing anthropogenic CO_2 emissions with regional climate trends and enhanced climate variability (Martin). Some parts of the globe are getting warmer and some colder; some parts are getting wetter and some drier. The magnitude of the changes varies greatly from region to region.

Important interactions between climate and air pollutant effects were illustrated at the meeting. During the course of some of the experiments, natural variations in climate occurred, especially in terms of precipitation. The variations in precipitation introduced an unexpected variable into the experiments, often producing different responses depending on whether drought or moist conditions occurred. This was the case with responses of black oak to N treatments in the California experiments (Grulke), the responses of trees to Ca limitations in acidic soils in Croatia (Potocic), and the O_3 effect on adult beech in Germany (Löw, Blumenröther). As a general rule, both O_3 and drought stresses affected tree growth, and compensation mechanisms resulted in shifts in internal resource allocation, as shown in Aleppo pine (Inclan).

Small changes in the gaseous composition of the atmosphere have significant impacts on trees. Some of the most important but least studied involve changes in stomatal responses. Rising atmospheric CO_2 concentrations are suggested to ameliorate the harmful O_3 effects, by reducing stomatal conductance and thus the potential O_3 flux into the leaf. Confirmation that stomatal acclimation to elevated CO_2 does or does not lessen over time, is critical for developing meaningful O_3 flux models (Paoletti). Contrasting responses emerge from the literature and may arise from: stomata responding much more slowly to changes in environmental conditions (scale of hours) than photosynthesis (scales of minutes); from species-specific responses; and from the network organization of guard cell signaling components (alteration in sensitivity to one signal affects other pathways and sensitivity to other signals).

Five years of free-air CO_2 enrichment on young poplar trees showed no acclimation of gas exchange except for the lower layer of the canopy and only by the end of the season (Calfapietra). Nitrogen fertilization increased N concentration in the leaves but did not significantly influence photosynthetic properties.

Long-term experiments on impacts of ambient O_3 and CO_2 enrichment on growth, productivity competitive interactions and fitness of trembling aspen in the FACE study in Rheinelander, Wisconsin, suggested that even relatively low ambient levels of O_3 can negate positive effects of CO_2 enrichment on biomass production of trees (Karnosky).

Root and mycorrhizal responses to pollutant inputs were measured in several studies (Braun, Tarvainen, Sheppard), including the effects of CO_2 and O_3 (Kasurinen), with negative responses to O_3.

New research tools

The progress in the field of forest genetics supplies a variety of measures, criteria and indicators related to stress response of populations and individuals. Particularly, the rapid progress in the fields of molecular genetics and proteomics creates new interdisciplinary links in the study of the response of plants to stress and corresponding causal relationships (Müller-Starck). A variety of advanced tools is available to combine genome, transcript and protein analyses with studies on metabolic pathways and the expression of traits. These new tools will allow a better bridging between genetics, physiology and other disciplines related to trait expression.

Corticular (bark) CO_2 refixation should be considered when measuring respiratory fluxes from branches and stems of trees (Pfanz). Accurate estimates are important not only for measuring bark respiration rates, but also because cortical CO_2 fixation can significantly contribute to the carbon economy of trees. Accurate estimates of cortical fixation are needed when scaling responses to ecosystems and for successful modeling efforts.

PAM imaging is a novel technique to map and quantify changes in chlorophyll fluorescence (Löw). In conjunction with traditional microscopy techniques, it should allow new advances in functional anatomy of chloroplasts.

Diagnosis of foliar symptoms in the field can be verified in the laboratory. As shown in several presentations during the microscopy workshop (Günthardt-Goerg, Kivimänpää, Oksanen, Vollenweider), the principal role of microscopy in stress physiology is to link stress factors and visible symptoms with cellular and biochemical effects. Microscopy has proved its value in diagnosing several stress factors and therefore ascertaining the observed visible symptoms to render them suitable for a bioindication purpose (in particular for O_3), irrespective of species and site. Physiological and biochemical processes could be localised by cytochemistry and their mechanisms of action better understood. Increasing experience in electron microscopy has been used to detect stress associated structural changes as occurring in conifer chloroplasts on a subcellular level, potentially before visible symptoms appear. The number of peroxisomes, mitochondria and chloroplast plastoglobuli in conifer species is associated with light and/or O_3. Exposure of the organ and tissue to light is an important factor enhancing O_3 injury (Günthardt-Goerg, Vollenweider, Kivimäenpää), and has to be considered in assessments, sampling and microscopical analysis. The latest results scale the mechanisms of O_3 action from the structural down to the biochemical and molecular level. For example, O_3-induced increase of H_2O_2 (detected with a specific staining) and transcript levels of catalase appeared to be restricted to the apoplast without ultrastructural injuries in tolerant aspen clones, whereas in sensitive plants they continued to the plasma membrane, cytosol and chloroplasts (Oksanen). The latter effect was absent in the treatment with elevated CO_2, showing the possible influence of the carbon balance in the response. At a cellular and ultrastructural level, the compartmentation plays a vital role; this was also evident in the investigation of products from the secondary metabolism. Their presence, quantity and site within tissue cells and cell compartments give important indications for detecting different stress factors. An innovation was the detection of heavy metals in situ by cytochemical methods (Vollenweider). The influence of leaf anatomy on the relative O_3 sensitivity of native vegetation was shown (Koivisto, Maninnen, Timonen), however woody plants are more easily used for O_3 monitoring than herbaceous species, which have very variable life circles, tend to senescence after flowering and are more often infected by fungi. In contrast to O_3, increased UV-B did not have significant effects so far in a Finnish peatland experiment (Haapala, Mörsky), but increased flavonoids in birch seedlings (Keski-Saari). Microscopy was used to explain the anatomical features induced by nutrient deficiency (as shown for boron by Sutinen). Traditional methods used for long term monitoring could now show structural ameliorations in the vegetation around industries after changing production techniques in Eastern European countries (Kupcinskiene).

