CENTRE FOR ECOLOGY AND HYDROLOGY
(Natural Environment Research Council)
Defra Contract EPG 1/3/205
CEH Project No. C02309

The UNECE International Cooperative Programme on Vegetation

Final Report

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May 2006

Defra Contract EPG 1/3/205: The UNECE International Cooperative Programme on Vegetation

Executive Summary
(April, 2003 – March, 2006)

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Background

The ICP Vegetation[1] of the Convention on Long-range Transboundary Air Pollution (LRTAP) has studied the impacts of air pollutants on crops and (semi-)natural vegetation in the UNECE (United Nations Economic Commission for Europe) region for almost two decades. In the last five years, the ICP Vegetation has focussed on two air pollution problems of particular importance: quantifying the risks to vegetation posed by ozone pollution and the atmospheric deposition of heavy metals to vegetation. In addition, two other pollution problems have recently been considered by the programme: the impacts of nitrogen pollutants on vegetation and plant responses to pollutant mixtures. The results of studies conducted by the ICP Vegetation are reported to the Working Group on Effects of the LRTAP Convention, where they are used in assessments of the current, and predictions of the future, state of the environment. Currently, the work of the ICP Vegetation is providing information for the revision of the Gothenburg Protocol (1999) designed to address the problems of acidification, nutrient nitrogen and ground-level ozone, and the Aarhus Protocol (1998) designed to reduce emissions of heavy metals.

Coordination of the programme

During the course of the contract, participation has increased by 25% to over 180 scientists from 35 Parties to the LRTAP Convention. The programme of work has been coordinated from the Programme Coordination Centre at CEH Bangor. Experimental protocols have been produced for both the ozone effects work and the heavy metals in mosses surveys, results have been collated and analysed, and state of knowledge and progress reports have been provided for Defra, WGE, ICP Vegetation participants and the public via the web-page (icpvegetation.ceh.ac.uk). Three successful Annual Task Force Meetings have been organised by the Coordination Centre and held in Greece (2004), Spain (2005) and the UK (2006) involving 50-60 participants each year.

As part of this contract, the text related to ozone critical levels in the previous version of the Mapping Manual (LRTAP Convention, 1996) was substantially revised to reflect the decisions made at the Gothenburg critical levels for ozone II workshop (2002) and subsequent Task Force Meetings of the ICP Vegetation and ICP Modelling and Mapping. For the first time, stomatal flux-based critical levels were included in the Mapping Manual (Chapter 3; LRTAP Convention, 2004, available at www.icpmapping.org) together with the benefits of using this method for quantifying the impacts of ozone in the ECE region

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[1] The International Cooperative programme on Effects of Air Pollution on Natural Vegetation and Crops

compared to using AOT40[2]-based critical levels. The text related to the critical levels of SO_2, NO_x and NH_y, and in some cases the values, were also revised in the chapter. The new Mapping Manual was accepted at the 23rd Session of the Working Group on Effects (WGE), September 2004. In 2005, the Coordination Centre assisted the local organisers of the most recent critical levels workshop "Critical levels of ozone: further applying and developing the flux-based concept" (Obergurgl, Austria, 15-19 November 2005). The workshop made recommendations for further revision of chapter 3 of the Mapping Manual and at the 19th ICP Vegetation Task Force Meeting it was decided to include the new text as an annex. Drafting of the text was coordinated from CEH Bangor, and the annex included a simplified flux-modelling approach for integrated assessment modelling plus a new critical level for (semi-)natural vegetation communities dominated by perennial species and new data to support the choice of communities for mapping purposes.

The impacts of ambient ozone on biomonitors

Each year, the participants of the ICP Vegetation conducted a series of standardised experiments to monitor the effects of ambient ozone pollution on vegetation. The concentration-based long-term critical level for (semi-)natural vegetation and agricultural crops (a three-month AOT40 of 3 ppm h) was exceeded at approximately 80% of sites each summer. Ozone concentrations were highest in the hot, dry summer of 2003 when over 50% of the mean daily maximum ozone concentrations for each of the 28 day harvest periods were ≥ 60ppb; in 2004 and 2005 the proportion was 19% and 20% respectively. Although ozone concentrations were lower in 2004 and 2005 than in 2003, visible ozone injury was still recorded widely across sites in Europe, but at a lower intensity than in 2003. It is of note that visible injury was also recorded at sites where the long-term critical level of ozone was not exceeded, including at both Bangor (UK) and Ebro Delta (Spain) in 2003, where there were moderate injury scores (5-25% of leaves affected) at two or more harvests. Biomass reductions in the ozone-sensitive biotype relative to the resistant one were observed at sites where the three month AOT40 was in excess of ca. 6 ppm h. A carry-over effect of ozone exposure on the clover plants was identified. There was significant improvement to the relationship between ozone exposure and NC-S/NC-R biomass ratio when the AOT40 over time periods prior to the start of the current harvest interval were taken into account.

At 12 – 17 participating sites each year, plants were grown from seeds collected by colleagues from Switzerland from an ozone sensitive and an ozone resistant population of *Centaurea jacea*. Ozone-specific visible injury was observed at approximately two thirds of these sites, including Ostad (Sweden) in the north and Rome (Italy) and Velenje (Slovenia) in the south of Europe. As a contribution in kind to the ICP Vegetation, colleagues in Switzerland are developing a biomonitoring system using cloned material of ozone-sensitive and –resistant plants of *Centaurea jacea* that are initially grown using tissue culture with the aim of reducing the inherent variation associated with using seeds.

Flux-effect relationships

In the last two years, stomatal-flux-based critical levels have been included in the revised Mapping Manual for wheat and potato. Although these two crops are very important in Europe, the need to expand the range of crops for which flux-effect relationships exist was

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[2] The sum of the differences between the hourly mean ozone concentration (in ppb) and 40 ppb each hour when the concentration exceeds 40ppb, accumulated during daylight hours.

addressed within this contract. Firstly, a canopy flux-effect relationship was developed for clover using data from the ICP Vegetation ambient air experiments. The dose-response function using canopy flux had a lower r^2 value of 0.3 than that based on AOT40 for the same three month dataset ($r^2 = 0.53$), and also fitted less well than the single leaf flux model ($r^2 = 0.45$). This may reflect uncertainty in estimating the development of leaf area index for clover during each 28d period together with the use of the 28d harvest biomass to estimate leaf area index. Secondly, the literature on effects of ozone on crops other than wheat and potato has been reviewed together with data on stomatal conductance-environmental parameter relationships. Parameterisation of the DO_3SE (**D**eposition of **O**zone for **S**tomatal **E**xchange) flux model was possible for four additional crops species (tomato, grapevine, maize and sunflower). Applying these flux models to five representative 50 x 50 km grid squares using surface ozone and meteorological data provided by EMEP MSC-W, showed the importance of g_{max} and phenology in determining accumulated flux.

In addition, a flux model was derived for *Centaurea jacea* using data collected in 2004 and 2005 at sites in the UK (Bangor), Switzerland (Cadenazzo), France (Grignon) and Italy (Rome). Different parameterisations were needed for central European compared to western European sites. Applying the models to five representative EMEP grid squares indicated that accumulated fluxes were approximately twice as high for the Atlantic Central European square than for the Western Mediterranean square.

Mapping semi-natural vegetation at risk from ozone

The results from over 60 papers were examined, and data for 83 species of (semi-) natural vegetation were included in a database named OZOVEG (ozone impacts on vegetation). Meta-analysis of the ozone sensitivity data showed a wide inter-specific range in response to ozone from strong inhibition to stimulation of growth. Some relationships with plant physiological and ecological characteristics were identified. Plants of the therophyte lifeform were particularly sensitive to ozone. Plants with higher mature leaf N concentration were more sensitive to ozone than those with lower leaf N concentration. Some relationships between relative sensitivity to ozone and Ellenberg habitat requirements were also identified. In contrast, no relationships between relative sensitivity to ozone and mature leaf P concentration, Grime's CSR strategy, leaf longevity, flowering season, stomatal density and maximum altitude were found.

A regression-based model was developed for predicting changes in biomass of individual species exposed to ozone (RS_p), based on their Ellenberg Indicator values. The model was applied to plant communities to develop two further predictive tools. The first tool, percentage change in biomass (ORI%) was tested on data from a field-based ozone exposure experiment conducted in Switzerland and predicted a 27% decrease in biomass over 5 years compared with an observed decrease of 23%. The second tool, an index of community sensitivity to ozone (CORI), was applied to 48 grassland communities and suggests that community sensitivity to ozone is primarily species-driven.

For wider application to mapping risk within Europe, a simpler method based on the proportion of ozone-sensitive species was developed. Using data within OZOVEG, 54 EUNIS (European Nature Information System) level 4 communities with six or more ozone-sensitive species (%OS) and c. 20% or more species tested for ozone sensitivity, were identified as potentially ozone-sensitive. The largest number of these communities (23) was associated with Grasslands, with Heathland, scrub and tundra, and Mires, bogs and fens having the next highest representation at 11 and 8 level 4 communities each respectively. Within the grasslands classification, E4 (Alpine and sub-alpine grasslands), E5 (Woodland fringes and clearings) and E1 (Dry grasslands) were the most sensitive with 68, 52 and 49 %OS respectively. Methods for mapping the location of these communities in Europe were developed by harmonising the Stockholm Environment Institute landcover map with the European Environment Agency Corine landcover map.

As a first step towards flux-effect relationships for (semi-)natural vegetation, a climate-dependent simulation of canopy growth and transpiration was integrated with the DO_3SE model of ozone flux. The focus of the analysis was the interaction of three factors: nutrient status, soil moisture and management regime. A key conclusion was that these factors need to be considered in combination rather than in isolation. Actively managed mesic and wet grasslands are likely to have a high ozone flux and experience a greater impact of ozone. Nutrient-poor dry grasslands may have lower flux and be less sensitive *in situ* than the examination of the responses of individual species suggests.

Heavy metal concentration in mosses

Preparations for the 2005/6 moss survey started in year 1, and in year 2 of the contract the Manual for collecting and analysis of moss samples was reviewed and revised by the Coordination Centre and the participants in the moss survey. Certified moss reference material was supplied to participants from Finland for quality assurance purposes. The Coordination Centre has also been assisting participants to gain funding for the 2005/6 survey by writing letters of support to funding bodies. In all, participants from 32 countries are contributing to the 2005/6 moss survey (with the Netherlands awaiting approval of funding) and will sample mosses from over 7,000 sites across Europe. Of these, 18 countries will also analyse the nitrogen concentration in mosses at ca. 3,200 sites.

As part of this contract, the data from all the pan-European moss surveys (1970 – 2000) were standardised to a common format. Statistical analysis of the temporal trends were conducted for five selected European countries: Norway, Slovakia, Sweden, Switzerland and the United Kingdom. The concentrations of almost all metals were higher in Slovakia than in the other countries. Only cadmium and lead concentrations in mosses showed a consistent decline between 1990 – 2000 in all countries studied. This decline was in general agreement with that modelled by EMEP for lead and cadmium deposition. Further comparisons with the EMEP model indicated that the model mimicked the spatial pattern of lead concentration in mosses across Europe. No clear temporal trends were identified for arsenic, chromium, copper, iron, mercury, nickel, vanadium and zinc.

Uncertainty in economic estimates of ozone-induced crop yield loss

Using AOT40-based methods, a range for ozone-induced losses for 23 crops in 47 countries has been quantified for Europe of €4.4 to 9.3 billion/year, around a best estimate of €6.7 billion/year for year 2000 emissions. This core estimate represents losses equal to 2% of arable agricultural production in Europe. Results for a series of scenarios considered for 2020, by when all current legislation should be fully in place, range from 2.4 to 5.5 Billion Euro per year for three of the EU's recent CAFE (Clean Air For Europe) Programme scenarios, and from 1.1 to 2.3 for the maximum feasible reduction according to the RAINS model. These estimates do not account for damage via visible injury, changes in crop quality, or interactions with pests. The largest sources of uncertainty are, in order of decreasing importance: Response function for vegetables, variation in ozone concentration with height, crop yield estimates and the response function for potato. Results based on the use of flux-based methods for five grid cells representing each of five European climate zones indicates both increases (e.g. in Northern Europe) and decreases (e.g. in Continental Central Europe) in flux-based yield loss estimates relative to concentration-based estimates, depending on climatic zone.

Nitrogen concentration in mosses as an indicator of nitrogen pollution

Nutrient nitrogen has been incorporated into the remit of the ICP Vegetation by exploiting the existing heavy metals in mosses sampling network. Firstly, moss samples from five countries that have had long involvement in the European heavy metals in moss survey (at least 1980 – 2000) were analysed for total N concentration. There were higher N concentrations in mosses collected in Germany than in Scandinavian countries and values for Spain were intermediate (except in the 1970s), but no significant temporal trends within countries were observed. When compared with the N deposition rates (reduced, oxidised and total N) in Scandinavia, good positive correlations existed within individual countries. Secondly, historic herbaria moss samples were received from the Czech Republic, Finland, France and Switzerland and analysed for total N concentration. When pooled together, there was a trend towards a small increase in the total N concentration in mosses of ca. 0.2% over the last century. When the data were grouped into different time periods, there was no significant change in the total N concentration in mosses up to 1960. However, beyond 1960 there was overall a significant ($P < 0.001$) increase in the total N concentration in mosses compared to earlier years. There was a significant country effect on the total N concentration in the mosses, with the average values being 0.89%, 1.06%, 1.10% and 1.20% for France, the Czech Republic, Finland and Switzerland, respectively.

The impacts of nitrogen on the responses of vegetation to ozone

The literature on the impacts of nitrogen pollution on the responses of vegetation to ozone was reviewed. Nitrogen can affect the sensitivity of species to ozone by altering uptake *via* physiological and morphological parameters and by altering the capacity for detoxification and repair. There are numerous ways in which N may alter exposure to ozone. These include geographical exposure, life strategy, growth form, and the timing of key events through the year such as seedling emergence, the main period of vegetative growth, flowering and seed set. Changes in resource partitioning and in tissue chemistry due to both N and ozone may have indirect effects on wider components of the ecosystem such as mycorrhizal and symbiotic relationships, soil microbial activity and rates of litter decomposition and N mineralisation. Other effects may be mediated by external factors such as herbivory, disease, management and limitation of other resources such as soil phosphorus, while competitive interactions may affect individual plant exposure and community sensitivity as a whole.

The evidence above suggests that ozone and nitrogen can have both synergistic and antagonistic effects on species and ecosystem processes, and that they may interact in unpredictable ways to affect plant communities. Three EUNIS communities have been identified which are potentially at risk of exposure to both elevated nitrogen and ozone. These are: E1 - Dry grasslands; E4 - Alpine and sub-alpine grasslands; and F4 - Temperate shrub heathland. Geographical co-occurrence of both pollutants is greatest in southern Germany and parts of northern Italy. Co-exposure is most likely to affect E1 and E4 grasslands.

Results from experiments with *Centaurea jacea* suggest that nitrogen-limited plants are unable to allocate sufficient resources to ozone detoxification and cell repair. Elevated levels of N can reduce ozone injury but can also increase injury in very young fresh shoot material.

The influence of climate change on the impacts of ozone on vegetation

A review of the literature has revealed that it is important when predicting future impacts of O_3 to consider O_3 effects within the context of global climate change. The overall impact of warming on the canopy flux of O_3 is difficult to predict and will depend on the location of the vegetation, severity and timing (e.g. summer or winter) of warming, its impacts on soil water potential and phenology of the vegetation. Each plant species has its own optimum temperature for g_s and the impact of warming on g_s will depend on which part of the temperature response function corresponds with the current ambient temperature. Indirect effects of warming via changes in vapour pressure deficit and soil water potential and enhance plant development will also alter stomatal flux of O_3 into leaves. In general, elevated CO_2 ameliorates O_3-induced stress and the combined effects of O_3 and CO_2-enrichment on plant growth and physiology has often been near neutral as elevated CO_2 and O_3 affect vegetation in opposite ways. Effects of changes in precipitation patterns are likely to be mediated directly through (a) effects of VPD on g_s with increasing VPD causing a decrease in flux and (b) changes in SMD, with increasing SMD resulting in decreased stomatal flux and vice versa. The little data that is available on interactive impacts of combined global climate change factors has indicated that the effects are complex and that generalisations might be difficult.

Future research

The following have been identified as research aims for study in the next three years:

- An evidence-based assessment of the impacts of current ambient ozone on vegetation in Europe.

- To quantify the risk of ozone effects on (semi-)natural vegetation in Europe by (i) further developing the Ellenberg method and applying it across Europe, and (ii) including the modifying influence of nitrogen.

- To develop a flux-based assessment of the risk of ozone damage to (semi-) natural vegetation in Europe.

- To analyse the temporal trends of heavy metals in European mosses across Europe and compare the trends with deposition trends based on the EMEP model.

- To determine the nitrogen concentration in mosses across Europe, analyse spatial trends and compare these trends with deposition trends based on the EMEP model.

- To assess the evidence for impacts of nitrogen on vegetation in areas of Europe with high nitrogen deposition and identify areas where nitrogen critical loads are exceeded for specific EUNIS communities.

- To predict ozone effects on crops in a changing climate by modifying and applying the stomatal flux model (taken climate change factors such as atmospheric CO_2 enrichment into account) and predict crop yield losses in a changing climate.

- To develop methods to integrate our knowledge of the mechanisms of flux and flux-effect models (which exist only for a limited number of crops) to understand how best to modify concentration-effect models (which exists for a wide range of crops) and apply this to improve the economic impact assessment of ozone on crops in Europe.

ACKNOWLEDGEMENTS

We wish to thank the UK Department for Environment, Food and Rural Affairs (Defra) for the continued financial support of the ICP Vegetation (Contract EPG 1/3/205). The support of the Scottish Executive, the National Assembly for Wales and the Department of Environment in Northern Ireland is also acknowledged. The contributions of the ICP Vegetation Steering Committee (Professor Jürg Fuhrer, Dr Ludwig De Temmerman, Dr Ben S. Gimeno and Professor Mike Ashmore) and of all the ICP Vegetation participants are gratefully acknowledged. Finally we wish to thank the following people for their help with the project: Jackie Cooper, David Norris, Patrick Bűker, Georgia Koeber, Andrew Lloyd and Rose Macmillan.

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1. INTRODUCTION

1.1 Overview of the ICP Vegetation

Reporting to the Convention on Long-range Transboundary Air Pollution, the ICP Vegetation is an International Cooperative Programme investigating the effects of air pollutants on vegetation in countries in the geographical region of the United Nations Economic Commission for Europe (UNECE), including Europe, USA and Canada. The Convention secretariat is with the UNECE. The main activities of the ICP Vegetation are as follows:

- Conducting coordinated experiments to determine the effects of ozone pollution on crops and (semi-)natural vegetation in Europe and North America;
- Developing models to quantify and interpret the influence of climatic conditions and environmental stresses on the responses of plants to ozone;
- Developing maps showing where vegetation is at risk from ozone pollution within the ECE region;
- Assessing the economic losses caused by the effects of ozone on crops;
- Collating and reviewing information on the effects of ozone on plant biodiversity;
- Considering the evidence for the modifying effects of nitrogen deposition on the impacts of O_3 on communities of (semi-) natural vegetation in Europe;
- Collating and reviewing monitoring data on the atmospheric deposition of heavy metals, and subsequent accumulation by plants including a European survey of heavy metal concentrations in mosses;
- Studying the extent of nitrogen deposition in Europe by determining the nitrogen concentration in mosses at an extensive network of monitoring sites.

The work of the ICP Vegetation currently aims to provide information for the proposed revisions of the Gothenburg Protocol (1999) designed to address the problems of acidification, nutrient nitrogen and tropospheric ozone, and the Aarhus Protocol (1998) designed to reduce emissions of heavy metals. Over 180 scientists from 35 countries of Europe and North America contribute to the programme by conducting experiments, sampling vegetation or modelling pollutant deposition and effects. The Programme Coordinating Centre is at the Centre for Ecology and Hydrology Bangor and the coordination is supported by the UK Department for Environment, Food and Rural Affairs (contract EPG 1/3/205). Dr Harry Harmens is the chair of the ICP Vegetation and Dr Gina Mills is the Head of the Programme Coordinating Centre at CEH Bangor. Sub-contracts are held by the Stockholm Environment Institute – York, EMRC – Reading and Imperial College.

1.2 Objectives of Contract EPG 1/3/205

The objectives for this contract are listed below and fall into four distinct work packages and from additional options. A brief introduction for each area of work is provided at the beginning of the text for each package, option or subject area. The Institutes, Universities or Consultancies contributing to the work for each package or option are described within each introduction.

Work Package 1: Coordination of the Programme

1. To chair the programme and to manage the reporting of the results of the programme to Defra, WGE, Scientific Press and the public.
2. To manage the experimental programme of the ICP Vegetation, including data collation and analysis, and running the UK sites.
3. To organise the annual Task Force Meeting, and to attend UNECE meetings and conferences, as appropriate.

Work Package 2: Critical levels of ozone for crops

4. To further parameterize and validate ozone flux-effect models for white clover.
5. Further parameterisation of flux models for crop species other than wheat, clover and potato.

Work Package 3: Quantifying the effects of ozone on semi-natural vegetation in Europe

6. Development of a flux-effect model for *Centaurea jacea*.
7. To identify, and to further develop, methods for mapping semi-natural community types at risk from ozone including the link to flux-effect modelling.

Work Package 4: Heavy Metals

8. To complete the reporting of the 2000/01 metals in mosses survey, and to coordinate the 2005 survey.

Options: Additional work funded to support the programme

9. To calculate the economic losses associated with exceedance of the critical levels/fluxes agreed by the ICP Vegetation.
10. Incorporation of the effects of nutrient nitrogen within the remit of the ICP Vegetation.
11. An analysis of the spatial and temporal trends of heavy metals in mosses, using data available to the ICP Vegetation from moss surveys (1980-2000).
12. Impacts of pollutant mixtures.

2. WORK PACKAGE 1: COORDINATION OF THE PROGRAMME

2.1 Introduction

The objectives for work package 1 describe the ongoing activities of the Coordination Centre for the ICP Vegetation at CEH Bangor. These include the coordination of the ozone biomonitoring experiments, reporting to the LRTAP Convention and Defra and organising the annual Task Force Meeting for the programme. Within this package, the funding also covers experimental work at CEH Bangor and Imperial College and attendance of sub-contractors from SEI-York, University of York, Imperial College and EMRC at the annual Task Force Meeting.

2.2 Programme coordination and reporting (Objective 1)

2.2.1 Programme management

In February 2004 Dr Harry Harmens took over the chair of the ICP Vegetation from Dr Gina Mills, and has since then been the official representative of the programme at LRTAP Convention meetings. In close collaboration with Dr Gina Mills, he has managed the work programme for the ICP Vegetation during the last two years and has been the initial contact person for participants. Sub-contractors have attended the annual Task Force Meetings (see section 2.5), have attended project meetings at CEH Bangor and have been in regular email contact with Dr Gina Mills and Dr Harry Harmens.

Table 2.1 Countries participating in the ICP Vegetation.

Austria
Belarus
Belgium
Bosnia and Herzegovina
Bulgaria
Czech Republic
Denmark
Estonia
Finland
FYR of Macedonia
France
Germany
Greece
Lithuania
Hungary
Iceland
Ireland
Italy
Latvia
Netherlands
Norway
Poland
Portugal
Romania
Russian Federation
Serbia and Montenegro
Slovakia
Slovenia
Spain
Sweden
Switzerland
Turkey
United Kingdom
Ukraine
USA

During the three years of this contract, participation in the ICP Vegetation increased to over 180 scientists from 35 Parties to the Convention (Table 2.1). It should be noted that in many countries, several other scientists (too numerous to mention individually) also contribute to the biomonitoring programmes, analysis and modelling procedures that comprise the work of the ICP Vegetation.

2.2.2 Reporting to the LRTAP Convention

Each year the Chair of ICP Vegetation attended the Extended Bureau meetings (twice per year, February/March and August), the joint WGE/EMEP Bureau meetings (February/March) and the 22nd – 24th sessions of the WGE meeting (August/September) in Geneva. At the WGE meetings the Chair of the ICP Vegetation presented an overview of the results and activities in the previous year regarding the air pollutants ozone and heavy metals, and recently also nitrogen. In addition, deliverables to the WGE and the medium-term work plan were presented. Full details of the reports to the WGE are given below for each contract year:

2003/4:

Results of the heavy metals in mosses survey 2000/2001. ICP Vegetation, Technical Report prepared for the 22nd Session of the Working Group on Effects, September 2003. (EB.AIR/WG.1/2003/8).

Contribution of ICP Vegetation to 2003 joint report of the International Cooperative Programmes and the Task Force on the health aspects of air pollution. Technical Report prepared for the 22nd Session of the Working Group on Effects, September 2003. (EB.AIR/WG.1/2003/3 and Add. 1).

Buse, A., Mills, G., Harmens, H., Büker, P., Hayes, F., Williams, P., Emberson, L., Cinderby, S., Ashmore, M., Holland, M. and the participants of the ICP Vegetation (2003). Air pollution and vegetation. UNECE ICP Vegetation Annual Report 2002/2003. ISBN: 1 870393 71 6.

2004/5:

The scientific basis for the new flux-based critical levels of ozone. ICP Vegetation, Technical Report prepared for the 23nd Session of the Working Group on Effects, September 2004. (EB.AIR/WG.1/2004/8).

Contribution of ICP Vegetation to 2004 joint report of the International Cooperative Programmes and the Task Force on the health aspects of air pollution. Technical Report prepared for the 23nd Session of the Working Group on Effects, September 2004. (EB.AIR/WG.1/2004/3 and Add. 1).

Harmens, H., Buker, P., Mills, G., Buse, A., Cinderby, S. (2004). Factors influencing the concentrations of heavy metals in mosses across Europe. Contract report to the UNECE Trust Fund.

Harmens, H., Mills, G., Hayes, F., Williams, P. and the participants of ICP Vegetation (2004). Air pollution and vegetation. UNECE ICP Vegetation Annual Report 2003/2004. ISBN: 1 870393 75 9.

Mills, G. (2004). Mapping critical levels for vegetation. In: UNECE Mapping Manual 2004. This chapter was edited by Gina Mills using text provided by: Ashmore, M., Bermejo, V., Broadmeadow, M., Danielsson, H., Emberson, L., Fuhrer, J., Gimeno, B., Holland, M., Karlsson, P.E., Mills, G., Pihl Karlsson, G., Pleijel, H. and Simpson, D. Additional editorial advice was provided by: S. Braun, H. Harmens, M. Johansson, U. Lorenz, M. Posch, T. Spranger, and A. Vipond.

Sliggers, J., Kakebeeke, W. (2004) Clearing the air: 25 years of the Convention on Long-range Transboundary Air Pollution. With contributions from H. Harmens and G. Mills. United Nations, Geneva. ISBN 92 1 116910 0.

Working Group on Effects (2004) Review and assessment of air pollution effects and their recorded trends. Working Group on Effects, Convention on Long-range Transboundary Air Pollution. With contributions from and editing by G. Mills and H.

2005/6:

An overview of the impacts of ambient ozone on white clover at the ICP Vegetation sites (1996-2003). ICP Vegetation, Technical Report prepared for the 24th Session of the Working Group on Effects, September 2005. (EB.AIR/WG.1/2005/8).

Contribution of ICP Vegetation to 2005 joint report of the International Cooperative Programmes and the Task Force on the health aspects of air pollution. Technical Report prepared for the 24th Session of the Working Group on Effects, September 2005. (EB.AIR/WG.1/2005/3 and Add. 1).

Contribution of ICP Vegetation to draft 2006 work plan for the effects-oriented activities. Technical Report prepared for the 24th Session of the Working Group on Effects, September 2004. (EB.AIR/WG.1/2005/4).

Harmens, H., Mills, G., Hayes, F., Williams, P., De Temmerman, L. (2005) and the participants of ICP Vegetation. Air pollution and vegetation. ICP Vegetation Annual Report 2004/2005. ISBN: 1 870393 80 5.

2.2.3 Papers, conference proceedings and other publications

2003/4:

Conference proceedings:

Harry Harmens, Alan Buse, Patrick Büker, David Norris, Gina Mills, Bronwen Williams, Brian Reynolds, Trevor W. Ashenden, Åke Rühling, Eiliv Steinnes (2004). Heavy metal concentration in European mosses: 2000/2001 survey. Third International Workshop on Biomonitoring of Atmospheric Pollution, 21-25 September 2003, Bled, Slovenia. Published on CD Rom. Also in book of abstracts. Invited keynote presentation.

Others:

Mills, G., Harmens, H., Hayes, F., Büker, P., Williams, P. (2003). ICP Vegetation Experimental Protocol for the 2003 season. ICP-Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK.

2004/5:

Scientific papers:

Harmens, H., Buse, A., Büker, P., Norris, D., Mills, G., Williams, B., Reynolds, B., Ashenden,, T.W., Rühling, Å., Steinnes. E. (2004). Heavy metal concentration in European mosses: 2000/2001 survey. Journal of Atmospheric Chemistry 49: 425-436.

Conference proceedings:

Johansson, M., Posch, M., Gregor, H.-D., Achermann, B., Conway, F., Farrett, R., Forsius, M., Harmens, H., Haußmann, T., Hettelingh, J.-P., Jenkins, A., Johannessen, T., Krzyzanowski, M., Kucera, V., Kvaeven, B., Lorenz, M., Lundin, L., Mill, W., Mills, G., Skjelvkvåle, B.L., Spranger, T., Johannessen Ulstein, M., Bull, K. (2004) Effects research for the air pollution convention during 25 years. 13th World Clean Air and Environmental Protection, 22 – 27 August, London, UK.

Others:

Harmens, H., Hayes, F., Mills, G., Williams, P. (2004). ICP Vegetation Experimental Protocol for the 2004 season. ICP-Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK.

2005/6:

Scientific papers:

Harmens, H., Mills, G., Emberson, L., Ashmore, M. Implications of climate change for the stomatal flux of ozone: a case study for winter wheat. Environmental Pollution (accepted with minor revions).

Hayes, F., Jones, M.L.M., Ashmore, M. Mills, G. Meta-Analysis of the relative sensitivity of semi-natural vegetation to ozone. Environmental Pollution (accepted with minor revions).

Jones, M.L.M., Hayes, F., Mills, G., Sparks, T.H., Fuhrer, J. Predicting community sensitivity to ozone, using Ellenberg Indicator values. Environmental Pollution (accepted with minor revisions).

Mills, G.,Hayes, F., Jones, M.L.M., Cinderby, S. (2006). Identifying ozone-sensitive communities of (semi-)natural vegetation suitable for mapping exceedance of critical levels. Environmental Pollution (in press).

Mills, G, Buse, A, Gimeno, B , Bermejo, V, Holland, M, Emberson, L, Pleijel, H. AOT40-based response functions and critical levels for agricultural and horticultural crops. To be submitted to Atmospheric Environment, April 2006.

Conference proceedings/background papers:

Holland, M., Emberson, L., Mills, G., Ashmore, M., Harmens, H. (2005). Quantifying uncertainty in AOT40 and flux based estimates of crop losses from ozone exposure. Background paper workshop on ‘Critical levels of ozone: further applying and developing the flux-based concept’, 15-19 November 2005, Obergurgl, Austria.

Mills, G., Jones, M.L.M., Hayes, F., Fuhrer, J. (2005). Identifying ozone-sensitive communities of (semi-) natural vegetation for mapping exceedance of critical levels. Background paper workshop on ‘Critical levels of ozone: further applying and developing the flux-based concept’, 15-19 November 2005, Obergurgl, Austria.

Harmens, H., Mills, G., Emberson, L., Ashmore, M. (2005). Implications of climate change for the stomatal flux of ozone. Background paper workshop on ‘Critical levels of ozone: further applying and developing the flux-based concept’, 15-19 November 2005, Obergurgl, Austria.

Mills, G., Hayes, F., Williams, P, Jones, M.L.M., Macmillan, R., Harmens, H., Lloyd, A., Büker, P. (2005). Should the effects of increasing background ozone concentration on semi-natural vegetation communities be taken into account in revising the critical level? Background paper workshop on ‘Critical levels of ozone: further applying and developing the flux-based concept’, 15-19 November 2005, Obergurgl, Austria.

Emberson, L.D., Massman, W.J., Büker, P., Soja, G., van de Sand, I., Mills, G., Jacobs, C. (2005). The development, evaluation and application of O_3 flux and flux-response models for additional agricultural crops. Background paper workshop on ‘Critical levels of ozone: further applying and developing the flux-based concept’, 15-19 November 2005, Obergurgl, Austria.

Fuhrer, J., Bassin, S., Volk, M., Mills, G., Jones, M.L.M., Hayes, F., Ashmore, M. (2005). Impacts of ozone on communities of (semi-)natural vegetation. Background paper workshop on ‘Critical levels of ozone: further applying and developing the flux-based concept’, 15-19 November 2005, Obergurgl, Austria.

Harmens, H., Mills, G. and the participants of the ICP Vegetation (2005). The UNECE ICP Vegetation: impacts of ozone on vegetation. In: Conference abstracts, Acid Rain 2005, 7th International conference on acid deposition. Prague, Czech Republic, June 12-17, 2005.

Gregor, H.-D. *et al.* (including Harmens, H., Mills, G.) (2005). Trends in observed effects of air pollution in Europe. In: Conference abstracts, Acid Rain 2005, 7th International conference on acid deposition. Prague, Czech Republic, June 12-17, 2005.

Gregor, H.-D. *et al.* (including Harmens, H, Mills, G.) (2005). Monitoring and assessment of air pollution effects on a European scale. In: Conference abstracts, Acid Rain 2005, 7th International conference on acid deposition. Prague, Czech Republic, June 12-17, 2005.

Others:

Mills, G., Hayes, F., Williams, P., Harmens, H. (2005). ICP Vegetation Experimental Protocol for monitoring the incidences of ozone injury on vegetation 2005. ICP-Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK. Available on http://icpvegetation.ceh.ac.uk

Harmens, H. and the participants of the European moss survey (2005). Heavy metals in European mosses: 2005/2006 survey. Monitoring manual. ICP Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK. Available on http://icpvegetation.ceh.ac.uk

In addition to the above publications, several oral and poster presentations were given each year at national and international conferences.

2.2.4 Reporting to Defra

Each project year, quarterly reports were sent to Defra and at the end of year 1 and 2 a progress report, followed by a final report in year 3 were submitted. Details of the annual reports and any additional reports are given below:

2003/4:

Mills, G., Harmens, H., Hayes, F., Williams, P., Emberson, L., Cambridge, H., Cinderby, S., Terry, A., Ashmore, M., Holland, M., Green, E., Power, S. (2004). The UNECE International Cooperative Programme on Vegetation. Defra contract EPG 1/3/205. Progress report (April, 2003 – March, 2004).

2004/5:

Mills, G., Harmens, H., Hayes, F., Williams, P., Emberson, L., Cinderby, S., Terry, A., Ashmore, M., Holland, M., Green, E., Power, S. (2005) The UNECE International Cooperative Programme on Vegetation. Defra contract EPG 1/3/205. Progress Report (April, 2004 – March, 2005).

2005/6:

Mills, G., Harmens, H., Hayes, F., Williams, P., Emberson, L., Cinderby, S., Terry, A., Ashmore, M., Holland, M., Green, E., Power, S. (2006) The UNECE International Cooperative Programme on Vegetation. Defra contract EPG 1/3/205. Final Report (April, 2003 – March, 2006).

Holland, M., Kinghorn, S., Emberson, L., Cinderby, S., Ashmore, M., Mills, G., Harmens, H. (2006). Development of a framework for probabilistic assessment of the economic losses caused by ozone damage to crops in Europe. Contract report to Defra, contract EPG 1/3/2005.

Harmens, H., Mills, G. (2005). Review of the influences of climate change on the impacts of ozone on vegetation. Variation to Defra contract EPG 1/3/205: The UNECE International Cooperative Programme on Vegetation. September 2005.

2.2.5 Web site

The ICP Vegetation web-page http://icpvegetation.ceh.ac.uk is a source of information for the participants in the programme and the general public and was updated regularly. Documents such as the ICP Vegetation Annual Reports, other glossy reports, experimental protocols and the minutes of the annual Task Force Meeting were made available via the web-page. Links are provided to other relevant web pages such as Defra and the subsidiary bodies under the LRTAP Convention.

2.3 Revision of Chapter 3 "Mapping critical levels for vegetation" of the Mapping Manual

During the first year of the contract, under the guidance of Dr Gina Mills (CEH Bangor), the text related to ozone in the previous version of the Mapping Manual (LRTAP Convention, 1996) was substantially revised to reflect the decisions made at the Gothenburg critical levels for ozone II workshop (Karlsson *et al*., 2003) and subsequent Task Force Meetings of the ICP Vegetation and ICP Modelling and Mapping. For the first time, stomatal flux-based critical levels were included in the Mapping Manual (LRTAP Convention, 2004) together with the benefits of using this method for quantifying the impacts of ozone in the ECE region compared to using AOTX-based critical levels. The text related to the critical levels of SO_2, NO_x and NH_y, and in some cases the values, were also revised in the chapter. The new Mapping Manual was accepted at the 23rd Session of the Working Group on Effects (WGE), September 2004, and can be downloaded from the following web site: www.icpmapping.org

The critical levels and methods described for ozone in chapter 3 were prepared by leading European experts from available knowledge on impacts of ozone on vegetation, and thus represent the current state of knowledge. The chapter provides an in depth description of the critical levels, their scientific bases and how to calculate exceedance. All of the indicators included in the chapter for ozone impacts are based on the accumulation of ozone (either as concentration or stomatal flux) above a predetermined threshold over a specified time period. The latest revision of the Mapping Manual provides concentration-based critical levels that are more closely defined for agricultural crops and (semi-) natural vegetation and substantially revised for forest trees. In addition, a concentration-based critical level has been defined for horticultural crops and a vapour pressure deficit-modified concentration-based short-term critical level for visible injury has been defined for crops. For the first time, stomatal flux-based critical levels are included for wheat, potato and provisionally for beech and birch. A technical report on the scientific basis of the new flux-based critical levels of ozone was prepared for the 23rd session of the WGE (EB.AIR/WG.1/2004/8).

In 2005, the Coordination Centre assisted the local organisers of the most recent critical levels workshop "Critical levels of ozone: further applying and developing the flux-based concept" (Obergurgl, Austria, 15-19 November 2005) and contributed to six background papers at the workshop and submitted four papers to a special issue of Environmental Pollution (see section 2.2.3 for details). In addition, Dr Gina Mills gave a keynote presentation and chaired the plenary sessions regarding the conclusions and recommendations from the three working groups (crops/application of the flux-based models; semi-natural vegetation and forest trees) at the workshop. The proceedings of the workshop will be available soon on the web at http://www.uni-graz.at/ ozone_workshop_obergurgl_2005/ and a technical report (EB.AIR/WG.1/2006/15) has been produced for submission to the 25th session of the WGE (Geneva, 30 August – 1 September 2006). The workshop made recommendations for the revision of chapter 3 of the Mapping Manual and at the 19th ICP Vegetation Task Force Meeting it was decided to include the new text as an annex to the Mapping Manual of which a draft version is presented in Annex 1. Dr Gina Mills coordinated drafting and editing of the text for the annex to the Mapping Manual.

In summary, the Obergurgl Workshop concluded:

- New data collated and compiled after the Gothenburg Workshop in 2002 continues to support the use of the flux-based approach.

- The flux-based approach should be used for risk assessment in integrated assessment modelling for crops and forest trees, and the AOT40-approach should be used for (semi-)natural vegetation.

- A new critical level was proposed for (semi-)natural vegetation communities dominated by perennial species and new data was provided to support the choice of communities that are potentially ozone sensitive for mapping purposes. No new critical levels were proposed for forest trees and crops.

- A simplified flux-modelling approach was proposed for integrated assessment modelling – the details of the parameterisation were discussed further at the ICP Vegetation Task Force Meeting (Caernarfon, January 2006).

2.4 ICP Vegetation experimental programme for ozone (Objective 2)

Each year, the participants of the ICP Vegetation conducted a series of experiments to monitor the effects of ambient ozone pollution on vegetation. The experimental objectives and design were agreed by the participants at the Annual Task Force Meetings. In 2003, 2004 and 2005, the ICP Vegetation ozone monitoring programme was conducted with white clover (*Trifolium repens* cv Regal) and brown knapweed (*Centaurea jacea*), following a standard protocol distributed by CEH Bangor. The aims were:

- To determine the geographical extent of the ozone problem within the UNECE area by identifying areas where the ozone concentration is sufficient to induce ozone-specific injury in a sensitive biotype of white clover and in *Centaurea jacea*;
- To determine the frequency of ozone injury occurring episodes at participating sites and to examine temporal trends;
- To contribute data to a future revision of the short-term critical level for visible injury.

2.4.1 The ICP Vegetation monitoring programme with white clover

Since 1996, the biomonitoring experiment of the ICP Vegetation has comprised of a comparison of the growth and physiology (stomatal conductance, photosynthesis etc.) of two biotypes of white clover (*Trifolium repens* cv Regal) that have been selected for sensitivity (NC-S) and resistance (NC-R) to ozone. The initial aims were to determine the effect of ambient ozone on the biomass relationship between the NC-S and NC-R clover and to determine a dose-response relationship for use in derivation of a critical level for this species. More recently, there has been an increased focus on conditions required to induce visible injury symptoms on the NC-S biotype, with many sites assessing plants on a weekly basis. Participants have also been performing stomatal conductance measurements according to a standard Protocol to develop a flux-effect relationship to use in conjunction with ambient ozone concentration data (see Section 3.2). In addition to clover, several participants have also been exposing plants of brown knapweed (*Centaurea jacea*) from ozone sensitive parent plants to monitor the effects of ambient ozone on a (semi-)natural vegetation species (see Section 2.4.3 and 2.4.4).

The experiments were coordinated from CEH-Bangor, including data collection, quality assurance checks and statistical analysis. Stock plants of each biotype were maintained by CEH Bangor and during the period March to May in each year, cuttings of either the sensitive biotype or both biotypes were sent to participants (with some participants having more than one biomonitoring site), with the timing being dependant on the growing season and occurrence of ozone episodes in each of the participating countries. In addition, fibre-glass wick material was sent to each participant to ensure that the same watering regime was used across the European sites. The clover experiment was conducted according to a standard experimental protocol (LRTAP Convention 2003, 2004, 2005) that was updated at CEH Bangor and distributed to all participants in early April. In addition to experimental data on plant growth and physiology, pollutant and climatic data such as atmospheric ozone concentration, air temperature, global radiation, relative humidity, wind speed and direction were collected for each site, added to the existing ICP Vegetation database, quality-checked and further processed.

The year 2003 had generally a hot, dry summer across Europe and ozone concentrations were higher than in both 2004 and 2005. This is illustrated by the mean daily maximum ozone concentrations for each of the 28 day harvest periods, where the percentage of 28 day harvest periods that had mean daily maximum ozone concentrations of >60 ppb was much higher in 2003 than for either 2004 or 2005 (Figure 2.1). However, in each year the long-term critical level for (semi-) natural vegetation and agricultural crops (a three-month AOT40 of 3 ppm h), was exceeded at approximately 80% of sites where ozone was continuously monitored (data for selected sites is presented in Figure 2.2). In 2005, an AOT40 of 10 ppm h was exceeded at 25% of sites. In 2003 this was exceeded at 60% of sites and at Neochorouda (Greece), the AOT40 during the clover growing period was 30 ppm h, which is ten times the current critical level for (semi-) natural vegetation and agricultural crops.

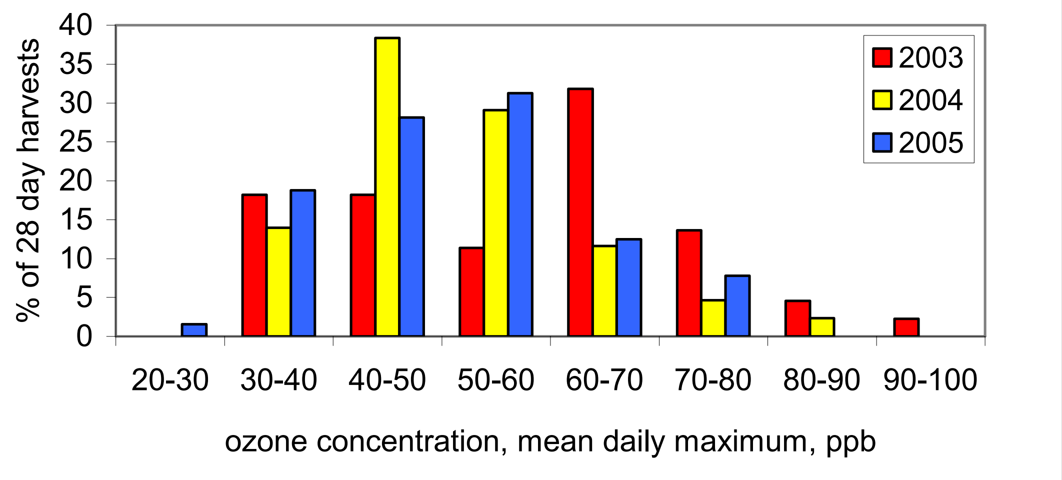

Figure 2.1 A comparison of the 28 day mean daily maximum ozone concentration at selected ICP Vegetation sites in 2003, 2004 and 2005.

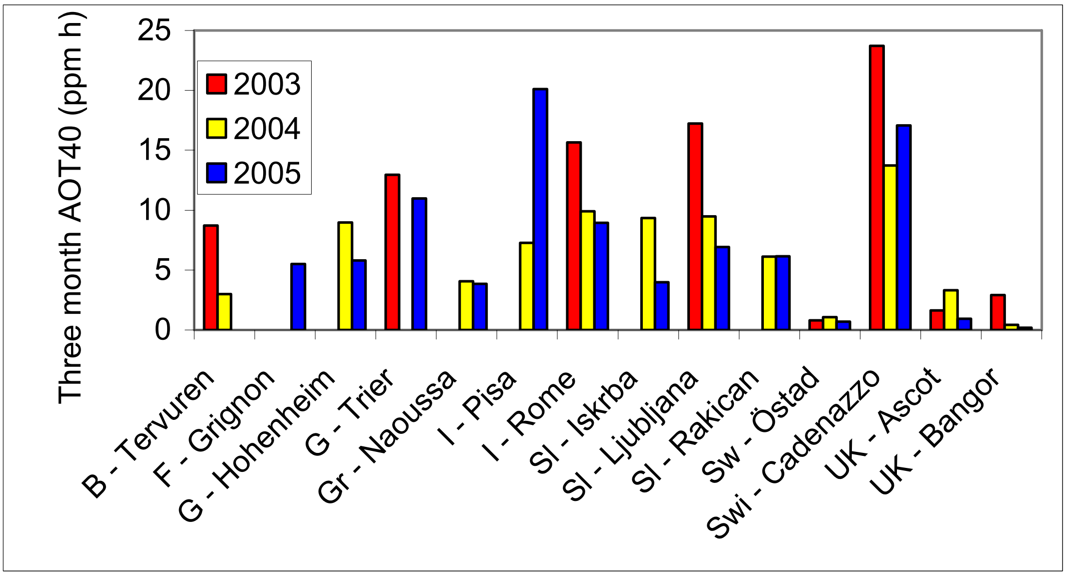

Figure 2.2 A comparison of three month AOT40 at selected ICP Vegetation sites in 2003, 2004 and 2005.