Several interesting directions for future research can be outlined in synthesis from the microscopy workshop.

(1) Microtechnical level:

- Importance of fixation, sampling, selection of staining and observing methods as a decisive step. For example, routine aldehyde fixation deactivates the proanthocyanidins (Vollenweider). As a consequence, the crucial role of the tannins in stress reactions has been often overlooked because of the widespread use of aldehydes in fixation pretreatments. A critical approach is required to examine the interactions between the preparation chemicals and the observed plant structures. New advances can be expected by research labs, having different technical specialities, joining in common research investigations.
- Extension of the list of bioindicators usable for differential diagnosis of more stress factors and in a widened list of plant

species. Investigations in this domain are urgently required with an increasingly stressed plant environment in a changing world. Joining microscopic and biochemical techniques is an efficient way of overcoming the limitations imposed by both approaches (respectively the significance and the specificity of the observed changes).

(2) Actuality of certain stress factors as outlined by recent advances in research and changes in the global situation:

- Effects of drought and heat in mesophilous and climacical species.
- Quantitative contribution of light with and without O_3 which leads to photo-bleeching and other visible symptoms.

(3) Biotic and abiotic stress interactions:

- Role of biotic stress (air pollution by fungal spores), which may be underestimated.
- Predisposition to biotic infection by anthropogenic pollution or environmental change.
- Cellular reactions against different biotic attacks, also as founding criteria of differential diagnosis.

(4) Understanding the changes in cell physiology underlying microscopical stress symptoms, noteably concerning:

- Vesiculations in the cytoplasm.
- Changes in chloroplast structure.
- Changes in stomatal apparatus anatomy.
- Changes in vein structure caused by nutrient deficiencies.

Conclusions and prospects

Monitoring is essential to document forest health's status and changes. The presentations given at the meeting pointed out strengths and weaknesses of forest monitoring as carried out at present. While it is important to acknowledge the achievements, questions related to the value of the response indicator used in multivariate analysis and to the importance of the monitoring design indicate potential problems with the results obtained to date. This means that indicator development, monitoring design and the evaluation of their combined effects on data analysis and on the strength of the conclusions represent areas where substantial improvements are needed.

In support of monitoring, microscopy techniques can be useful for validating the effects of different stress factors. Microscopical symptoms should be linked to higher observation levels, e.g. to scale the significance of leaf and needle symptoms to whole tree physiology, and to differentiate species- and climate-specific (North vs. South) responses to natural aging and senescence (the use of these terms is not clearly defined). In addition, it needs to be taken into account that abiotic environmental conditions can infer biotic stresses caused by pathogens and insects in many ways.

Most of attention at the meeting focused on ozone. Establishing a cause and effect relationship for O_3 and tree growth in forests is inherently difficult. For instance, extrapolation of the results obtained with open-top chambers to forest conditions is difficult (Manning). Experiments in which forest trees of known high and low sensitivity to O_3 are treated with O_3 -protective chemical such as ethylenediurea (EDU), while crucial environmental factors are monitored and biological responses are measured, could help in investigations of long-term cause and effect relationships (Manning). Free-air O_3 exposure gives valuable information in near-natural ecosystems (Karnosky, Nunn). Seedlings were not surrogates for adult trees even under identical climatic conditions, while twigs were suggested to act as surrogates for whole crown responsiveness to O_3 (Then). Leaf-level responses significantly reflect O_3 impact when standardizing sampling scheme. It seemed that adult trees in the field are less sensitive to O_3 than seedlings, based on free-air O_3 exposure studies; nonetheless, long-term risks cannot be ruled out (Nunn).

Despite topicality of the ozone issue, future work should address the synergistic effects of air pollution and climate change to a greater extent. Air pollution and climate change are closely related. The main driving force of climate change is the increase in atmospheric CO_2 concentrations; both CO_2 and air pollutants come largely from human activities. Research emphasis has shifted from single to multiple pollutants, from chamber to field investigations, and from simple reconstructed systems to complex field systems that include both air pollution and climate stresses. Overall, the joint long-term effects of CO_2 and O_3 on tree stomatal responses are still poorly understood. Combined exposures should also allow for scaling up the variation in stomatal density and size to the whole tree level. As larger stomata are slower to close (Aasamaa et al., 2001), an altered stomatal size-density relationship is critical for developing meaningful hydrological and ozone-flux models in a global change scenario.

Most of the results discussed at the meeting were obtained in North America and Europe. Investigation of plant reactions to air pollution and climate change in Africa, South America and Asia are needed. However, improving the research involvement of the under-represented countries also implies access to funding. Funding has become more international, despite decreasing support from conventional forest research institutions

(Seppälä). The growing share of private funding implies increasing result-oriented research and short-term thinking. This may put basic research and quality of research in danger.

More than in the past, researchers working with pollution and forests need to improve the interface between science and policy. How do we make research more useful? How do we go beyond passive dissemination and increase the influence and impact of research? The IUFRO President Risto Seppälä suggested the following guidelines: involve users; make your research results attractive to stakeholders; state your biases and paradigms; use multidisciplinary teams (holistic view); make your research more policy-oriented.

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