Figure 2.3 Ozone injury (fine cream coloured flecks) on white clover grown at the CEH Bangor site in July, 2003.

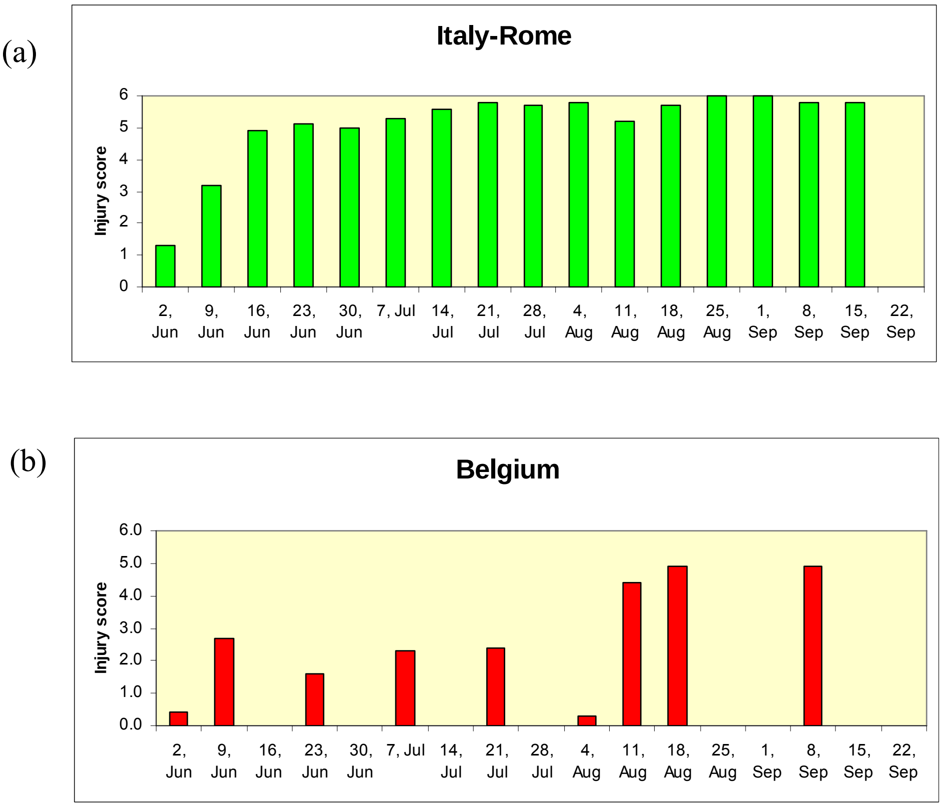

Figure 2.4 Weekly assessments of visible injury on *Trifolium repens* in 2003 at (a) Italy-Rome and (b) Belgium-Tervuren. (Injury score: 1= <1% of leaves affected, 2= 1%-5%, 3= 5%-25%, 4= 25%-50%, 5= 50%-90%, 6= 90%-100%)

A major component of the biomonitoring work with *Trifolium repens* in 2003 to 2005 was to monitor the incidences of visible injury by weekly recordings. The amount of visible injury recorded can be quite extensive – 50-90% of leaves affected in one week when there had been no injury present the previous week. An example of visible injury on white clover at CEH Bangor is shown in Figure 2.3. Visible injury due to ozone was observed almost every week in each of the years 2003 to 2005 during the main exposure period at sites including Rome (Italy, Figure 2.4a), Ljubljana (Slovenia) and Östad (Sweden). At other sites e.g. Belgium-Tervuren, visible injury was observed intermittently throughout the growing seasons (Figure 2.4b) reflecting the episodic nature of ambient ozone. Visible injury was also recorded at sites where the critical level of ozone for yield reduction was not exceeded, including at both UK-Bangor and Spain-EbroDelta in 2003, where there were moderate injury scores (5-25% of leaves affected) at two or more harvests. In contrast, some other sites showed less injury than expected, e.g. Slovenia-Ljubljana and Greece-Pilion (both in 2003), which both had 3-month AOT40s exceeding 15 ppm.h, but maximum injury scores of 3.1 and 4.2 respectively (equivalent to between approximately 5-25% and 25-50% of leaves being affected). Although ozone concentrations at individual sites were lower in 2004 and 2005 than in 2003, visible injury was still recorded widely across sites in Europe, but at a lower intensity than in 2003. An example is shown for the last week in July, where the ozone injury score on *Trifolium repens* recorded at ICP Vegetation sites during that week are shown for 2003, 2004 and 2005 (Figure 2.5). Although not all sites recorded the occurrence of visible injury in each of these years, the trend for reduced injury score across sites in 2004 and 2005 compared to 2003 can be seen.

Only a few sites were still recording development of ozone injury into October, but of these, Spain-EbroDelta and Italy-Rome showed new ozone injury developing on the leaves of the clover during this time in 2004. In both cases, the ozone concentrations were not particularly high (daily maximums in the range 40 – 60 ppb), but these small peaks persisted for several days. In addition, the night-time ozone concentrations at Spain-EbroDelta remained high (>30ppb) for some of these nights. An Autumn/Winter exposure of NC-S clover is currently taking place in Italy-Naples to see whether visible injury will occur over the winter months at this site.

The injury scores from individual assessment dates in 2004 were plotted against vapour pressure deficit modified (VPD) AOT30 ($AOT30_{VPD}$) over the previous 8 days, the parameter used to describe the revised short-term critical level for ozone (0.16 ppm.h) in the Mapping Manual (LRTAP Convention, 2004). However, no clear relationship was found between the injury score and $AOT30_{VPD}$ in daylight hours (Figure 2.6). For comparison with the short-term critical level, injury score was also converted into percent injury and plotted together with the function used to define the short-term critical level (Figure 2.7). The majority of the points fell below this line, largely reflecting the very low amounts of injury detected at those sites that could provide VPD data. The two points just above/close to the short-term critical level function are from Spain-Ebro Delta and Sweden-Ostad. In both cases the AOT30 over the previous 8 days was fairly low (367 and 27 ppb.h respectively), but due to the low VPD in each case, uptake of the ambient ozone into the leaf was likely to have been high. Thus, it wasn't possible to validate the new short-term critical level for visible injury as the vast majority of the sites providing adequate quality ozone and VPD data had less than 10% ozone injury at any individual assessment.

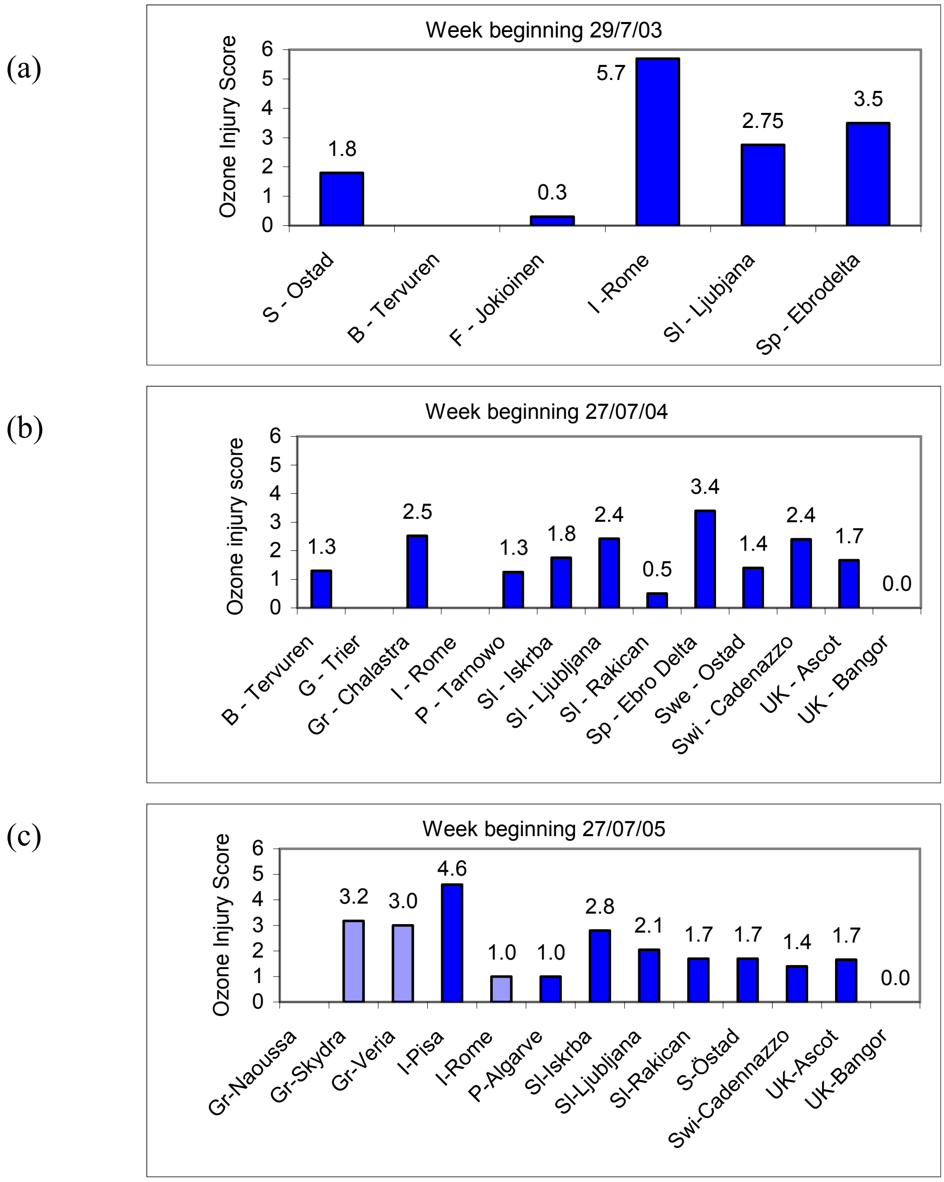

Figure 2.5 Ozone visible injury score on *Trifolium repens* recorded at ICP Vegetation sites during the last week in July in (a) 2003, (b) 2004 and (c) 2005. (Injury score: 1= <1% of leaves affected, 2= 1%-5%, 3= 5%-25%, 4= 25%-50%, 5= 50%-90%, 6= 90%-100%).

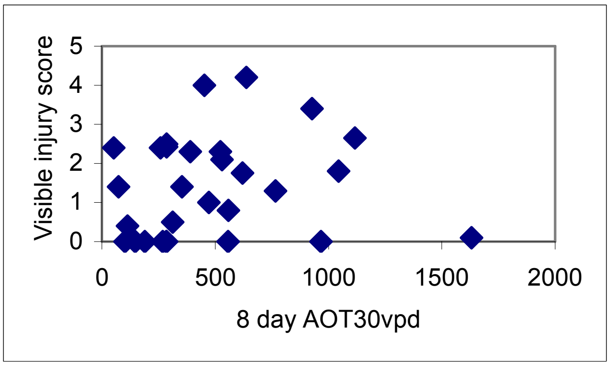

Figure 2.6 Ozone visible injury score plotted against the VPD-modified AOT30 for the 8 days prior to injury assessment.

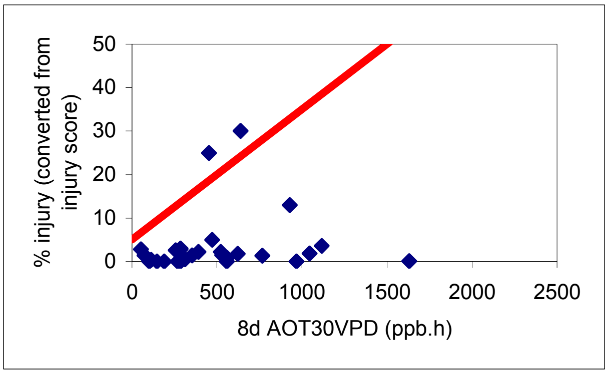

Figure 2.7 Ozone visible injury score converted into percentage injury and plotted together with the function (red line) used to establish the short-term critical level for ozone injury, as described in Section 3.2.4 of the Mapping Manual (LRTAP Convention, 2004).

Several sites monitored the NC-S/NC-R biomass ratio. The decrease in biomass ratio with increasing ozone exposure from the 2003, 2004 and 2005 data fits the same trend as data from 1996 to 2002 (Figure 8) and has increased confidence in the relationship as many of the new points are from the higher AOT40 region of the curve. The r^2 of the trendline for the combined dataset is 0.44. There was no significant difference in the slope of the trendline when the 2003-2005 data was added, compared to the trendline for 1996-2002. Approximately 40% of the datapoints showed NC-S biomass reductions of >10% compared to the NC-R biotype over a period of 3 months. Approximately 15% of the datapoints showed NC-S biomass reductions of >25% over 3 months. Although many of the datapoints that show large reductions in NC-S biomass compared to NC-R are from sites where the three-month AOT40 is high, this is not always the case. For example, clover plants at Italy-Pisa in 2004 showed a three-month biomass ratio of 0.65 with an AOT40 of 7.26 ppm.h. In Germany-TrierCity in 1999 the NC-S/NC-R biomass ratio was 0.89 when the AOT40 was only 1.36 ppm.h, which is below the critical level for ozone.

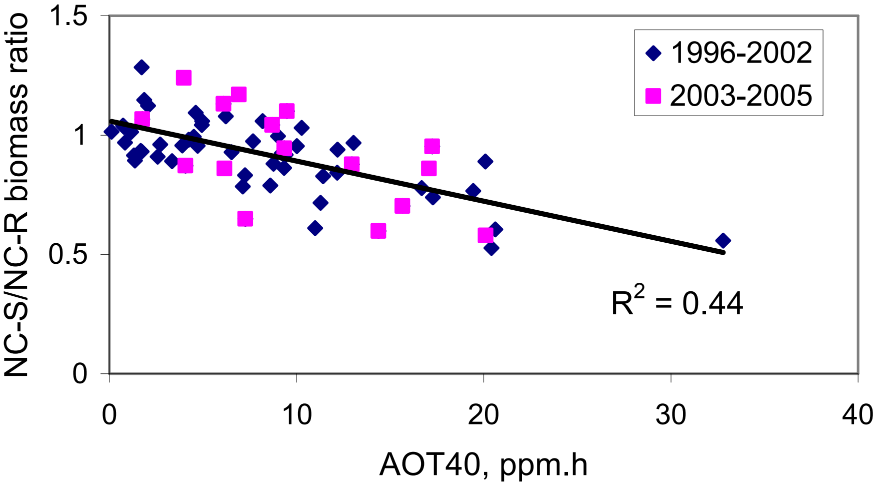

Figure 2.8 The relationship between three-month AOT40 and NC-S/NC-R biomass ratio of *Trifolium repens* from sites across Europe from 1996 to 2005.

A carry-over effect of ozone exposure on the clover plants has been identified. There was significant improvement to the relationship between ozone exposure and NC-S/NC-R biomass ratio when the AOT40 over time periods prior to the start of the current harvest interval were taken into account. Figure 2.9a-d illustrates how the r^2 value increases when the carry-over effect is taken into account from 0.3 (AOT40 in current month) to 0.61 (AOT40 in previous month) to 0.69 (AOT40 in previous 2 months), with a small decrease in r^2 back to 0.61 for previous three months, although the data availability is smaller for this category. These new results help to explain the previously puzzling results that AOT40-biomass response functions for this data for 28d periods have far more scatter (r^2 = 0.2 – 0.3) than those accumulated over three month periods (r^2 = 0.4 – 0.5).

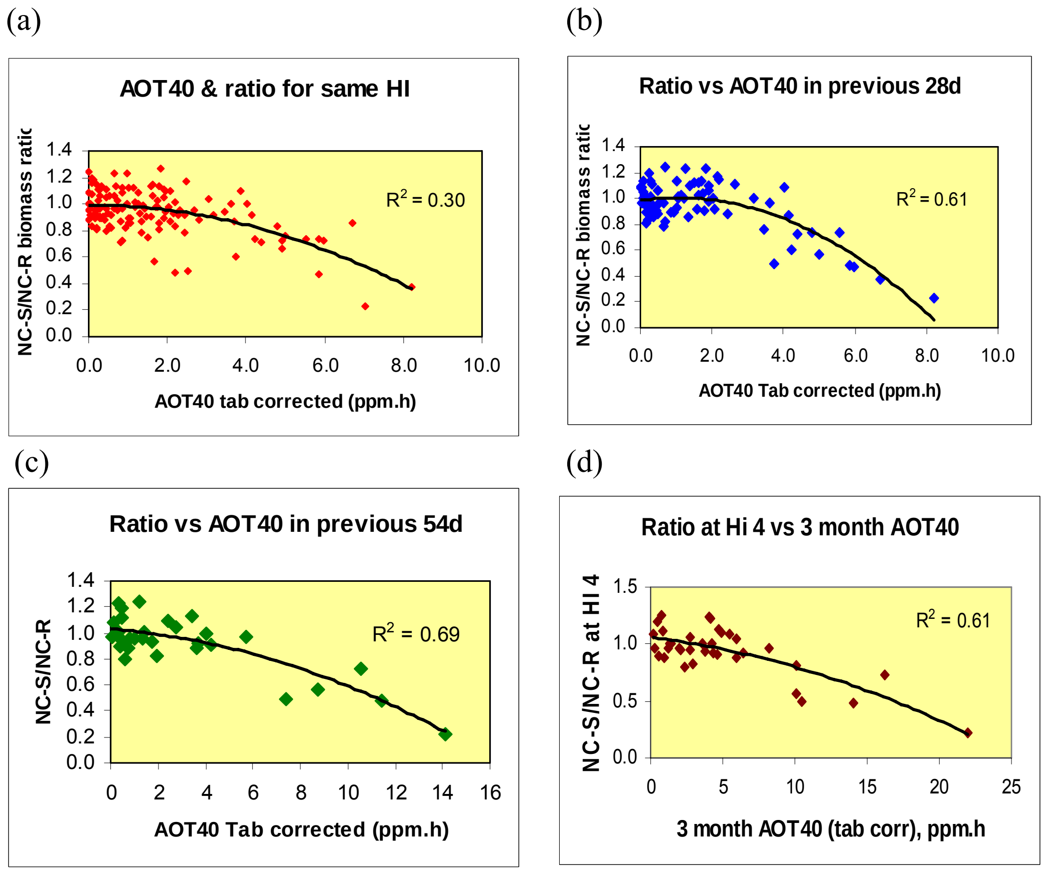

Figure 2.9 NC-S/NC-R biomass ratio in response to AOT40 accumulated over (a) the 28d for that harvest interval (b) the harvest interval before that used to calculate biomass ratio, (c) the current and previous harvest interval and (d) the current and two previous harvest intervals.

2.4.2 Monitoring data with white clover from UK sites

CEH (Bangor)

Clover plants were initially grown at the Pen-y-Ffridd Experimental Research Centre at Bangor from biotypes of the NC-S and NC-R *Trifolium repens* cv Regal. Two batches of plants were grown and placed out in the CEH Bangor ambient air experimental plot at Abergwyngregyn (8 miles east of Bangor) 14 days apart to ensure that there was always a mature canopy present. Commencing at the first 28d harvest for each batch of plants, ozone damage was assessed weekly. On the day of harvesting, the plants were assessed before the harvest. Assessments were carried out conforming to the ICP Vegetation Protocol. There were four harvests carried out each year. In 2004, additional plants of the NC-R biotype were grown in standardised soil received from Dr Ludwig De Temmerman (Belgium) and leaf samples of these plants were sent to Dr De Temmerman for analysis of heavy metal content (see Section 5.4).

Environmental measurements were made using a computer controlled monitoring system. The following physical aspects of the climate were continuously measured at the clover plot: air temperature, relative humidity, global radiation, rainfall, wind speed and direction at a height of 1 and 3 m. In addition continuous measurements of ozone were made at the experimental site at heights of 1 and 3 m.

Visible injury due to ozone was observed on the leaves of the sensitive biotype of *Trifolium repens* following two significant ozone episodes that occurred in July and August 2003. The mean ozone injury scores on the two batches of plants are presented in Table 2.2. These results show that the highest injury score of 3.6 was recorded for Batch A on the 20/08/03 indicating ozone injury was present on approximately 40% of the leaves. No visible injury due to ozone was observed on the *Trifolium repens* plants in either 2004 or 2005.

Table 2.2 The mean weekly ozone injury on plants of batches A (placed at site on 28/05/03) and B (placed at site at 11/06/03) in 2003, starting on the date at which ozone injury was first detected and finishing when the last ozone injury was detected.

Date	**Mean weekly Injury score**	
	Batch A	Batch B
17/7/03	2.5	3.0
23/7/03	3.3	3.0
30/7/03	1.4	1.7
6/8/03	0.3	1.1
13/8/03	3.5	1.0
20/8/03	3.6	2.4

In addition to assessments of visible injury, at each assessment in 2003 and 2004 the following canopy characteristics were determined to provide additional information needed for flux-effect modelling (Work Package 2, Objective 4):

1. Maximum canopy height measured from soil level.
2. Maximum canopy diameter and diameter perpendicular to that.
3. A photograph was taken from above and from the side using a ruler as a scale.

These measurements were used to calculate the volume of the clover canopy and results are shown in Figure 2.10. To assess the development of leaves within the canopy and distribution of leaf age classes, individual leaves were labelled as they emerged and their contribution to the canopy at intervals throughout one 28d harvest period was assessed. Data from each of these studies has been incorporated into the clover canopy flux model (see Section 3.2).

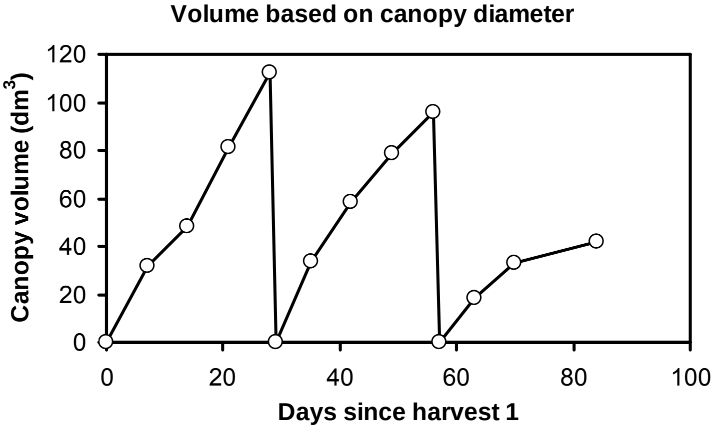

Figure 2.10 The response function of the volume of the white clover canopy with time. The canopy was harvested at 28 days intervals. The canopy height and the mean of the maximum canopy diameter and diameter perpendicular to that were used to calculate half the volume of a sphere (i.e. half of 4/3 π r^3).

Stomatal conductance measurements were made according to the ICP Vegetation protocol in 2003 and 2004. In addition the stomatal conductance of leaves at different positions of the plant was made: inside the canopy, outer edge of the canopy and at the top of the canopy, in addition to 'leaf 4' – the standard measured leaf for measuring stomatal conductance as part of the ICP Vegetation flux measurement work. An example of the variation in stomatal conductance in different regions of the canopy is shown in Figure 2.11, which shows that conductance was lowest in the centre of the canopy and greatest for the uppermost leaves and leaves from the canopy edge.

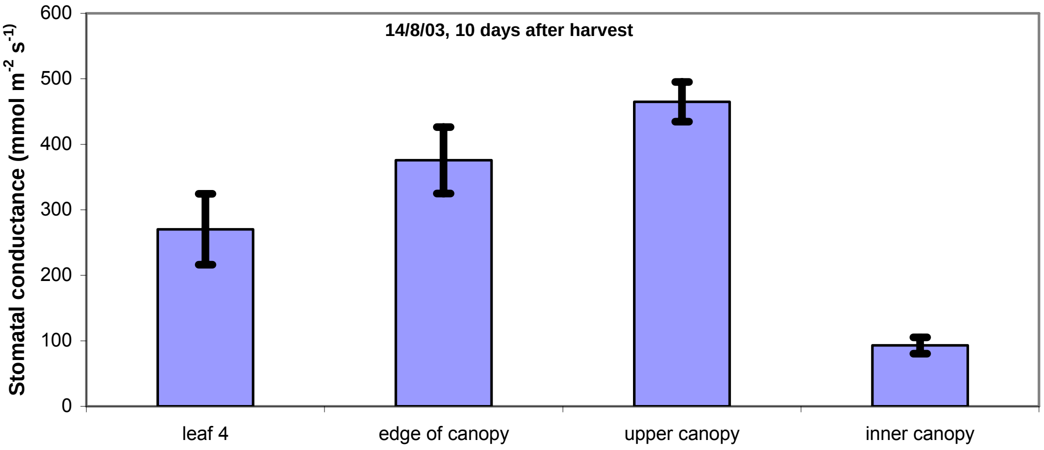

Figure 2.11 Stomatal conductance at different positions in the plant canopy. This example is from 14th August 2003, 10 days after harvest.

Imperial College (Ascot)

NC-S (sensitive) biotypes of white clover were grown in 2003, 2004 and 2005 according to the Protocol. Two batches of plants (six replicates per batch) were grown two weeks apart, and transplanted into 15 cm diameter pots and exposed to ambient air. Batches were assessed for visible injury weekly and above ground plant material was harvested at 28 day intervals over a four month period, providing four harvests per batch, per season. In 2003 and 2004, ozone injury on leaves was present from the middle of June till the end of September with mean injury scores at harvests ranging from 0.5 (early July) to 2.3 (end of August). Although visible injury was recorded during most weeks during the period middle of July to end of August in 2005, the injury scores recorded were lower than in 2004 and 2003. Visible injury scores during July and August for 2003, 2004 and 2005 are shown in Table 2.3. Mean daily maximum ozone concentrations for July and August at the UK-Ascot site are shown in Figure 2.12. The data for 2004 is not presented as some of this data originated from a different source. A large ozone episode, lasting for many days, occurred at the beginning of August in 2003. However, this did not result in a noticeable increase in injury score on the NC-S clover (Table 2.3) during this time. In contrast, during 2005 an ozone episode occurred during early-mid July (Figure 2.12) and increases in the mean visible injury score of NC-S clover during this period can be seen (Table 2.3).

Table 2.3 Mean injury score on NC-S clover during July and August in 2003, 2004 and 2005 at Ascot.

Week Beginning	2003	2004	2005
1 July	1.8	0.8	1.3
8 July	1.8	1.3	2.0
15 July	2.2	0.7	2.2
22 July	1.2	1.7	1.8
29 July	1.3	2.3	1.7
5 August	1.3	1.7	1.3
12 August	1.6	1.7	1.2
19 August	1.3	2.0	0.5
26 August	1.3	0.7	0.5

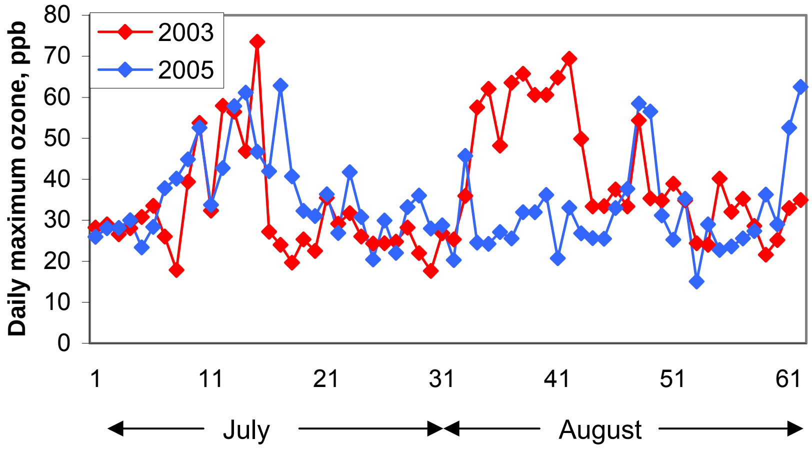

Figure 2.12 Daily maximum ozone concentrations (ppb) during July and August at the UK-Ascot site.

2.4.3 The ICP Vegetation monitoring programme with brown knapweed (Centaurea jacea)

Whilst there is considerable evidence for effects of ozone on a wide variety of crop plants, including clover, relatively few native plant species have been investigated. Existing evidence suggests that many species characteristic of (semi-) natural plant communities are at least as sensitive to ozone as the major crop plants. *Centaurea jacea* has been identified as one of several native species which is relatively sensitive to ozone, exhibiting characteristic symptoms of ozone injury following exposure. *Centaurea jacea* has been used as a model species to evaluate the relationship between ozone exposure and effects on the performance and injury symptoms of native plant species, along a naturally existing gradient of ozone exposure from the north to the south of Europe.

Seeds were collected from an ozone sensitive and ozone resistant population of *Centaurea jacea* by participants from Switzerland, led by Prof. Jürg Fuhrer. The seeds and wick material were distributed by the ICP Vegetation Coordination Centre to participants, who established and grew *Centaurea jacea* in glasshouses according to the experimental protocol (LRTAP Convention 2003, 2004, 2005), which included additional information provided by the participants from Switzerland. The plants were transferred to the field sites and exposed to ambient ozone concentrations when they had about 15 true leaves. From then onwards leaf injury due to ozone was recorded weekly and photographs were taken to document the growth stage and extent of injury (LRTAP Convention 2003, 2004, 2005). An example of a leaf showing ozone injury symptoms in Switzerland is shown in Figure 2.13. The extent of ozone injury on the stem leaves was recorded separately to the injury on the rosette leaves. The plants were harvested after eight or more weeks of exposure in the field.

Figure 2.13 Ozone injury on *Centaurea jacea*.

The number of sites participating in the *Centaurea* experiment ranged from twelve to seventeen between 2003 and 2005 (not all data is available yet for 2005, some participates receive pollutant data from local Governments and this can cause a delay in processing results at these sites). Ozone-specific visible injury has been observed at approximately two thirds of these sites. Whether or not ozone injury was observed on *Centaurea* in each year is indicated in Table 2.4. Leaves of *Centaurea jacea* plants do not get replaced at the same rate as those of *Trifolium repens.* This has the consequence that although the overall effects of ambient ozone exposure over a season can be identified, it is more difficult to identify individual episodes of ambient ozone that have caused ozone injury. The development of ozone injury over the course of the exposure period in 2004 is shown for Austria, Sweden, Switzerland-Cadenazzo and Italy-Rome for both rosette and stem leaves (Figure 2.14). There appears to be no consistent difference between the extent of ozone injury on stem and rosette leaves. Differences in the extent of injury at different sites can clearly be identified, for example, there was far more visible injury in Switzerland and Austria than in Sweden, however the effects of individual ozone episodes cannot be seen.

The ozone exposure to the *Centaurea jacea* plants at these participating sites in 2003 is shown in Table 4 and ranged from an AOT40 of 0.26 ppm.h (Finland) to 33.9 ppm.h (Switzerland). The 12-hour mean ozone concentration is also shown to give an indication of pollution levels at each site, as not all plants in this study were exposed to ambient air for the same length of time and this consequently affects the AOT40.

Table 2.4 Presence or absence of ozone injury on *Centaurea jacea* at sites across Europe in 2003, 2004 and 2005.

COUNTRY – SITE	INJURY IN 2003	INJURY IN 2004	INJURY IN 2005
Austria	Yes	Yes	
Finland	No		
France – Grignon			Yes
Germany – Hohenheim	Yes		
Germany – Trier	No		
Greece – Kalamata	Yes		
Greece – Naoussa			Yes
Ireland – Carlow	No	No	
Italy – Rome		Yes	
Slovenia – Velenje	Yes	Yes	Yes
Slovenia – Zavodnje		Yes	
Spain – EbroDelta	Yes	Yes	
Sweden	No	Yes	
Switzerland	Yes	Yes	Yes
UK – Ascot	Yes	Yes	Yes
UK – Bangor	No	No	No

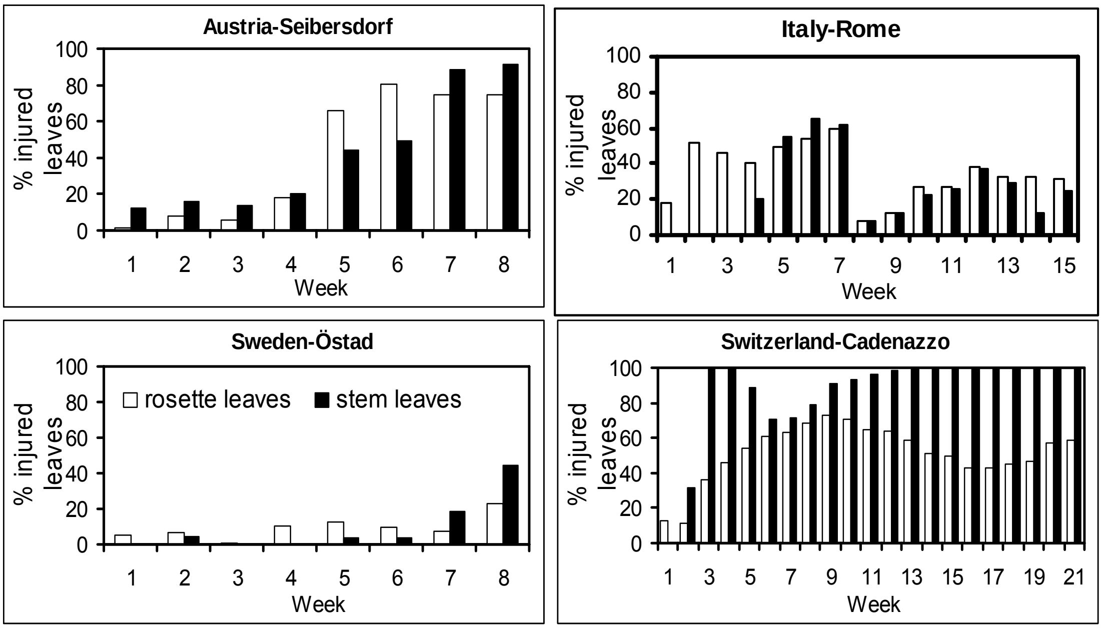

Figure 2.14 Development of ozone injury on rosette (white bars) and stem leaves (black bars) of *Centaurea jacea* at selected sites in Europe in 2004.

Table 2.4 Ambient ozone exposure of *Centaurea* at sites across Europe in the summer of 2003.

COUNTRY – SITE	12H MEAN	AOT40 (PPM H)
Austria – Seibersdorf	48.7	10.1
Finland – Jokioinen	27.1	0.3
Germany – Hohenheim	50.5	12.5
Germany – Trier	40.6	17.6
Greece – Kalamata	54.3	15.8
Ireland – Carlow	30.0	1.2
Spain – EbroDelta	38.0	2.5
Sweden – Östad	33.1	0.8
Switzerland – Cadenazzo	63.2	33.9
UK – Ascot	23.1	1.2
UK – Bangor	19.8	0.3

Stomatal conductance measurements on *Centaurea jacea* have been carried out at some sites. This data has been used to parameterise a stomatal conductance model for *Centaurea jacea* and this is described in Section 4.2. In addition, some participants of the ICP Vegetation have also carried out investigations of the interaction between ozone pollution and nutrient nitrogen by supplying different amounts of nitrogen to the plants during the exposure to ambient ozone (Section 9.5).

2.4.4 Centaurea experiments at UK sites

CEH (Bangor)
No visible leaf injury due to ozone was observed on *Centaurea jacea* plants at Bangor in any year. An investigation of whether nutrient nitrogen influenced the response of *Centaurea jacea* to ambient ozone concentrations was carried out in 2004. To provide additional supporting data for the ICP Vegetation programme, a small number of *Centaurea jacea* plants were exposed to ozone in the solardomes at CEH Bangor together with the upland vegetation funded under Defra contract EPG 1/3/201. These experiments provided the opportunity to take good quality photographs of the development of ozone injury on this species which have been incorporated into the 2005 and 2006 experimental protocol.

Imperial College (Ascot)
Dr Sally Power and Ms Emma Green from Imperial College contributed to Contract EPG 1/3/205 by growing *Centaurea jacea* in ambient air. In 2003 and 2004 plants were supplied with different levels of nitrogen fertiliser to represent three nitrogen deposition rates. At their field station at Silwood Park, two sequential batches of (sensitive) *Centaurea* seeds were germinated, 2-3 months apart, following surface sterilisation and pre-chilling. Seedlings were transferred into 30 cm diameter pots at the 8-10 leaf stage. Three nitrogen treatments were used in each batch: control (0 kg N ha^{-1}), low (30 kg N ha^{-1}) and high (80 kg N ha^{-1}) N treatments, with 8 replicates of each for each batch. Batch 1 was exposed to ambient air from June-July; batch 2 from September-October. Visible injury was assessed weekly, and plants were harvested, and the degree of insect injury and extent of flowering recorded, after 2 months. Ozone concentrations and climatic data were recorded on site, and submitted to the Coordination Centre. Leaf samples from each nitrogen treatment were also sent to CEH Bangor for C:N analysis.

A small amount of visible injury was observed in both 2003 and 2004, although it is possible that this was caused by high light conditions rather than ozone. There was insufficient evidence to verify whether or not the extent of ozone injury was influenced by the availability of nutrient nitrogen.

Despite large differences in nitrogen availability in the three N treatments, no significant effect of N was seen in terms of plant performance (biomass) or the extent of insect injury in either batch (Figure 2.15). Lower dry weight measurements, and a greater degree of insect injury in the second exposure batch confirm less favourable growth conditions later in the season. No flowering was recorded in the second batch, but there was an indication of a small increase in flowering at the highest N treatment in the first batch of plants - an average of 31 flowers were present in the high N treatment, as opposed to 18 in the low N and 23 in the control treatments (data not presented). In the absence of a growth response to N, no interactions between ozone and N deposition could be tested. Data from UK-Ascot has been combined with the datasets of other participants of the ICP Vegetation. This is presented in Section 9.5.

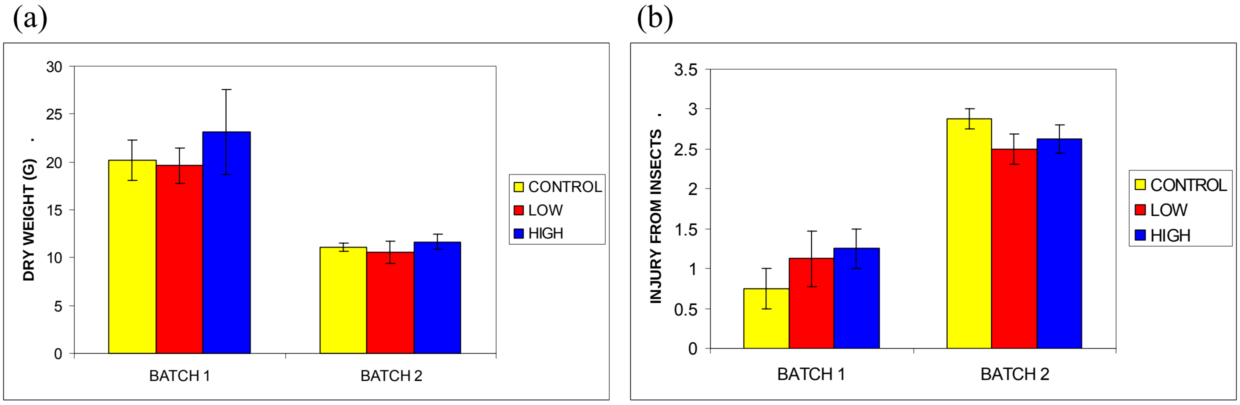

Figure 2.15 (a) Biomass of *Centaurea jacea* and (b) insect injury in relation to N treatment.

2.4.5 Conclusions

The Critical Level for ozone was exceeded at approximately 80% of ICP Vegetation sites in each year. Visible injury due to ozone was widespread across Europe on both *Trifolium repens* and *Centaurea jacea* in 2003, 2004 and 2005. During the years of 2004 and 2005, when ozone concentrations were lower than in 2003, visible injury on *Trifolium repens* was observed at a similar frequency, but a lower intensity. Further datapoints showing the relationship between the NC-S/NC-R biomass ratio of *Trifolium repens* in response to AOT40 increased confidence in this relationship because many of the new points were from sites in Europe with higher AOT40 values.

2.5 Organise the annual Task Force Meeting and attending meetings (Objective 3)

In 2004, 2005 and 2006, the ICP Vegetation organised very successful Task Force Meetings in collaboration with the local organisers in Kalamata (Greece), Almeria (Spain) and Caernarfon (UK), respectively. The meetings were attended by 50 – 60 participants from 15 – 19 Parties to the Convention, together with the Chairman and Secretary of the Working Group on Effects (WGE), representatives of other ICPs (Forests and Modelling and Mapping), EMEP/MSC-West, and representatives of each of the sub-contractors for contract EPG 1/3/205.

The results from the ICP Vegetation were reported to the participants at the Task Force Meetings and minutes of the meeting were distributed to all ICP Vegetation participants, Defra and WGE. CEH Bangor's contribution to the meeting was to collaborate with the local organisers throughout, send out invitations, collate information on participant's requirements, organise the scientific programme, chair the meetings as well as the ozone and heavy metals sub-groups, present oral papers and posters and to write and distribute the minutes (For example, the minutes of the 19th Task Force Meeting (2006) are presented in Annex 2). Sub-contractors contributed further oral papers and posters to the meetings.

In addition, representatives of the ICP Vegetation attended the Task Force Meetings of the ICP Modelling and Mapping and the ICP Forests and one or two relevant conferences/workshops each year (Mills *et al*., 2004; Mills *et al*., 2005). The Chair of the ICP

Vegetation attended annual WGE meetings as described in Section 2.2.2. In 2005 sub-contractors attended the 7th Acid Rain Conference (June 2005, Prague, Czech Republic), where the Coordination Centre presented a poster and contributed to two WGE posters. The Coordination Centre and sub-contractors attended the LRTAP Convention Ozone Critical Levels workshop (November 2005, Obergurgl, Austria; see section 2.2.3. for details on contributions) and kick-off meeting for a new EU Network of Excellence on ozone and vegetation (February 2006, Ispra, Italy).

3. WORKPACKAGE 2: OZONE FLUX-EFFECT MODELS FOR WHITE CLOVER

3.1 Introduction

In the last two years, stomatal-flux-based critical levels have been included in the revised Mapping Manual for wheat and potato (LRTAP Convention, 2004 – see section 2.2.3). Although these two crops are very important in Europe, there remains the need to expand the range of crops for which flux-effect relationships exist. The work conducted in this package attempts to do this, and falls into two areas. Firstly, flux-effect relationships are being developed at SEI-York for white clover using data from the ICP Vegetation ambient air experiments (described in Section 2.4.1) and using inputs from measurements at CEH Bangor to derive whole canopy flux models (see Section 2.4.2). Secondly, the literature on effects of ozone on crops other than wheat and potato has been reviewed at SEI-York together with data on stomatal conductance-environmental parameter relationships for these crops, with the aim of widening the range of crops for which flux-effect relationships can be derived.

The aims for this work package were:

- To further parameterize and validate ozone flux-effect models for white clover;
- Further parameterisation of flux models for crop species other than wheat, clover and potato.

3.2 Ozone flux-effect models for white clover (Objective 4)

3.2.1 Introduction

The stomatal flux - yield-response functions described in the Mapping Manual (LRTAP Convention, 2004) have been derived from ozone exposure experiments conducted in open-top chambers, mainly during the 1980s and 1990s. The disadvantage of using this data is that wind speed is constant inside OTCs and other climatic factors are modified to a certain extent (e.g. reduced rainfall usually supplemented with irrigation, 1-2 °C rise in temperature, Sanders et al, 1991). In contrast, the ambient air experiments of the ICP Vegetation described under Work Package 1 (Section 2.4.1) provide a unique database for developing flux-based critical levels. These data describe the responses of “real plants” to “real ozone episodes” in the wide range of climatic and pollutant conditions experienced in Europe, without the confounding effect of any chamber-based ozone exposure system. Stomatal conductance measurements were collected at nine sites during the period 1998-2001 for a range of climatic and ozone conditions; data from the 1998 and 1999 seasons were used under the previous contract to develop a stomatal conductance model based on the Emberson *et al.* (2001) approach. A flux-effect model for biomass reductions was also developed under the previous contract based on flux estimates to an individual leaf, but further refinements were required before the function could be used to map biomass reductions and incidences of ozone injury across Europe. During this contract, effort has concentrated on up-scaling from a single leaf flux model to a whole canopy flux model for the ICP Vegetation white clover.

3.2.2 Development of a whole canopy clover flux model

The whole canopy clover flux model has now been developed and estimates average canopy stomatal conductance (G_{sto}). It is based on the original leaf level multiplicative stomatal conductance (g_s) model (see Final Report of Contract EPG 1/3/170) and determines the variation in g_s with environmental variables. Scaling from the leaf to the canopy level is achieved on consideration of i) the penetration of irradiance into the canopy estimated using a canopy extinction algorithm; ii) the LAI of the canopy and; iii) the fraction of leaf age populations present in the canopy throughout the harvest period and their respective stomatal conductance g_s. The following text summarises the model formulations used to incorporate these individual components for the modelling of whole canopy stomatal ozone flux.

i) Canopy irradiance

The estimation of the penetration of irradiance into the canopy requires knowledge of the solar elevation (which is a function of time of day (in relation to solar noon), calendar day and latitude). Solar elevation was calculated according to standard principles of solar geometry as described in Jones (1992) and Campbell and Norman (1998). The fraction of the PAR received at the top of the canopy that is direct ($pPAR_{dir}$) and diffuse ($pPAR_{diff}$) must also be determined and this was calculated based on atmospheric principals according to the altitude of the site and the formulations of Weiss and Norman (1985). The potential total PAR ($pPAR_{total}$) is then simply the sum of the diffuse and direct components and this can be compared to the actual total PAR (PAR_{total}) that is measured at each site. The actual PAR_{dir} and PAR_{diff} can then be calculated by multiplying the respective direct and diffuse fractions with the actual total PAR (PAR_{total}).

Estimations of the diffuse and direct fractions are necessary to calculate the PAR incident on the sunlit and shaded portions of the canopy which are estimated as follows using algorithms to estimate irradiance penetration into the canopy. The sunlit leaf area (LAI_{sun}) and shaded portions of the canopy were calculated as follows:

$$LAI_{sun} = [1 - \exp(-0.5 * LAI / \sin\beta)] * 2 \sin\beta$$

$$LAI_{shade} = LAI - LAI_{sun}$$

PAR_{sun} and PAR_{shade}, which are dependant on the mean angle between leaves and the sun, is calculated using a modified "big leaf" version of the canopy radiation transfer model of Zhang *et al.* (2001).

The mean canopy leaf stomatal conductance determined by irradiance was calculated for sunlit ($F_{light}sun$) and shaded ($F_{light}shade$) leaves and weighted according to the fraction of sunlit and shaded leaf area and summed to give the total irradiance-dependant canopy conductance (F_{light}). This method has been simplified so that rather than integrating F_{light} over the canopy a "big leaf" approach has been employed. This means that F_{light} will be underestimated since the model does not allow for the variability in direct and diffuse irradiance within the canopy. However, since these parameters vary only slightly the model under-estimations are relatively small:

$F_{light}sun = [1 - \exp(-\alpha * PAR_{sun})]$

$F_{light}shade = [1 - \exp(-\alpha * PAR_{shade})]$

$F_{light} = F_{light}sun * LAI_{sun} / LAI + F_{light}shade * LAI_{shade} / LAI$

The above calculations require that leaf area index (LAI) is known. This has not been specifically measured on site so the assumptions described below have been used to attempt to derive the best estimate for the locations investigated.

ii) Leaf Area Index (LAI)

The previous calculations have shown how LAI is integral to estimating the irradiance penetration within the canopy and hence average canopy stomatal conductance ($\overline{G}$sto). Ideally, LAI would have been measured at each of the site locations since the evolution and final LAI value (LAI_{max}) is likely to be determined by local conditions (primarily climate). LAI development has not been measured at each site, so this has been estimated for each growth period at each site.

To date, two data sources have been identified that have observed LAI over the course of the growing season, from ICP Vegetation participants from Giessen, Germany collected by Gavriilidou *et al.* (2002) which provide three years of LAI measurements for three different harvest periods within each year and from Tervuren, Belgium, collected by De Temmerman *et al.* (pers. comm.) where LAI was measured for a single harvest period. These LAI observations are shown in Figure 3.1, the measurements have been standardised to allow the profile of the LAI over the 28 day period to be determined more easily. From these data, an LAI function has been established (fLAI) which allows estimation of LAI at any point during the harvest period from knowledge of the final LAI value (LAI_{max}). This function follows the formulation proposed by Gavriilidou *et al.* (2002):

$$fLAI = a\,(1 - e^{bx})^{c}$$

Where a, b and c are constants equal to 22.32, -0.0059 and 1.65 respectively. LAI is then simply fLAI multiplied by LAI_{max}. A minimum LAI was also defined to allow for residual LAI after harvesting prior to continuation into the next harvest period. This is currently set to 0.15 LAI_{max}.

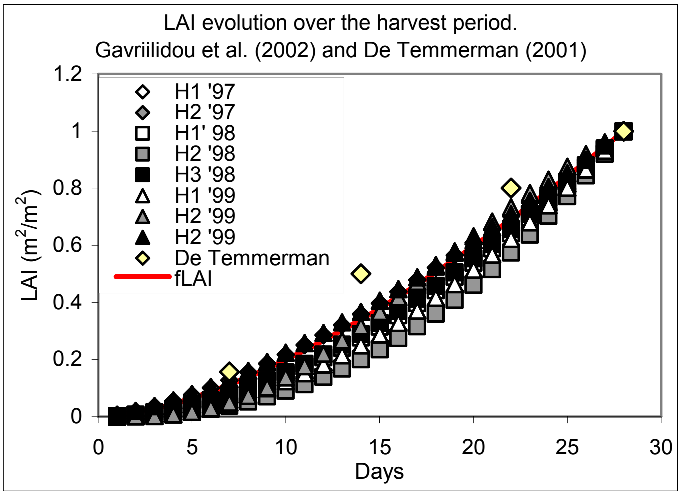

Figure 3.1 Variation of standardised LAI with time over the course of the 28 day harvest period. Data from Gavriilidou *et al*. (2002) shown as black and white symbols where H*x* is the harvest period number and '*x* is the year. fLAI is the function designed to estimate LAI for all sites on availability of the final LAI (LAI_{max}) value.

The LAI_{max} term also needed to be defined for each site. Since no observation data were available this was derived using the relationship between dry weight and LAI (Figure 3.2). This is based on data provided by De Temmerman *et al.* using plants from Belgium-Tervuren, as these provide a more conservative estimate of LAI than those reported by Gavriidilou *et al.* (2002) based on plants growing in Germany. LAI is one important component of phenology driving canopy conductance since LAI varies with the growth stage of the plant. However, as LAI changes, so will the age of the leaves within the canopy. Since leaf conductance varies with leaf age it is important to also incorporate this within the canopy model.

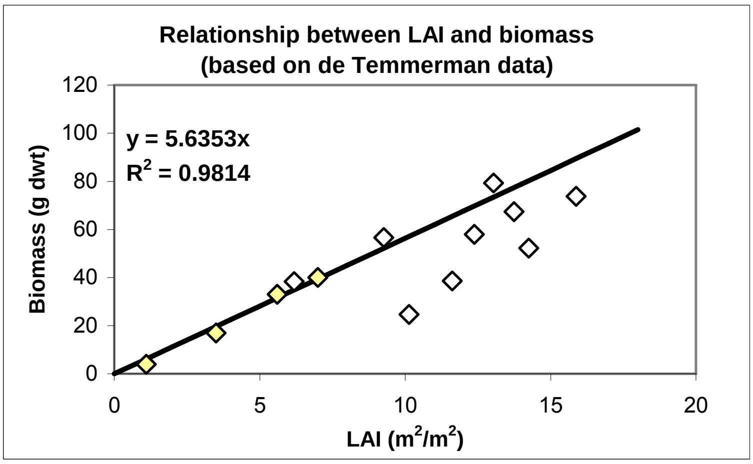

Figure 3.2 Relationship between leaf area index (LAI) and dry weight, g, as determined from data of Gavriilidou *et al*. (2002) and De Temmerman (pers. comm.).

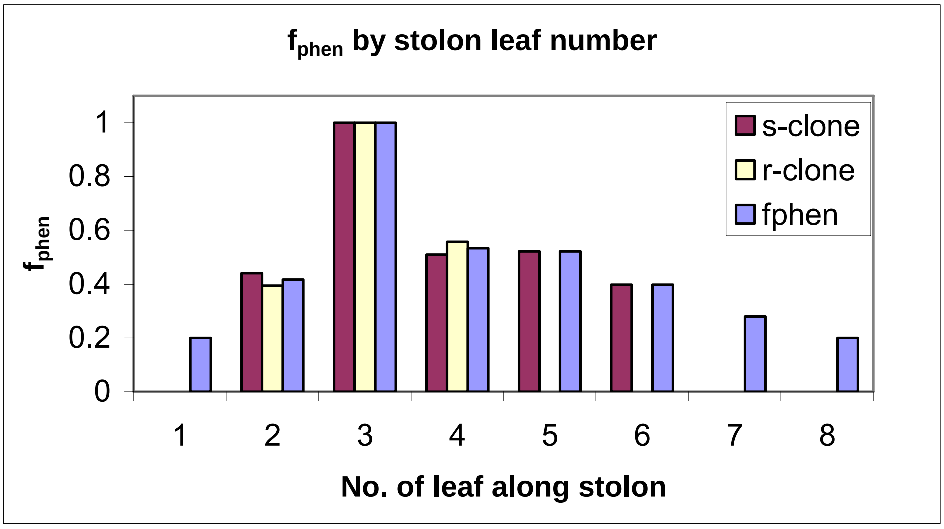

Figure 3.3 Variation in f_{phen} for both the sensitive (s-clone) and resistant (r-clone) with leaf number along a stolon. Data from Werner *et al.* (1998).

iii) Phenology

Evidence of the variation in leaf stomatal conductance (g_{sto}) with leaf age for clover plants was described by Werner *et al.* (1998) for clover plants grown in Trier, Germany. Figure 3.3 shows the variation in f_{phen} (standardised g_{sto}) with leaf number along a stolon. It was assumed that there was no difference between the clones in the g_s relationship with leaf age as suggested by measurements made on leaf numbers 2 to 4. Measurements were only made on leaf numbers 2 to 6, therefore extrapolation of f_{phen} for leaves 1, 7 and 8 has been necessary as up to 8 leaves may develop on a stolon over a 28 day harvest interval. This extrapolation, along with the average s- and r- clone measurement data is represented by f_{phen} in Figure 3.3.

To simplify the modelling it is assumed that each week two new leaves will appear on the stolon with f_{phen} being defined as their average. Data were collected at CEH Bangor, UK describing the leaf age canopy fraction over the growth period with leaf age being defined by week (e.g. less than 1 week old, 1 week old, 2 weeks old etc…) (see Figure 3.4).

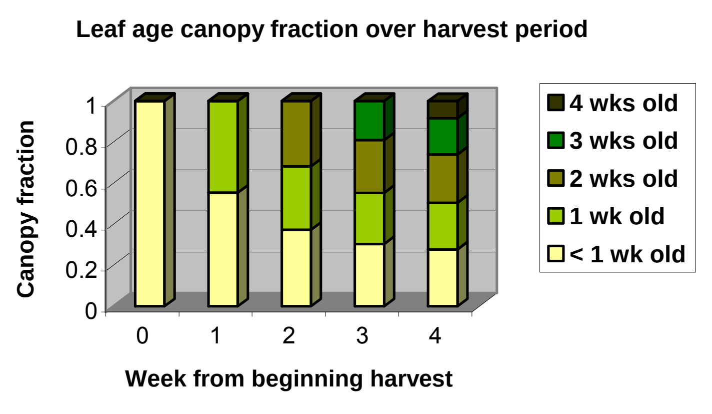

Figure 3.4 The variation in leaf age canopy fraction over the course of the 28 day harvest period. Data supplied from Bangor, UK.

These data were combined with the f_{phen} data to determine the canopy average F_{phen} according to:

$$F_{phen} = \sum_{i=0}^{4} f_{age\,i} * f_{phen\,i}$$

Where $f_{age\ i}$ is the fraction of leaves of age i within the canopy and $f_{phen\ i}$ is the f_{phen} of these leaves. Finally, the F_{phen} calculated above were interpolated to provide a representative F_{phen} for each day of the growth period.

3.2.3 Average leaf stomatal conductance of the canopy ($\overline{G}$ sto)

The average leaf stomatal conductance of the canopy can now be estimated using the multiplicative stomatal algorithm (e.g. Emberson *et al.* 2001) according to:

$$\overline{G}\ sto = F_{phen} * F_{light} * (\mathrm{Max}\{g_{min}, f_{temp}*f_{VPD}\})$$

Where f_{temp} is the clover g_s relationship with temperature (in this case the observed air temperature which is assumed equivalent to the leaf temperature) and f_{VPD} is the clover g_s relationship with vapour pressure deficit (the observed air vapour pressure deficit which is assumed equivalent to the leaf to air VPD). f_{temp} and f_{VPD} are calculated using the formulations and parameterisation established for a single leaf of the upper canopy since it is assumed that all canopy leaves will respond similarly to these environmental variables.

3.2.4 Canopy stomatal ozone flux

The calculation of $\overline{G}$ sto described above provides us with a stomatal conductance value of an average leaf of the clover canopy. In order to estimate canopy stomatal flux this value needs to be combined with the appropriate ozone concentration. Although a common Protocol is followed at each site, there are differences in both location of ozone monitors from the clover canopies and the height of the clover canopy above the ground (according to whether pots and water reservoirs are sunk into the ground or not). This means that there is not a closed canopy homogenous with the surrounding vegetation and therefore standard micro-meteorological principals for the evaluation of mass transfer to estimate the transfer of ozone from the monitoring height to the top of the canopy cannot be used. Instead, corrections for the different canopy and ozone monitoring heights have been made in accordance with the recently revised Mapping Manual (LRTAP Convention, 2004) which provides a "tabulated gradient" to infer the relationship between ozone concentrations at a number of different heights in the absence of appropriate meteorological data.

Ideally, to estimate ozone flux to the canopy leaves the canopy boundary layer resistance (Rb) would also be estimated, again, as a function of wind speed and leaf surface characteristics. Since wind speed data are unavailable and given the uncertainties in the estimation of $O_{3\ canopy}$ it was assumed that Rb (and also rb, the leaf boundary layer resistance) is negligible.

In effect, the O_3 canopy value was assumed to be the ozone concentration of the air immediately external to the stomatal pores and leaf cuticles. There are two primary parallel pathways by which this O_3 will be deposited, the first is to the external cuticle and the second is to the internal leaf structures via the stomatal aperture. Since we are concerned with estimating the ozone damage caused to the plants, and since only that ozone which is absorbed via the stomatal pores is considered capable of causing ozone damage, we only need to estimate the canopy stomatal ozone flux (F_{st}). This can be calculated as follows :-

$$F_{st} = (\overline{G}\,sto + \overline{G}\,ext) * (\overline{G}\,sto / (\overline{G}\,sto + \overline{G}\,ext)) * LAI * O_{3\ canopy}$$

Where Fst is the canopy stomatal flux in mol m^{-2} PLA s^{-1}, $\overline{G}$ sto is the average canopy leaf stomatal conductance in mol m^{-2} PLA s^{-1}, $\overline{G}$ ext is the average canopy leaf cuticular conductance and O_3 canopy is the ozone concentration at the leaf surface in nmol mol^{-1}. A value for $\overline{G}$ ext is selected that is consistent with European scale modelling of ozone deposition where Rext = 2500/SAI, SAI is the surface area index (green + senescent LAI). It was assumed that the clover canopy contains only green LAI so that SAI = LAI, SAI is simply scaled so that $\overline{G}$ ext = 1/2500, m s^{-1}.

The accumulated stomatal ozone flux over the growth period is then estimated according to:

$$AF_{st} = \sum_{i=1}^{672} Fst_i * 3600$$

Where multiplication by 3600 converts from per second to per hour and the sum over 672 hours represents the number of hours in the 28 day growth period.

3.2.5 Canopy flux-effect relationship

The clover canopy flux model was applied to data from selected ICP Vegetation sites that have quality assured three-month NC-S/NC-R biomass data. This comprises twelve different sites and five different years (1996 to 2000), with 23 datapoints in total. The relationship between measured three-month NC-S/NC-R biomass ratio and ozone flux to the canopy ($AF_{st}0$), calculated based on the climate and ozone data at each site (together with site-specific information such as height of the clover canopy above ground level, as indicated above) showed an r^2 of 0.32 (Figure 3.5a). This compares to an r^2 of 0.53 when relating three-month NC-S/NC-R biomass ratio to three-month AOT40 for the same data set (Figure 3.5b). There was little difference to the r^2 between canopy flux and biomass ratio when a threshold for canopy flux was incorporated.

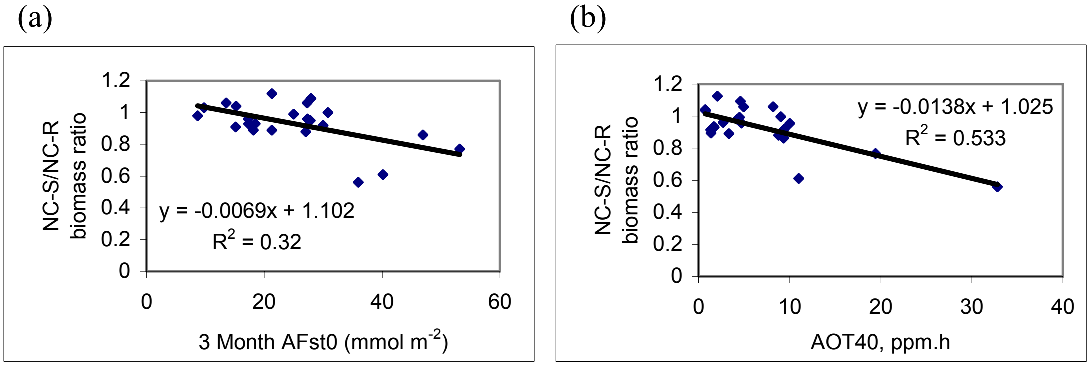

Figure 3.5 Relationship between three-month NC-S/NC-R biomass ratio and a) ozone flux to the canopy, $Af_{st}0$; b) AOT40.

When considering 28-day data at harvests 2,3 and 4 for the same sites that were used for the above 3-month relationships there is similarly no improvement to the relationship with NC-S/NC-R biomass ratio when using canopy ozone fluxes compared to use of AOT40 (data not presented). Again, there was little difference to the r^2 of the relationship when using a threshold for ozone flux to the canopy rather than total flux.

When clover canopy flux is used there was improvement to the relationship between 28 day canopy flux and 28 day NC-S/NC-R biomass ratio when the ozone flux over the previous harvest intervals was considered. This was also found using the 28 day AOT40 and has been described previously (using different data) in Section 2.4.1. This improvement to the relationship between clover canopy flux and NC-S/NC-R ratio is illustrated in Figure 3.6, where the r^2 value increased when the carry-over effect was taken into account from 0.08 (canopy ozone flux in current month) to 0.20 (canopy ozone flux in the previous month) to 0.31 (canopy ozone flux in the previous 2 months), with a small decrease in r^2 back to 0.23 when using the previous three months. The data presented is based on total canopy flux, $AF_{st}0$. When a threshold of 6 nmol m^{-2} s^{-1} was used, there was a very similar pattern, with r^2 values of 0.07 (canopy ozone flux in current month) to 0.20 (canopy ozone flux in the previous month) to 0.31 (canopy ozone flux in the previous 2 months), with a small decrease in r^2 back to 0.23 when using the previous three months (data not presented).

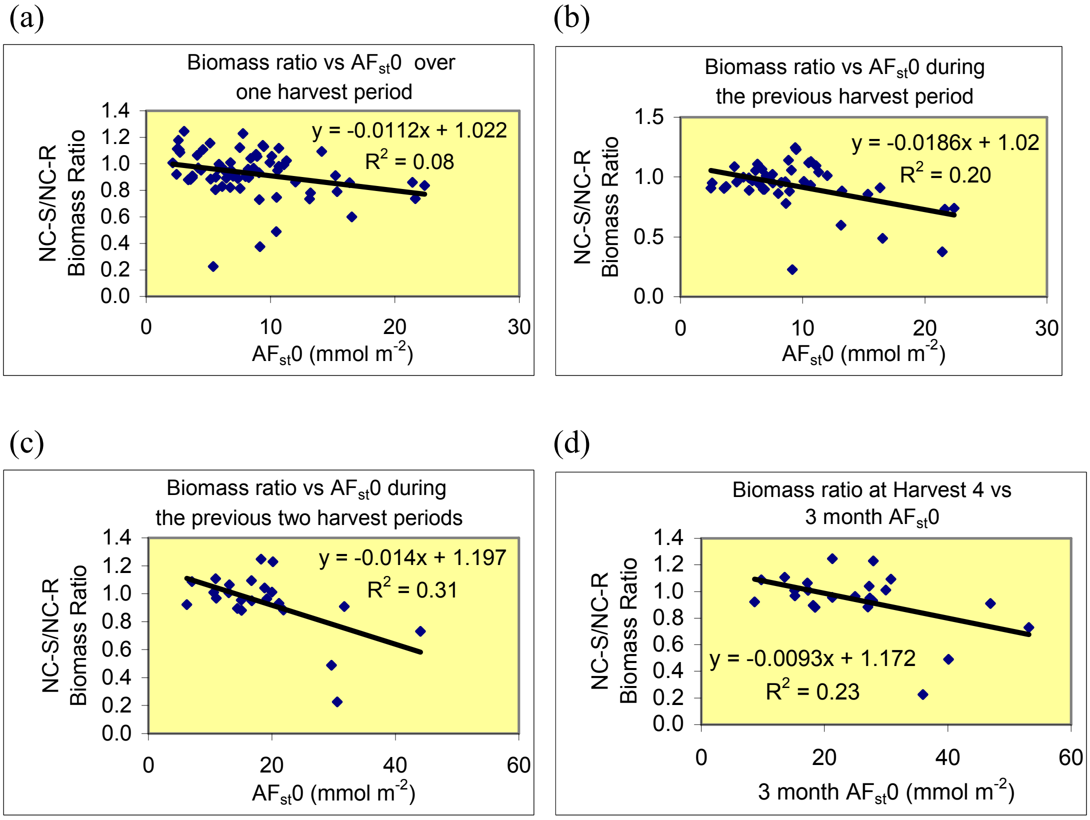

Figure 3.6 Biomass ratio in response to canopy ozone flux accumulated over (a) the 28d for that harvest interval (b) the harvest interval before that used to calculate biomass ratio, (c) the current and previous harvest interval and (d) the current and two previous harvest intervals.

The datapoints that are outliers in Figure 3.6 are from Italy-Isola-Serafini and Italy-Milan, where the NC-S/NC-R biomass ratio is very low even though the calculated ozone flux to the canopy is also low.

3.2.6 Modelling canopy flux for selected 50x50 km EMEP gridsquares

Model runs for *Trifolium repens* were performed for five grid locations representing the five European climate zones defined in the Mapping Manual. These used canopy height O_3 concentrations and surface meteorological data provided by EMEP MSC-W. In order to perform the model runs, assumptions had to be made for certain growth characteristics, such as the start and end of the growing season. For each gridsquare, the ICP Vegetation sites nearest to that gridsquare were identified. From these, the site that was closest to the mean start time for the growing season in each case was used to identify appropriate dates for that gridsquare for each 28 day harvest interval.

The model also requires LAI, which is derived using the relationship between LAI and biomass as described above. For each gridsquare, three sites were identified to run the model, one with biomass at harvest 3 close to the median for that gridsquare, and the sites with minimum and maximum biomass (based on the biomass at harvest 3). The sites used to set the growing season and the sites used to set the biomass for each gridsquare are shown in Table 3.1.

Table 3.1 Sites used to set the growing season and biomass for each gridsquare.

Gridsquare	Growing season	Low biomass	Median biomass	High biomass
UK	UK-Bangor '98	Ire-Carlow '96	B-Tervuren '98	B-Tervuren '01
Sweden	Sw–Ostad '99	F-Jokioinen '96	Sw-Ostad '99	Sw-Ostad '97
Germany	Ger–TrierCity '97	Ger-Essen '97	A-Seibersdorf '98	Ger-Braunschweig '97
Slovenia	Slo – Ljubljana '00	Gr-Athens '04	Slo-Iskrba '04	Gr-Thessaloniki '04
Spain	Sp – Ebrodelta '00	I-Rome '00	I-Naples '01	Sp-Ebrodelta '00

The model was run for each gridsquare using the dates and biomass data as indicated in Table 3.1. Stomatal fluxes for each of the gridsquares, calculated using these data are shown in Table 3.2. The calculated AOT40 over the same time period is also indicated. The importance of the LAI in determining stomatal flux is demonstrated, as changing the input biomass (and therefore the LAI that is derived from this) can have a large influence in some cases, for the example UK (Edinburgh) and Slovenia gridsquares at harvest 2, where the stomatal flux was approximately doubled using high biomass compared to low biomass.

The model was also run using thresholds for stomatal flux. The results when using a threshold of 6 nmol m^{-2} s^{-1} are shown in Table 3.3. In this case, the influence of the biomass used for calculations of LAI is even greater than when no threshold is used in some cases, for example the Slovenina gridsquare at harvest 2, where the cumulative stomatal flux over a threshold of 6 nmol m^{-2} s^{-1} is four times greater using high biomass compared to low biomass.

The results also show that regardless of whether or not a threshold is used in the calculation of cumulative stomatal ozone flux, the calculated fluxes do not correlate very well with the AOT40 values calculated using the same data over the same time period. This is illustrated using data with no threshold for accumulation of flux (Figure 3.7), although the pattern is the same when a threshold is used (data not presented).

The modelled fluxes also illustrate that fluxes of ozone into the plants (and therefore the potential biological impact on the plant) do not always match the concentrations of ozone in the air. In particular, for the northern Europe gridsquare for harvest 2, the calculated ozone fluxes are higher than for the Germany gridsquare, even though the AOT40 is much lower in northern Europe than that of Germany (Table 3.2). These differences in ozone fluxes could explain why *Trifolium repens* plants at some northern European sites e.g. Sweden – Östad often exhibit ozone injury symptoms to an extent similar to that of mid-Europe, even when the ozone concentrations at Sweden – Östad are considerably lower than in mid-Europe.

For each harvest in each gridsquare, an NC-S/NC-R ratio was calculated using biomass data from the site that represented the 'median biomass' (as indicated in Table 3.1). These 15 NC-S/NC-R biomass ratios were compared to the calculated canopy ozone flux (which incorporated the same biomass data to calculate LAI) for the relevant period. With the current model, the relationship between calculated canopy ozone flux and NC-S/NC-R biomass ratio using the 28 day data does not have a very good fit (Figure 3.8), with an r^2 of 0.13. However, the gridsquares used all have a comparatively low AOT40, up to a maximum of approximately 4 ppm.h over 28days, and with six of the 28-day harvest periods having an AOT40 of less than 1 ppm.h. In this range there is comparatively little response of the plants to ozone (see Figures 2.8 and 3.5). These EMEP gridsquares used do not adequately represent the ozone climate of Europe, particularly the Mediterranean region, where ozone concentrations are often much higher than those used in this modelling work and different gridsquares for the Mediterranean region may give a clearer indication of the relationship between NC-S/NC-R ratio and canopy stomatal flux.

Table 3.2 Calculated canopy stomatal fluxes ($AF_{st}0$, mmol m^2) for the gridsquares at harvests 2-4 for the NC-S biotype of *Trifolium repens*. 'Low', 'median' and 'high' indicate the relative biomass of the plants used in model calculations. AOT40 (ppb.h) accumulated over the same time period is also indicated.

Gridsquare	Harvest	Canopy stomatal flux ($Af_{st}0$, mmol m^{-2})			AOT40 (ppb h)
		Low	Median	High	
Northern Europe	2	16.5	16.3	16.3	667
	3	16.9	16.3	16.6	586
	4	5.7	7.3	6.5	222
UK (Edinburgh)	2	7.6	12.5	12.7	365
	3	13.3	14.8	13.5	567
	4	7.8	8.1	8.0	264
Germany	2	11.9	9.3	13.4	1618
	3	15.1	20.0	18.7	1194
	4	14.4	21.3	20.7	4002
Slovenia	2	10.4	23.3	23.1	2084
	3	11.0	16.1	25.0	4292
	4	7.9	8.9	19.5	2947
Spain (Madrid)	2	16.0	18.5	19.2	1058
	3	11.5	17.1	21.9	2389
	4	6.3	14.1	19.1	3627

Table 3.3 Calculated canopy stomatal fluxes over a threshold of 6 nmol m^2 s^{-1} ($AF_{st}6$, mmol m^{-2}) for the gridsquares at harvests 2-4 for the NC-S biotype of *Trifolium repens*. 'Low', 'median' and 'high' indicate the relative biomass of the plants used in model calculations (see table 3.2). AOT40 (ppb h) accumulated over the same time period is also indicated.

Gridsquare	Harvest	Canopy stomatal flux ($Af_{st}0$, mmol m^{-2})			AOT40
		Low	Median	High	(ppb h)
Northern Europe	2	9.4	9.2	9.1	667
	3	10.3	9.5	9.9	586
	4	1.0	2.2	1.5	222
Edinburgh	2	2.8	6.7	6.8	365
	3	6.9	8.6	7.8	567
	4	2.7	2.7	2.7	264
Germany	2	6.1	4.0	7.1	1618
	3	9.1	13.3	11.7	1194
	4	8.7	15.2	14.3	4002
Slovenia	2	4.9	16.6	16.4	2084
	3	5.6	10.3	18.4	4292
	4	3.3	4.1	13.7	2947
Madrid	2	9.9	12.2	12.7	1058
	3	5.8	11.0	15.2	2389
	4	1.6	8.5	12.9	3627

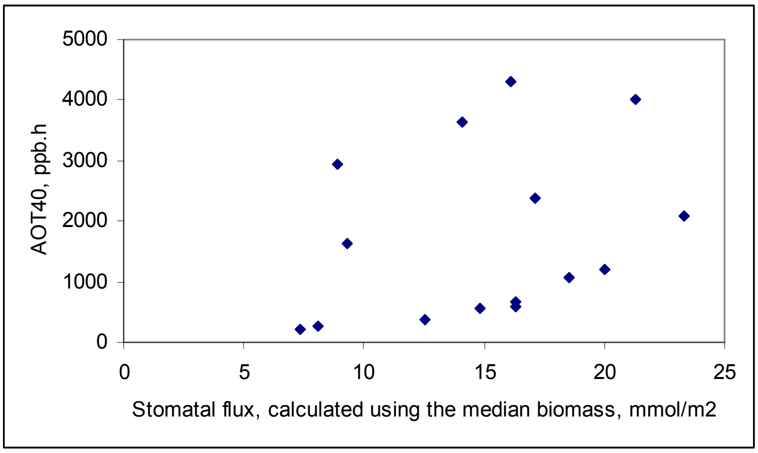

Figure 3.7 28-day stomatal flux versus AOT40, using the median biomass data for each gridsquare for the NC-S biotype of *Trifolium repens.*

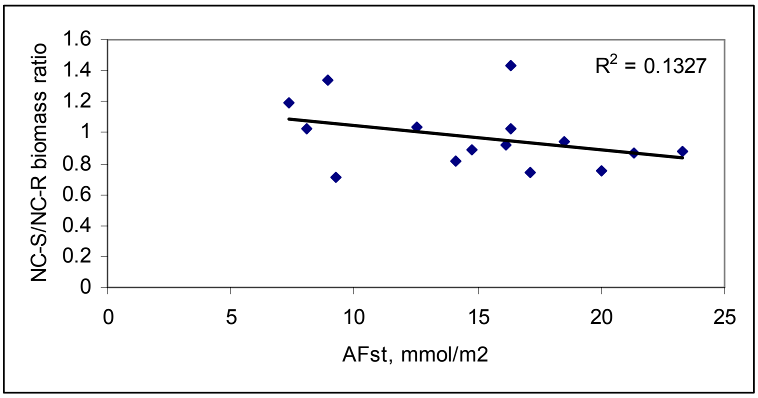

Figure 3.8 Relationship between 28-day calculated canopy stomatal flux ($AF_{st}0$) and NC-S/NC-R biomass ratio.

3.2.7 Conclusions

A large amount of data has been collected from participants of the ICP Vegetation and this has been used to create a stomatal flux model for an individual leaf of *Trifolium repens*, and then from this, a stomatal flux model for the canopy. This has highlighted the differences between some climates, where it has been demonstrated that fluxes of ozone into the plants in regions of low ozone concentrations can be higher than for regions of high ozone concentrations. In addition, a carry-over effect of ozone exposure on biomass production of *Trifolium repens* has been demonstrated, with ozone or stomatal flux data from previous harvest intervals influencing the biomass of the current harvest. The flux modelling work to date has indicated some important features that influence the uptake of ozone into *Trifolium repens* plants, however, the current model of canopy ozone flux does not explain the NC-S/NC-R biomass ratio better than a simple measure of ozone concentration in the air surrounding the plant.

3.3 Development of flux and flux response models for agricultural crops (Objective 5)

3.3.1 Development of flux models

Five species (tomato, grapevine, sugar beet, maize and sunflower) were selected for investigation as to the possibility of establishing robust stomatal flux models. These species were selected on consideration of their distribution across Europe, sensitivity to ozone and economic value. The intended stomatal flux models would be based on the DO_3SE (**D**eposition of **O**zone for **S**tomatal **E**xchange) models stomatal conductance (g_s) multiplicative algorithm as described in the revised Mapping Manual (LRTAP Convention, 2004) and hence require a number of different g_s parameters and g_s relationships with environmental variables to be identified. To establish the availability of such data a comprehensive literature search was conducted for each species, the results of this search are provided in Table 3.4 (a-d).

On the basis of this work it is considered possible to develop robust flux models for four species:-

i) grapevine
ii) sunflower
iii) tomato
iv) maize

For sugar beet the current data availability is considered too limited to define a robust flux model.

From Table 3.4 (a-d) it is clear that it is not possible for all species to parameterise the model for thermal time determined phenology (indicated by f_{phen_e} and f_{phen_f}) since the data needed to parameterise such relationships are not available in the literature; only analysis of datasets that have recorded g_s over entire growing seasons in conjunction with associated mean daily temperatures would offer the possibility of deriving these coefficients. Similarly, it has not been possible to identify the ΣVPD since derivation of this parameter requires sufficient diurnal g_s measurements and associated VPD values. As such, these parameters are presented in grey font in Table 3.4 (a-d).

Previous evaluations of the multiplicative g_s models have found the identification of an appropriate value for g_{max} to be crucial in deciding the predictive abilities of the model. In view of this we present the data describing g_{max} for the four species in Figure 3.9, Table 3.4 (a-d) also provides details of the standard deviation (s.d.) of the species' g_{max}. Maize has the fewest observations useful for g_{max} determination, and perhaps due to this has one of the lowest s.d. values at only 27 mmol O_3 m^{-2} s^{-1}. The other species have more observations contributing to the derivation of g_{max}, for tomato and grapevine, the s.d. is still within an acceptable range (50 to 75 mmol O_3 m^{-2} s^{-1}). Sunflower g_{max} is based on nearly as many observations as grapevine but in contrast has a s.d. of 230 mmol O_3 m^{-2} s^{-1}; this is largely due to two observations which could be considered outliers. The use of median values to derive g_{max} from such datasets reduces the influence of such potential outliers in determining the final value selected. The following summarises the other key issues regarding the establishment of flux models for each of the 3 agricultural crops species.

i) Grapevine

The parameterisation of the flux model for this species is considered reasonably robust. This is largely due to the use of both published data and g_s measurement datasets that have been kindly donated by a number of scientists who have worked with this species in the past. The main uncertainty lies in the parameterisation of f_{VPD} since there is a lot of scatter in the g_s data when plotted against VPD. Clarification of this relationship should be made in the next year as more g_s measurement datasets become available.

ii) Tomato

No data have been found to date to parameterise the phenological function. In addition, there is some inconsistency in the data that has been collected and used to derive the f_{light} and f_{VPD} relationships.

iii) Sunflower

The flux model established for sunflower is reasonably robust with the exception that it has not been possible to find any information describing the g_s relationship with temperature.

iv) Maize

The parameterisation for this species is arguably the least robust of all species for which models have been developed. No new data have been found to parameterise the phenological relationship so here we use the default parameterisation provided by Simpson *et al.* (2003). f_{light} is parameterised with the most amount of data that is also consistent in terms of the relationship derived. In contrast, f_{temp}, f_{VPD} and f_{SWP} are parameterised each based only on one or two studies. This lack of corroboration by different datasets reduces the certainty of the flux model for maize.

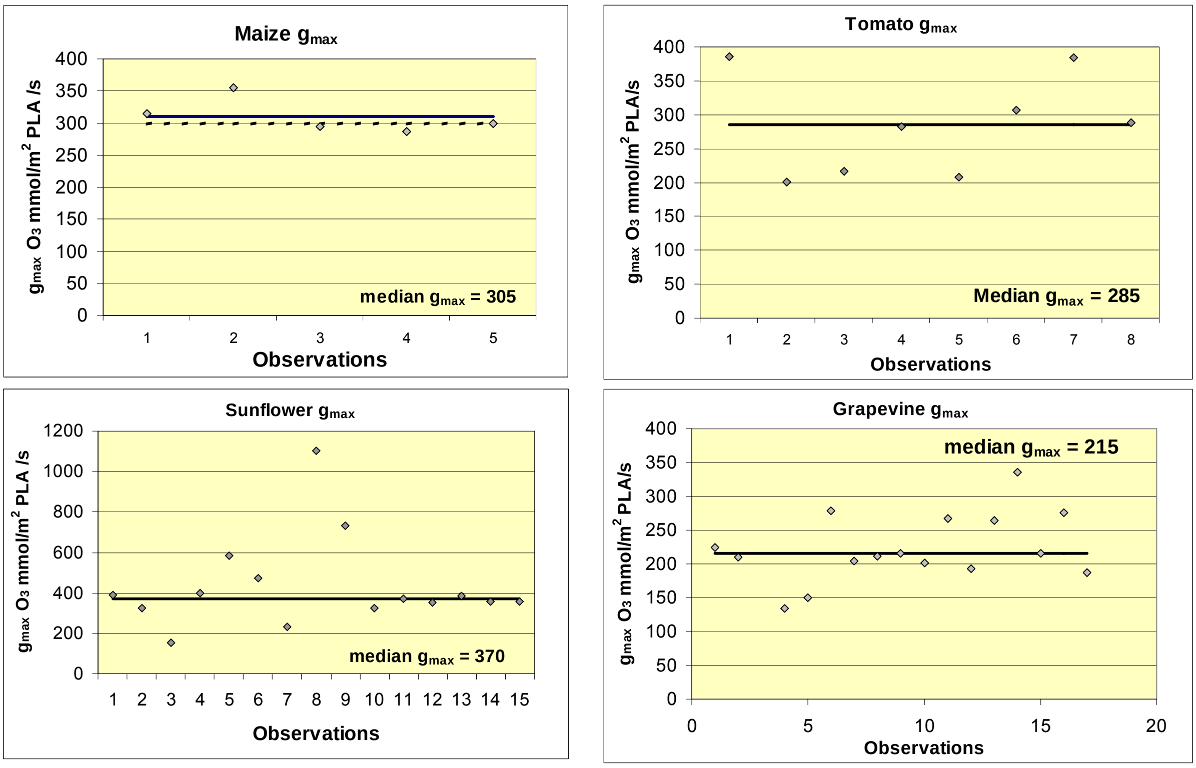

Figure 3.9 Data used to establish g_{max} for 4 agricultural crop species.

Table 3.4a Maize DO$_3$SE model parameterisation.

Parameter	Units	Maize (*Zea mays*)	Reference
SGS	dd	130	FAO, AGL http://www.fao.org/ag/agl/aglw/cropwater/maize.stm
EGS	dd	250	
Astart	dd	as SGS	
Aend	dd	as EGS	
LAI_max	m^2/m^2	3.0	
LAI_min	m^2/m^2	0	
Ls	dd	40	
Le	dd	30	
Albedo	fraction	0.2	
Lm	m	0.1	
h	m	2.0	Simpson *et al* (2003)
root	m	1.0	Simpson *et al* (2003)
g_{max}	mmol O_3 m^{-2} PLA s^{-1}	305 (27)	Korner *et al* (1979) [315]; Sinclair et a (1975) [355]; Stigter & Lammers (1974) [295] ; Tardieu *et al* (1991) ; Ozier-Lafontein *et al* (1998) [300]
f_{min}	(fraction)	0.05 (2)	Bethenod & Tardieu (1996); Sanguinetti *et al* (1999)
f_{phen_a}	(fraction)	0.1	after Simpson *et al* (2003)
f_{phen_b}	(fraction)	0.3	
f_{phen_c}	days	15	
f_{phen_d}	days	45	
f_{phen_e}	°C days	-	
f_{phen_f}	°C days	-	
$light_a$	(constant)	0.0048	Bethenod & Tardieu (1990) ; Turner & Begg (1973); Rochette *et al.* (1991) ; Machado & Lagoa (1994) ; Guilioni *et al*.(2000) ; Olioso *et al*.(1995) ; Ozier-Lafontein *et al* (1998)
T_{min}	°C	2	Turner & Begg (1973); Rodriguez & Davies (1982)
T_{opt}	°C	25	
T_{max}	°C	48	
VPD_{max}	kPa	0	Olioso *et al.* (1995)
VPD_{min}	kPa	5.0	
ΣVPD_{crit}	kPa	-	
SWP_{max}	MPa	-0.12	Tardieu *et al* (1992a); Davies *et al* (1994)
SWP_{min}	MPa	-0.8	

N.B. g_{max} values are median values from collated studies, values in brackets represent the standard deviation.

Table 3.4b Grapevine DO$_3$SE model parameterisation.

Parameter	Units	Grapevine (*Vitis vinifera*)	Reference
SGS	dd	105	Jones *et al* (2005)
EGS	dd	270	
Astart	dd	as SGS	
Aend	dd	as EGS	
LAI_max	m^2/m^2	3.0	Padro (1994), Mascart *et al* (1991)
LAI_min	m^2/m^2	0	
Ls	dd	20	
Le	dd	20	
Albedo	fraction	0.2	Simpson *et al* (2003)
Lm	m	0.15	Massman *et al* (1994)
h	m	1.7	
root	m	1.0	Simpson *et al* (2003)
g_{max}	mmol O$_3$ m^{-2} PLA s^{-1}	215 (51)	Schultz (2003) [225], Naor & Wample (1995) [210]; Schultz (2003a) [134, 150], Medrano *et al* (2003) [279, 204]; Patakas *et al* (2003) [211, 216, 201]; Winkle & Rambal (1993) [267, 193]; Winkle & Rambal (1990) [264, 336, 216]; Correia *et al* (1995) [276]; Jacobs *et al* (1996) [188]; Massman *et al* (1994) [315]
f_{min}	(fraction)	0.01	Jacobs *et al* (1996)
f_{phen_a}	(fraction)	0.2	Winkle & Rambal (1993); Correia *et al* (1995)
f_{phen_b}	(fraction)	0.2	
f_{phen_c}	days	60	
f_{phen_d}	days	45	
f_{phen_e}	°C days	-	
f_{phen_f}	°C days	-	
$light_a$	(constant)	0.0076	Schultz (2003); Winkle & Rambal (1993); Winkle & Rambal (1990); Lu *et al* (2003); Massman *et al* (1994); Jacobs *et al* (1996)
T_{min}	°C	9	Correia *et al* (1995); Flexas *et al* (1999); Schultz (2003); Schultz (2003a); Massman *et al* (1994); Jacobs *et al* (1996)
T_{opt}	°C	30	
T_{max}	°C	43	
VPD_{max}	kPa	1.6	Correia *et al* (1995); During (1987); Massman *et al* (1994); Jacobs *et al* (1996); Medrano *et al* (2003); Schultz (2003)
VPD_{min}	kPa	6.2	
ΣVPD_{crit}	kPa	-	
SWP_{max}	MPa	-1.2	Winkle & Rambal (1993); Quick (1992); Correia *et al* (1995)
SWP_{min}	MPa	-0.35	

N.B. g_{max} values are median values from collated studies, values in brackets represent the standard deviation.

Table 3.4c Tomato DO$_3$SE model parameterisation.

Parameter	**Units**	**Tomato (*Lycopersicum esculentum*)**	**Reference**
SGS	dd	180	The British Tomato Growers Association http://www.britishtomatoes.co.uk
EGS	dd	300	
Astart	dd	180	
Aend	dd	300	
LAI_max	m^2/m^2	4.5	Mulholland *et al* (2000)
LAI_min	m^2/m^2	0	
Ls	dd	90	
Le	dd	0	
Albedo	fraction	0.2	Simpson *et al* (2003)
Lm	m	0.05	
h	m	1.0	
root	m	1.0	
g_{max}	mmol O_3 m^{-2} PLA s^{-1}	285 (74)	Duniway (1971) [385], Morshet & Yocum (1972) [200]; Katerji *et al* (1998) [216]; Bakker (1991) [283]; Boulard *et al* (1991) [208]; Pirka *et al* (2003) [307, 384, 288]
f_{min}	(fraction)	0.01	Boulard *et al* (1991)
f_{phen_a}	(fraction)	0.1	Provisional values after Simpson *et al* (2003)
f_{phen_b}	(fraction)	0.3	
f_{phen_c}	days	15	
f_{phen_d}	days	45	
f_{phen_e}	°C days		
f_{phen_f}	°C days		
$light_a$	(constant)	0.0175	Boulard *et al* (1991); Bakker (1991)
T_{min}	°C	0	Starck *et al* (2000); Boulard *et al* (1991)
T_{opt}	°C	21	
T_{max}	°C	35	
VPD_{max}	kPa	1	Boulard *et al* (1991); Bakker (1991); Romero-Aranda *et al* (2001)
VPD_{min}	kPa	2.7	
ΣVPD_{crit}	kPa		
SWP_{max}	MPa	-0.3	Katerji *et al* (1998); Haupt-Herting & Fock (2000); Makela *et al* (1998)
SWP_{min}	MPa	-1.0	

N.B. g_{max} values are median values from collated studies, values in brackets represent the standard deviation + growing season assumes outdoor conditions (i.e. not under glasshouse or plastic).

Table 3.4d Sunflower DO$_3$SE model parameterisation.

Parameter	**Units**	**Sunflower (*Helianthus annuus)***	**Reference**
SGS	dd	150	Alternative Field Crops Manual http://www.hort.purdue.edu/newcrop/afcm/sunflower.html
EGS	dd	250	
Astart	dd	150	
Aend	dd	250	
LAI_max	m^2/m^2	5	Sims *et al* (1999)
LAI_min	m^2/m^2	0	
Ls	dd	30	
Le	dd	0	
Albedo	fraction	0.2	Simpson *et al* (2003)
Lm	m	0.25	Alternative Field Crops Manual http://www.hort.purdue.edu/newcrop/afcm/sunflower.html
h	m	2	
root	m	1	
g_{max}	mmol O_3 m^{-2} PLA s^{-1}	370 (230)	Ward & Bunce (1986) [390]; Connor & Jones (1985) [325]; Wookey *et al* (1991) [153]; Schurr *et al* (1992) [397]; Rivelli *et al* (2002) [586]; Hirasawa *et al* (1995) [473]; Wample & Thornton (1984) [233]; Fay & Knapp (1996) [1104]; Steduto *et al* (2000) [732]; Turner *et al* (1984) [323]; Turner *et al* (1985) [372]; Quick *et al* (1995) [350]; Koerner *et al* (1979) [385; 355; 355]
f_{min}	(fraction)	0.05	Hirasawa *et al* (1995)
f_{phen_a}	(fraction)	0.6	Connor & Jones (1985); Wookey *et al* (1991); Angadi & Entz (2002)
f_{phen_b}	(fraction)	0.4	
f_{phen_c}	days	34	
f_{phen_d}	days	34	
f_{phen_e}	°C days		
f_{phen_f}	°C days		
$light_a$	(constant)	0.002	Turner (1991); Fay & Knapp (1996)
T_{min}	°C	2	Maize parameterisation used as default
T_{opt}	°C	25	
T_{max}	°C	48	
VPD_{max}	kPa	1.2	Ward & Bunce (1986); Turner *et al* (1984); Tuebner, F. (1985)
VPD_{min}	kPa	4.0	
ΣVPD_{crit}	kPa		
SWP_{max}	MPa	-0.25	Quick *et al* (1992); Hirasawa *et al* (1995); Fambrini *et al* (1994); Sadras *et al* (1993); Zhang & Davies (1989); Gollan *et al* (1986)
SWP_{min}	MPa	-1.65	

N.B. g_{max} values are median values from collated studies, values in brackets represent the standard deviation.

3.3.2 Application of flux models across Europe

The flux models described above have been applied for the four crops grapevine, tomato, sunflower and maize using surface ozone and meteorological data provided by EMEP MSC-W. The meteorological data represent 1997 whilst the ozone concentration data are based on current day (year 2000) emissions data. The models are only applied for those crops likely to be grown commercially in the region; the model runs that have been performed (blue shaded cells) are shown in Table 3.5.

Table 3.5 Input data selected EMEP grid squares (lat. / long. location) by climate zone. Blue shaded cells indicate grid squares for which model runs were performed for each species.

Country	Climate zone	Latitude	Longitude	Grapevine	Tomato	Sunflower	Maize
Sweden	Northern Europe	57° 54' N	12° 24' E		🟦		
UK	Atlantic Central Europe	55° 19' N	3° 12' W		🟦	🟦	🟦
Germany	Continental Central Europe	52° 48' N	10° 45' E	🟦	🟦	🟦	🟦
Slovenia	Eastern Mediterranean	46° 7' N	15° 6' E	🟦	🟦	🟦	🟦
Spain	Western Mediterranean	40° 26' N	3° 42' W	🟦	🟦	🟦	🟦

All runs were performed using fixed dates for the start and end of growing season. The models were run to provide $AF_{st}6$ for an upper canopy leaf over the entire growth period following methods provided in the Mapping Manual (LRTAP Convention, 2004). The corresponding AOT40 values were also estimated over the growth period, i.e. these were not calculated using fixed time windows, as suggested in the Mapping Manual, since this would not have allowed comparison between flux and concentration based methods.

The analysis provided below compares the general patterns of the AOT40 and $AF_{st}6$ indices found for the different crops for all sites for which model runs were performed. A summary of these model results is provided in Figure 3.10.

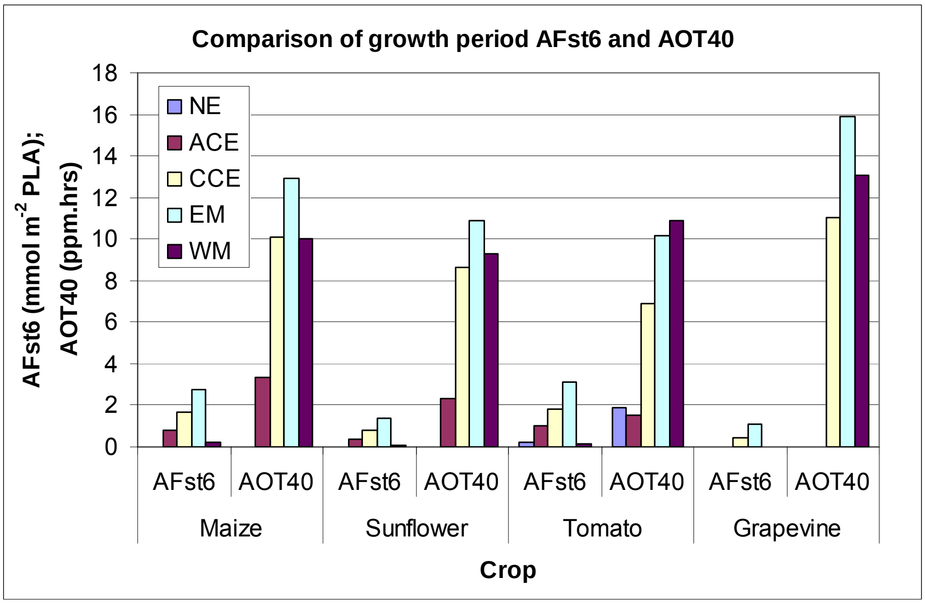

Figure 3.10 Comparison of the AOT40 (ppm h) and $AF_{st}6$ (mmol O_3 m^{-2} PLA) indices found for each of the different crops at all sites for which model runs were performed. All indices were accumulated over the entire growth period defined for each crop.

Maize and tomato have similar g_{max} values and the same growing season length (hence the same exposure period). Comparison of the AOT40 shows that maize, which has a forward shifted growth period, is generally exposed to higher ambient ozone concentrations across the different sites. However, this does not translate into higher AFst6 values, due to the tomato g_{max} being slightly lower (285 rather than 305 mmol O_3 m^{-2} PLA s^{-1} for maize).

In comparison with maize and tomato, sunflower has a lower $AF_{st}6$ even though it has a significantly higher g_{max} (at 370 mmol O_3 m^{-2} s^{-1}) and only a slightly reduced growth period (20 days less than tomato and maize). In addition, the AOT40s (and hence ozone exposure) are approximately the same as tomato due to the slightly later growth period. The reason for the reduced $AF_{st}6$ values is due to the f_{light} relationship, for sunflower this saturates only at very high irradiances (above 1500 PPFD µmol m^{-2} s^{-1}). As such, the maximum f_{light} during the bulk of the sunflower growth period rarely exceeds 0.8 which translates into a maximum potential g_s (before moderation by phenology or the other three environmental variables) of 296 mmol O_3 m^{-2} s^{-1}, i.e. similar to the g_{max} values of maize and tomato.

Finally, the modelling for grapevine has only been performed for three of the climate sites, CCE, EM and WM since it is not considered to be grown extensively for commercial purposes in the other locations. Grapevine has the lowest g_{max} of all the crops modelled (at only 215 mmol O_3 m^{-2} s^{-1}) although in compensation it has the longest growth period. Due to this low g_{max} the F_{st} rarely exceeds 6 nmol O_3 m^{-2} s^{-1} (Figure 3.11) leading to the low (almost insignificant) values for $AF_{st}6$.

Figure 3.11 shows F_{st} profiles and the evolution of $AF_{st}6$ and AOT40 for maize, tomato and sunflower for CCE; this site is chosen to represent an intermediate location in terms of both $AF_{st}6$ and AOT40 indices.

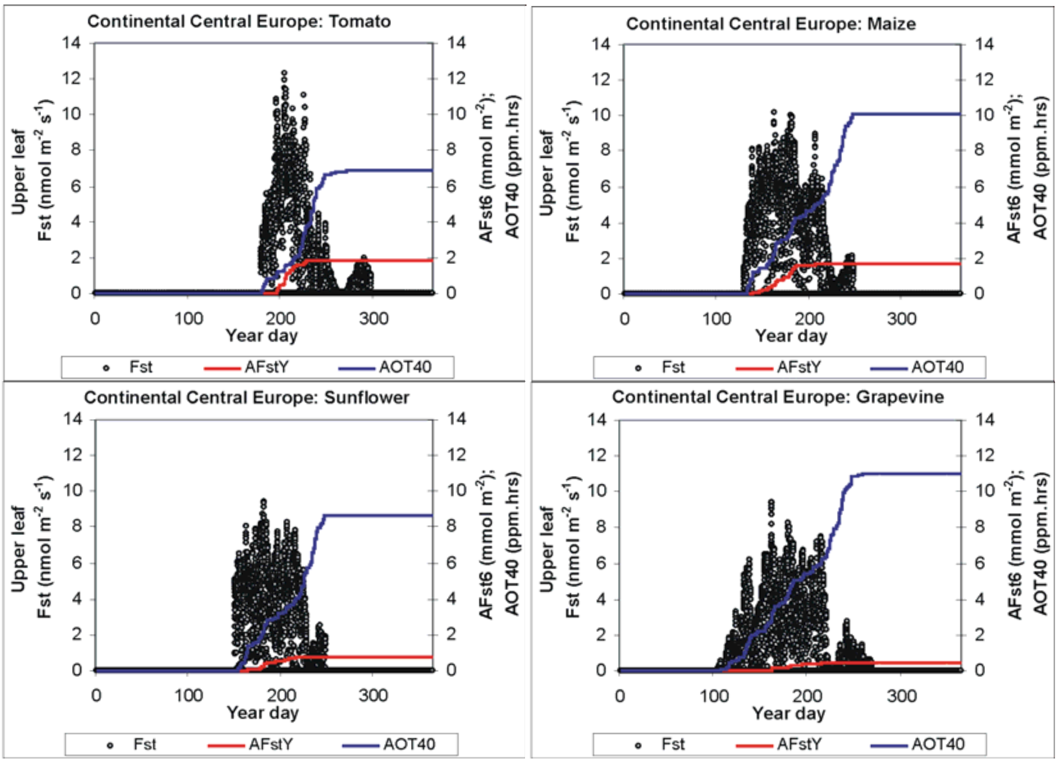

Figure 3.11 Stomatal ozone flux (F_{st}, nmol O_3 m^{-2} PLA s^{-1}) profiles and accumulating $AF_{st}6$ (mmol O_3 m^{-2} PLA) and AOT40 (ppm h) for maize, tomato and sunflower for CCE.

It is important to be clear of the limitations of this current study. The flux models for all species have been parameterised for four crops species based on both primary (un-analysed experimental data) and secondary (analysed data published in the literature) data. The number of datasets available for each species, and for the specific species parameters varies and related to this variation is certainty in the resulting models. In addition, to run the models for sites across Europe, certain parameters not directly related to the stomatal conductance modelling have also been assumed (e.g. the length and timing of the growing season and the associated profiles of LAI). In actuality, these will vary with climate and agricultural management practices but is outside the scope of this research to attempt to include such variation here.

As such, the modelling presented above has been performed using parameterisation that is as consistent as possible between species (i.e. fixed growing seasons, and similar LAI and f_{phen} profiles associated with these growth periods) to allow the core components of the multiplicative model to be compared. The results have highlighted the importance of the timing and length of the growth period (in determining the ozone concentrations which are potentially available for plant uptake as $AF_{st}6$); the importance of g_{max} (in determining the potential maximum flux) and finally the importance of the more extreme parameterisation (e.g. the high light saturating f_{light} relationship).

The modelling also compares flux and concentration based indices. Since flux-response relationships are currently unavailable it is not possible to compare the absolute index values since this would require translation into damage (requiring a dose-response relationship). As such, here we can only compare the relative indication of risk provided by the modelling. This is achieved by calculating relative risk for each index as a fraction of the highest risk across all sites. The relative risk indicated by the indices can then be evaluated by calculating the ratio between the indices, here relative $AF_{st}6$ / relative AOT40. As such, values above 1 are where $AF_{st}6$ is indicating a greater relative risk than AOT40, values below 1 are where $AF_{st}6$ indicates a lower relative risk to AOT40. Figure 3.12 shows the results of this analysis.

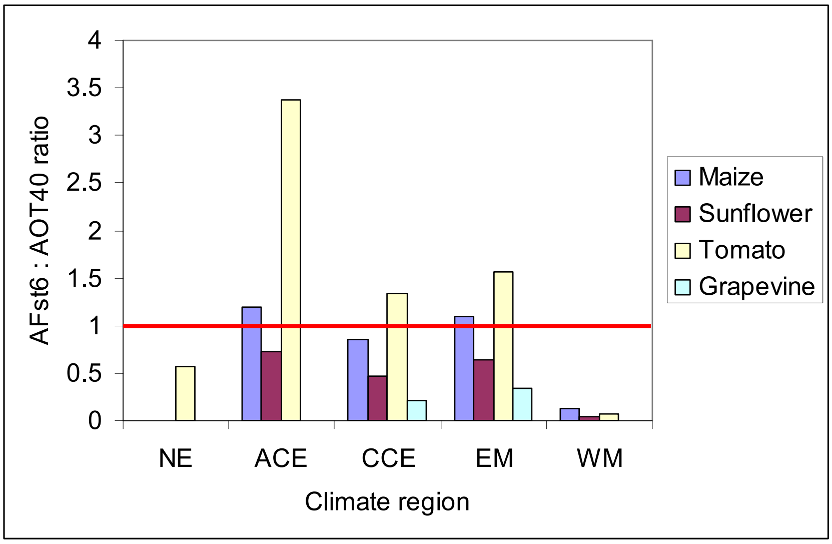

Figure 3.12 The relative risk indicated by $AF_{st}6$ and AOT40 across all species and sites.

Figure 3.12 shows that in relative terms, $AF_{st}6$ indicates a lower relative risk compared to AOT40. This is consistent with recent mapping exercises that have compared $AF_{st}Y$ and AOT40 indices across Europe and found that the spatial pattern of risk is far more homogenous when using the flux-based index (Simpson *et al.*, submitted).

3.3.3 Development of flux-response models

The establishment of flux models for maize, grapevine, tomato and sunflower identify these species as those to concentrate on in the development of flux-response models.

A potentially appropriate dataset was been obtained for grapevines from Gerhard Soja (Austrian Research Centers, Vienna), full details of this dataset are provided in Soja *et al.* (2004). In summary, the data describe experiments conducted on grapevines (*Vitis vinifera* L, cv. Welschriesling) that had been pre-cultivated for two years under field conditions in Eastern Austria, 30 km south of Vienna. The plants were then transplanted into containers and moved to open top chambers (OTCs), ozone fumigation was started in 1994 and was continued during the growth periods until 1996. Four fumigation regimes were compared: charcoal filtered air, non-filtered air, non-filtered air + 25 nmol mol^{-1}, non-filtered air + 50 nmol mol^{-1}. In 1997, the plants were removed from the OTCs and transferred to the field for one season. Response parameters investigated were grape yield and sugar yield, the latter being defined on chemical analysis of grape juice for soluble carbohydrates. As such this dataset would seem to provide an excellent opportunity to develop a flux based response relationship for grapevine. However, further analysis of the dataset found that the ozone and meteorological data provided were not recorded hourly or for conditions within the OTC. Rather, these data had been derived from mean monthly values. This means that is not possible to accurately model ozone flux since the data which would be used to assess the co-occurring stomatal conductance and ozone concentrations are not available. As such, it was not at this stage deemed appropriate to develop a flux-response model from these data. If additional data were to be obtained (e.g. meteorological and ozone data from a local monitoring station) then it may be possible to reconsider the potential of developing the flux-response model.

To date, no datasets that may be appropriate for the derivation of flux-response relationships for maize, tomato or sunflower have been identified. However, it is possible that a tomato dataset may be available from our Spanish colleagues (Victoria Bermejo, CIEMAT, Madrid)) and a maize dataset from our French colleagues (Didier Le Thiec, INRA, Nancy). As such, as new datasets become available in the future, the opportunities to produce flux models for additional crops species using the methods outlined here may arise.

4. WORK PACKAGE 3: QUANTIFYING THE EFFECTS OF OZONE ON SEMI-NATURAL VEGETATION IN EUROPE

4.1 Introduction

In 2003, 2004 and 2005, the ICP Vegetation ozone monitoring programme was conducted with a species of (semi-)natural vegetation, i.e. brown knapweed (*Centaurea jacea*), following a standard protocol distributed by CEH Bangor (see Section 2.4.3). In addition to recording the occurrence of visible injury, several participants also measured stomatal conductance of *Centaurea jacea* in ambient ozone in order to provide data to parameterise an ozone flux model for this species. The species (or close relatives) grows across much of the ECE region and in addition, unlike *Trifolium repens,* the *Centaurea jacea* is not harvested at 28-day intervals, allowing parameterisation according to phenology which is comparable to that of naturally occurring plants.

4.2 Development of a flux-effect model for Centaurea jacea (Objective 6)

4.2.1 Parameterisation of the DO$_3$SE model for Centaurea jacea

In order to estimate the ozone flux to upper stem leaves of brown knapweed (*Centaurea jacea*), stomatal conductance (g_s) and meteorological data collected during the pan-European Centaurea experiment in 2004 and 2005 have been used to parameterise the stomatal component of the DO$_3$SE model for this species using boundary line analysis techniques. The dataset consisted of 1840 data points from four sites, Bangor (UK), Cadenazzo (Switzerland), Grignon (France), and Rome (Italy).

The boundary line analysis for the derivation of the model functions for temperature (f_{temp}), Vapour Pressure Deficit (f_{VPD}) and phenology (f_{phen}) revealed a clear difference in the dependence of g_s on these parameters between the Central European (CE) (i.e. the UK, Switzerland and France) and Mediterranean European (ME) sites (represented by the site in Italy; Figure 4.1). The optimum temperature for stomatal opening differed by 12°C between these two climatic zones (with T_{opt} being 22 and 34°C for Central Europe and Mediterranean Europe respectively), the VPD function was much less sensitive at the Mediterranean site (with VPD_{min} being 3.5 and 6.0 kPa for Central Europe and Mediterranean Europe respectively). The difference in the phenology function possibly reflects the influence of the prevalent climate on the rate of plant development. These findings identified the need for two separate model parameterisations for Central Europe and Mediterranean Europe as listed in Table 4.1.

In order to exclude outliers, the maximum g_s (g_{max}) was defined as the 95-percentile of the French (g_{max} = 390 mmol O$_3$ m^{-2} s^{-1}) and Italian (g_{max} = 370 mmol O$_3$ m^{-2} s^{-1}) g_s-data to represent Central and Mediterranean European climates respectively. The functions describing the g_s relationships with environmental variables (PPFD, temperature and VPD) are established using all datasets to objectively define the boundary lines by eye. The French and Italian datasets are also used to adjust the f relationships so as to improve the correlation coefficient for observed against modelled g_s. The resulting functions are shown in Figure 4.1 for Central Europe and Mediterranean Europe in relation to g_s data collated from all sites.

f_{light} is identical for both climatic zones, whereas f_{temp}, f_{VPD} and f_{phen} clearly differ between central and southern European climate regions.

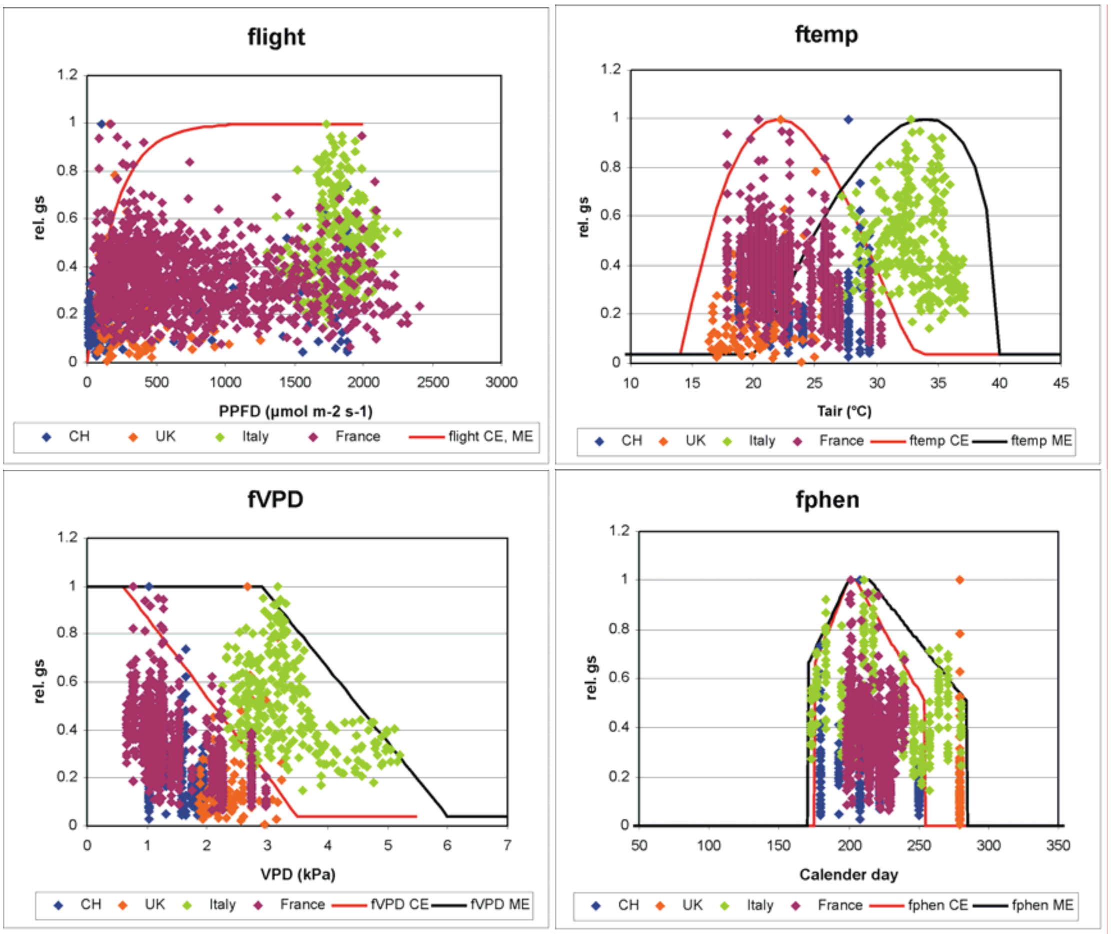

Figure 4.1 Observed g_s (expressed in relative terms, i.e. g_s/g_{max}, on a site-specific basis) of *Centaurea jacea* at four European sites in relation to the g_s-modifying factors PPFD, air temperature, VPD and calendar day. Functions are shown for Central Europe (CE, France, Switzerland and UK) and Mediterranean Europe (ME, Italy). Wheras f_{light} is identical for both climatic zones, f_{temp}, f_{VPD} and f_{phen} clearly differ between central and southern European climate regions.

Table 4.1 *Centaurea jacea* DO$_3$SE parameterisation.

Parameter	**Definition**	**Units**	**Central Europe**	**Mediterranean Europe**
SGS	Start of growing season	year day	early May; 120	mid April; 100
EGS	End of growing season	year day	mid October; 290	mid Sept; 250
LAI_max	Maximum LAI	$m^2 m^{-2}$	3.5 *	3.5 *
LAI_min	Minimum LAI	$m^2 m^{-2}$	0	0
Lm	Cross wind leaf dimension	m	0.025	0.025
h	Canopy height	m	1.0	1.0
root	Root depth	m	1.0	1.0
g_{max}	Maximum g_s	mmol O_3 m^{-2} PLA s^{-1}	390	370
f_{min}	Minimum g_s	(fraction)	0.04	0.04
f_{phen_a}	Minimum start of season f_{phen}	(fraction)	0.65	0.65
f_{phen_b}	Minimum end of season f_{phen}	(fraction)	0.5	0.5
f_{phen_c}	Days from f_{phen_a} to 1	days	25	30
f_{phen_d}	Days from 1 to f_{phen_b}	days	50	70
$light_a$	Constant	(constant)	0.005	0.005
T_{min}	Minimum temperature for g_s	^oC	14	20
T_{opt}	Optimum temperature for g_s	^oC	22	34
T_{max}	Maximum temperature for g_s	^oC	34	40
VPD_{max}	VPD for maximum g_s	kPa	0.6	2.9
VPD_{min}	VPD for minimum g_s	kPa	3.5	6
SWP_{max}	SWP for minimum g_s	MPa	-0.05	-0.05
SWP_{min}	SWP for minimum g_s	MPa	-1.5	-1.5

* Value taken from Simpson *et al*., 2003.

4.2.2 Application of Centaurea ozone flux model

Model runs for *Centaurea* were performed for five grid locations representing the five European climate zones defined in the Mapping Manual (LRTAP Convention, 2004). These runs used canopy height O_3 concentrations and surface meteorological data provided by EMEP MSC-W. In order to perform the model runs, assumptions had to be made for certain growth characteristics, namely the start and end of the growing season, the seasonal profile of LAI, the rooting depth and g_s relationship with soil moisture as detailed below and in Table 4.1.

The model is set up to estimate flux to the stem leaves (assumed to have a cross wind leaf dimension of 0.025 m) since these will tend to be higher in the canopy than the rosette leaves and hence associated with the higher ozone concentrations due to the nature of the vertical ozone profile within grassland canopies. The first stem leaves of *Centaurea jacea* are considered to develop in early May in Central Europe and hence this is defined as the start of the growing season (SGS). For southern Europe, SGS is approximately two weeks later. The end of the growing season (EGS) is mid October and mid September for central and southern Europe respectively. The maximum LAI was set to 3.5 m^2 m^{-2} as indicated for grassland communities in Simpson *et al*. (2003). The soil water potential function was parameterised with SWP_{min} = -1.5 MPa since this is a well established value for the permanent wilting point for a range of species. The model runs were performed for a medium soil texture assuming a rooting depth of 1 metre.

In making these assumptions it was possible to run the model to provide a simulation of stomatal flux for *Centaurea* for an entire growth period under field conditions. The hourly ozone fluxes were calculated using the appropriate parameterisation (Central or Mediterranean Europe) dependent upon the location of the grid square.

Key results of these runs are shown in Figures 4.2 and 4.3 which concentrate on the Atlantic Central European and Western Mediterranean grid, because they represent typical geographical distribution of *Centaurea* and also clearly demonstrate the difference in the seasonal time courses of g_s and accumulated flux between central and southern Europe. Modelled g_s does not differ substantially between the two grids in terms of its absolute range and maximum, with both locations showing higher g_s values to be in the range of 300 to 350 mmol O_3 m^{-2} PLA s^{-1} (Figure 4.2). However, the pattern of g_s over the course of the growing season shows higher g_s values for the Alantic Central European site at the beginning and end of the growing season when compared with the Western Mediterranean site. The lower g_s values towards the end of the growing season at the Western Mediterranean site reflect the limiting effect of temperature and soil moisture deficit. Depending on the distribution of ozone summer episodes, this will have an effect on the calculation of the seasonal accumulated ozone flux.

Figure 4.3 shows an almost doubling of accumulated ozone flux with a threshold of 6 nmol m^{-2} PLA s^{-1} ($AF_{st}6$) for the Atlantic Central European site (3.2 mmol m^{-2} PLA) in comparison with the Western Mediterranean site (1.75 mmol m^{-2} PLA). The difference in the $AF_{st}6$ between the two sites is mainly driven by the g_s-limiting effect of soil moisture deficit at the Mediterranean site. Furthermore, Figure 4.3 depicts the accumulated AOT40 for the two grids and reveals a more than three-times higher value for the Western Mediterranean (11.8 ppm.h) than for the Atlantic Central European site (3.75 ppm h). This highlights the difference between the concentration and flux-based approach, with the latter taking into account g_s-limiting parameters such as soil moisture deficit which will lower the potential phytotoxic effect of ambient ozone concentrations.

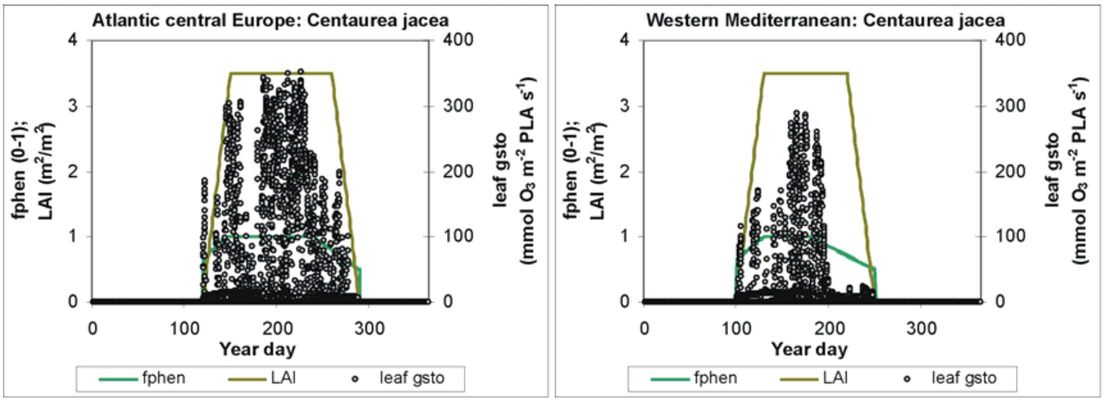

Figure 4.2 Time course of modelled leaf-level stomatal conductance (mmol O_3 m^{-2} PLA s^{-1}) of *Centaurea jacea* calculated for the entire growing season for locations in Atlantic Central Europe and the Western Mediterranean. Also shown are the seasonal time courses of f_{phen} and LAI.

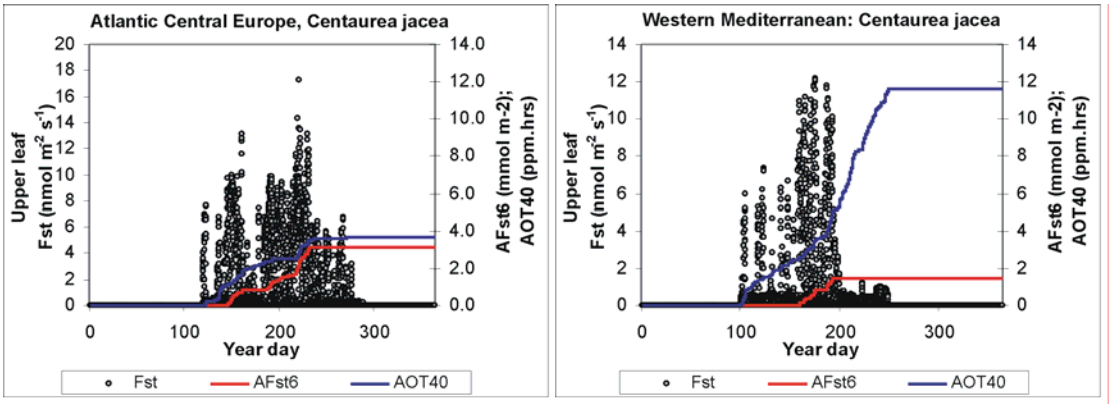

Figure 4.3 Time course of accumulated AOT40 (ppm h) and modelled $AF_{st}6$ (mmol m^{-2} PLA) for a stem leaf of *Centaurea jacea* at a Atlantic Central European and Western Mediterranean site.

Similar results are found for the model runs conducted for the remaining three grid squares (results not shown) which tend to show lower rates of accumulated fluxes in the south (especially where SMD is limiting) and lower rates of accumulated AOT40 in the middle and north of Europe. Hence, a direct comparison of modelled fluxes at different locations in Europe indicates the importance of soil moisture deficit, temperature and prevailing ozone concentrations on ozone uptake of *Centaurea jacea*. However, since the modelling of f_{SWP} and LAI were based on previous DO_3SE grassland parameterisations due to the lack of measured data, these model outputs have to be interpreted with caution.

The results presented above are the outcome of DO_3SE model runs using parameterisation based on data from a limited number of sites and assuming a monoculture stand of *Centaurea jacea*. *Centaurea jacea* is known to vary quite markedly even in its phenotypic characterisation across Europe. As such, large differences in the genotypes of plants from different provenances would be expected which would ordinarily translate into differences in plant physiological behaviour. In addition, *Centaurea jacea* will grow in communities characterised by a range of different forbs, legumes and grass species, all with different canopy structural (e.g. LAI and height) characteristics, and stomatal behaviour. As such the results presented here should not be taken out of context. These results only provide an indication of how *Centaurea jacea*, found at different locations under different climatic regimes, may be expected to behave in relation to stomatal conductance and associated stomatal ozone flux.

4.3 To identify, and to further develop methods for mapping semi-natural vegetation community types at risk from ozone including the link to flux-effect modelling (Objective 7)

The text in this section comprise in part of text extracted from or summarised from the following papers submitted by CEH Bangor and accepted for publication in Environmental Pollution; the reader is referred to the papers for the full text and for further detail. The research conducted by CEH Bangor was supported by additional funding from NERC which facilitated a more comprehensive study than originally proposed for contract EPG1/3/205.

Additional work conducted by SEI-York as part of this objective is indicated in the text. The papers, in press are:

1. *Hayes, F., Jones, M.L.M., Ashmore, M. Mills, G. Meta-Analysis of the relative sensitivity of semi-natural vegetation to ozone. Environmental Pollution, in press 2006.*
2. *Jones MLM, Hayes F, Mills G, Sparks TH, Fuhrer J. Predicting community sensitivity to ozone, using Ellenberg Indicator values. Environmental Pollution, in press, 2006.*
3. *G. Mills, F. Hayes, M.L.M. Jones, S. Cinderby. Identifying ozone-sensitive communities of (semi-) natural vegetation suitable for mapping exceedance of critical levels. Environmental Pollution, in press, 2006.*

4.3.1 Aims

- To extend the existing ICP Vegetation database to include new experimental data on species and community response
- To develop distribution maps of different European grassland communities
- To assess possible sensitivity based on species composition of different communities
- To develop the basis for modelling ozone flux to these communities.

4.3.2 Introduction

Numerous studies have indicated that many of Europe's natural and (semi-)natural vegetation species are potentially at risk from damage by ozone pollution. These studies have primarily involved exposure of plants to ozone pollution in solardomes (e.g. Hayes *et al.*, in press (a)) and open top chambers (e.g., Pleijel and Danielsson, 1997, Gimeno *et al.*, 2004a) with one study using an open field exposure system (Volk *et al.*, 2006). Regardless of exposure system used, the experiments have shown that a significant proportion of the species tested respond to ozone by developing one or more of the following: visible injury, premature and enhanced senescence, changes in biomass, resource allocation and/or seed production. Since each of these effects might impact on the vitality of plant communities, there has been a growing need to draw the published information together to identify which communities across Europe are potentially sensitive to ozone and to develop methods for mapping their location in relation to ozone exposure. In this study, existing datasets have been collated from over 60 papers into a database named OZOVEG (**Ozo**ne effects on **veg**etation) to allow identification of ozone-sensitive species and analysis of relationships between ozone sensitivity and plant characteristics. Data was selected for inclusion from field-release, open-top chamber or solardome experiments involving seasonal ozone exposure. Ozone-response functions were derived for the 83 species within OZOVEG that have three or more data points. The geographical coverage of the database reflects the sources of published data. Thus, it has a central and northern European bias since over 95% of the data OZOVEG contains is from experiments conducted in Sweden, Denmark, UK, Netherlands, Germany and Switzerland. OZOVEG has been analysed to develop methods of identifying ozone-sensitive communities that is applicable at the European level where limited data is available on the species present and at a regional scale where more detailed information is available on species present and abundance.

The physiological and ecological characteristics of each species were investigated as potential predictors of sensitivity of plants to ozone because some factors such as stomatal density and stomatal conductance may affect the amount of ozone that enters the plant. For example, Uddling *et al.* (2004) and Bermejo *et al.* (2002) on birch trees and white clover respectively, found that stomatal uptake of ozone was better related to biomass changes than the external ozone exposure. Other factors, such as the season in which the plants flower may be correlated with sensitivity to ozone, for example, species that flower in spring have been considered to be particularly sensitive to ozone because they are actively growing while ozone concentrations are relatively high (Fuhrer *et al.*, 2003). Meta-analysis of the OZOVEG database also considered ecological factors factors, including Grime's CSR strategy (Grime, 1973) and Ellenberg Indicators which have been widely used in the ecological literature to interpret responses to environmental gradients e.g. (Sparks *et al.*, 1996), or changes in vegetation communities over time (van der Maarel *et al.*, 1985).

During the 1980s, the Convention on Long-range Transboundary Air Pollution (LRTAP Convention) adopted the critical loads/critical levels approach to defining sensitivity of receptors to pollutants (Bull, 1991). The critical level for semi-natural vegetation is currently an AOT40[3] of 3 ppm h over three months and is applicable for growth reduction in perennial species and growth reduction and/or seed production in annual species when grown in a competitive environment (LRTAP Convention, 2004). Preliminary recommendations were suggested for mapping exceedance based on expert knowledge of those communities most likely to be sensitive to ozone. These were classified using the European Nature Information System, EUNIS (http://eunis.eea.eu.int/index.jsp) which has been adopted by the LRTAP Convention for mapping impacts of pollutants in Europe. This system provides a hierarchical approach to classification, with the broadest classes e.g. "Heathland, scrub and tundra" at level 1 denoted by a letter, in this case F, through to relatively specific descriptions of community types at level 4 denoted by the letter followed by "number. number number". As the full names are frequently long and wordy, these will be provided here at the first mention, and abbreviated names will be used thereafter. Using the EUNIS approach, ozone-sensitive communities were indicated in the 2004 revision of the Mapping Manual as Dry grasslands (EUNIS code E1), Mesic grasslands (E2), Seasonally-wet grasslands (E3) and Dehesa grasslands (E7.3) with Woodland fringes, clearings and tall forb habitats (E5, henceforth described as "Woodland fringes") also being considered likely to be sensitive. Such recommendations were based on peer review of available knowledge and were not based on any in depth analysis. Within this study, we have provided support for these choices as well as recommended other communities that are potentially at risk from ozone pollution. Methods of mapping the location of ozone-sensitive communities have also been developed from the harmonised CORINE and SEI-York land-cover maps.

Currently, it is not possible to apply a flux-based approach to assessing critical levels of ozone for semi-natural vegetation, primarily because of the lack of data for parameterisation of flux models and the range of different plant communities that are found across Europe. Nevertheless, consideration of parameters linked to stomatal flux may be of value in identifying communities and species of high sensitivity to ozone. Therefore, a further aim of this study was to quantify flux-related parameters for a range of grassland communities that could be linked to the OZOVEG database.

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[3] The sum of the differences between the hourly mean ozone concentration (in ppb) and X ppb when the concentration exceeds X ppb during daylight hours

4.3.3 Development of the OZOVEG database
(CEH Bangor)

The results from over 60 papers were examined. To be included in the database, the data needed to include biomass measurements and be from field-based experiments (open-top chambers (OTCs), field release systems, solardomes), with an exposure duration of at least three weeks and mean maximum hourly ozone concentration of less than 100 ppb. Those papers that met these requirements and provided data for the ozone sensitivity database (OZOVEG) are listed in Table 7.1.

AOT40, the ozone concentration accumulated over a threshold ozone concentration of 40 ppb during daylight hours, over the duration of the exposure period was used as the measure of ozone exposure. Where AOT40 information was not provided, this was calculated using the exposure information available (Mills *et al.*, 2003). To standardise the biomass responses, for each treatment within an experiment the above-ground biomass was expressed relative to that of the charcoal-filtered air (or occasionally non-filtered air if a charcoal filtered control was not used and the ozone concentrations in the non-filtered air was very low), which was considered to be 1.

For each of the 98 species where there were three or more data-points, (derived from several different investigations in many cases), an ozone dose-response relationship was derived using linear regression.

Table 4.2 Papers used in the development of the OZOVEG database.

	Exposure method	Exposure duration (days)	No. of species exposed	Country
Ashmore *et al.*, 1995	OTCs	21	38	UK
Bergmann *et al.*, 1995	OTCs	36-88	11	Germany
Bergmann *et al.*, 1996	OTCs	28-55	6	Germany
Bungener *et al.*, 1999	OTCs	160	24	Switzerland
Danielsson *et al.*, 1999	OTCs	50	2	Sweden
Foot *et al.*, 1996	OTCs	84-168	1	UK
Franzaring *et al.*, 2000	OTCs	71-142	10	Netherlands
Gimeno *et al.*, 2004a[1]	OTCs	43-69	19	Spain
Hayes *et al.*, submitted (b)	Solardomes	70	33	UK
Hayes *et al.*, unpublished data	Solardomes	84	6	UK
Kohut *et al.*, 1988	OTCs	83	2	USA
Mortensen and Nilsen, 1992	OTCs	33-66	16	Denmark
Pleijel and Danielsson, 1997	OTCs	34	27	Sweden
Power and Ashmore, 2002	OTCs	23-53	12	UK
Tonneijck *et al.*, 2004	OTCs	28	4	Netherlands

[1] including additional unpublished data, personal communication.
OTC = open-top chambers.

A sensitivity index was derived from the regression line of the dose-response relationship for individual species. Using the regression equation, the relative biomass at 3 ppm.h and 15 ppm.h ozone was calculated. The relative sensitivity of each species was calculated as:

relative biomass at 15 ppm.h/relative biomass at 3 ppm.h

This index forms the basis of all subsequent analysis. Values of <1 indicate above-ground biomass reductions in response to ozone whilst a relative sensitivity greater than 1 indicates a stimulation of above-ground biomass in response to ozone. A species was categorised as sensitive to ozone if the sensitivity index was less than 0.9, insensitive to ozone if the sensitivity index was between 0.9 and 1.06, or stimulated by ozone if the sensitivity index was greater than 1.06. These limit values represent the median relative sensitivities of those species which have values of <1 and >1 respectively. This database of 83 species (OZOVEG database) was used in subsequent analysis to identify traits associated with sensitivity to ozone.

4.3.4 Identification of traits associated with ozone-sensitivity

(CEH Bangor)

Approximately one third of the species in OZOVEG show above-ground biomass reductions equating to over 10% at 15 ppm.h AOT40 compared to 3 ppm.h AOT40 (Figure 4.4). A further 15 species showed above-ground biomass stimulation by ozone (sensitivity index >1.06), equating to a stimulation in above-ground biomass of over 5% at 15 ppm.h AOT40 compared to 3 ppm.h AOT40. The remaining 41 species were considered insensitive to ozone (sensitivity index between 0.9 and 1.06).

Available data indicate that plants of the *Fabaceae* family were more sensitive to ozone than plants of the families *Asteraceae, Caryophyllaceae* and *Poaceae* ($p<0.01$, Figure 4.5), with mean relative sensitivity 0.70 for the *Fabaceae* family, 0.88 for the *Asteraceae*, 0.95 for the *Poaceae* and 0.98 for *Caryophyllaceae*. However, these 'less sensitive' families still contain some species which are sensitive to ozone, for example *Leontodon hispidus* from the family *Asteraceae*, is the 6th most sensitive species in the OZOVEG database. Fifteen other families are included in the ozone sensitivity database, but these were only represented by one or two species each and therefore no conclusions could be made about them. An investigation of the relationship between sensitivity to ozone and Raunkiaer life-form showed that the mean relative sensitivity of the therophytes included in the database was 0.77, which was significantly lower ($p<0.001$) than for chamaephytes and hemicryptophytes, which had mean relative sensitivities of 1.02 and 0.96 respectively, indicating that therophytes are more sensitive to ozone (Figure 4.6).

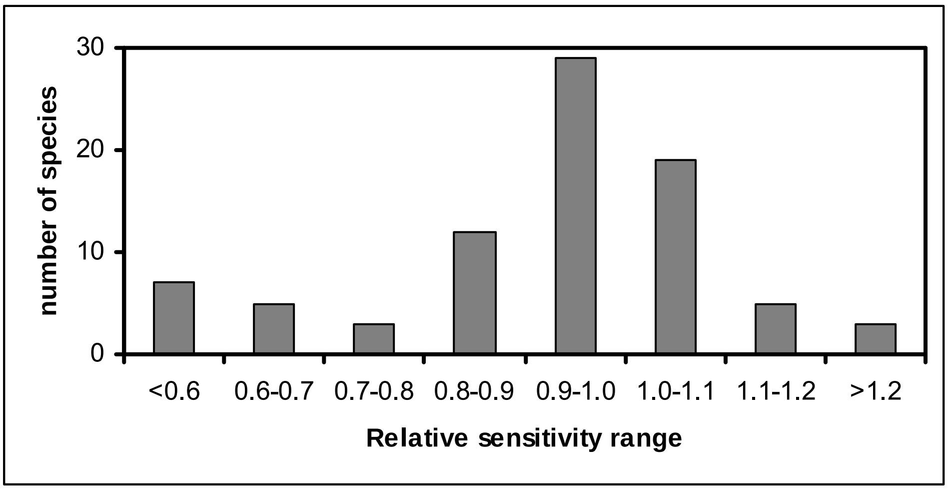


Figure 4.4 Range of relative sensitivity to ozone for the 83 species included in the OZOVEG database.

Helophytes, geophytes and phanerophytes were not well enough represented in the OZOVEG database to include in the comparisons. Limited data availability of mature leaf nitrogen concentrations (data is only available for 20 of the 83 species in the database) indicated that species with a mature leaf N concentration of 20-30 mg g^{-1} were more sensitive to ozone than those with concentrations of 10-20 mg g^{-1} (mean relative sensitivity of 0.80 and 1.03 respectively, $p<0.05$, data not presented). The following parameters showed no relationship with ozone sensitivity for those species for which data exists (data not presented): flowering season, leaf longevity (aestival, evergreen or semi-evergreen), mature leaf phosphorus concentration, stomatal density of the lower or upper leaf surfaces and the maximum altitude at which a species has been found.

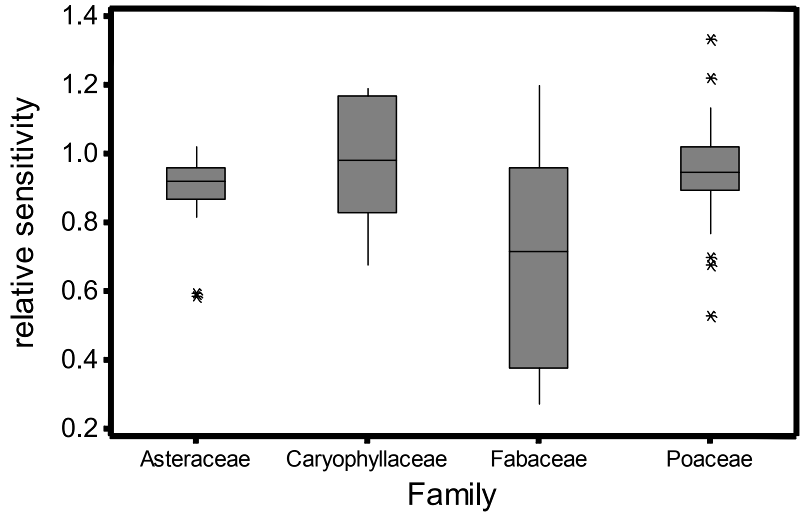


Figure 4.5 Boxplot of relative sensitivity to ozone for families that have ≥5 species with relative sensitivity information. Box shows median, upper and lower quartiles, whiskers show adjacent values, asterisks show potential outliers. (*Asteraceae* n=16, *Caryophyllaceae* n=6, *Fabaceae* n=12, *Poaceae* n=31). P=0.002.

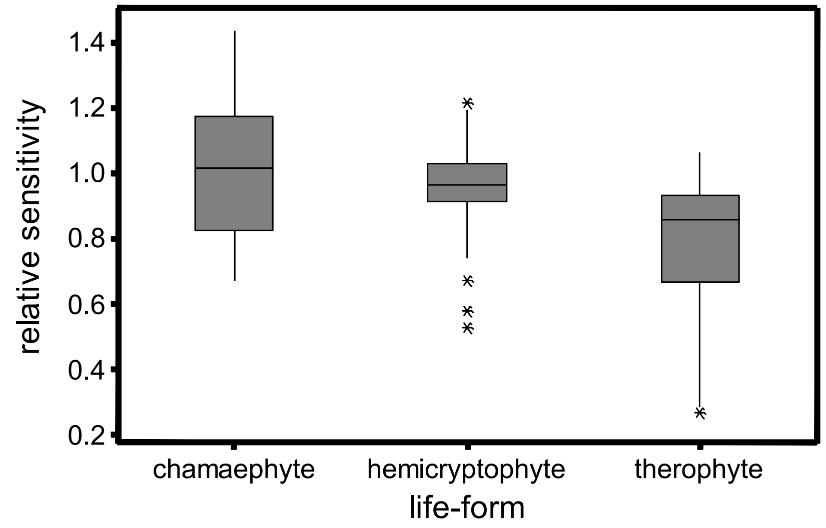


Figure 4.6 Relationship between life form and relative sensitivity to ozone. Box shows median, upper and lower quartiles, whiskers show adjacent values, asterisks show potential outliers. N = (in order of x-axis category) 6, 51, 22. Phanerophytes and helophytes were not included as they had only 1 and 2 replicates respectively. P<0.001.

Comparison of relative sensitivity to ozone with Ellenberg ecological habitat scores (the 'original' classification and the 'final' values modified for the UK flora, Ellenberg, 1991; Hill *et al.*, 1999) showed that light-loving plants tended to be more sensitive to ozone than plants that normally occur in the shade (Figure 4.7a, p<0.05 for original, p<0.001 for final). However, species representing the most shade-tolerant Ellenberg scores (1-4) were not represented in the OZOVEG database. Plants of Ellenberg moisture score 3 (dry site indicator) tended to be more sensitive to ozone than those found in more moist soils (Figure 4.7b, ns for original, p<0.01 for final). Plants which can tolerate moderately saline conditions (Ellenberg salt value of 1) were more sensitive to ozone than those of non-saline habitats (Figure 4.7c, p<0.05 for original, ns for final). It should be noted, however, that species with Ellenberg salt values of 2-9 were not represented in the OZOVEG database. There were no relationships between either Ellenberg nitrogen score and relative sensitivity to ozone or Ellenberg 'reaction' score and ozone sensitivity (data not presented). An investigation of the relationship between relative sensitivity to ozone and Grime's CSR strategy showed no significant differences between the overall classifications of each species (Figure 4.8).

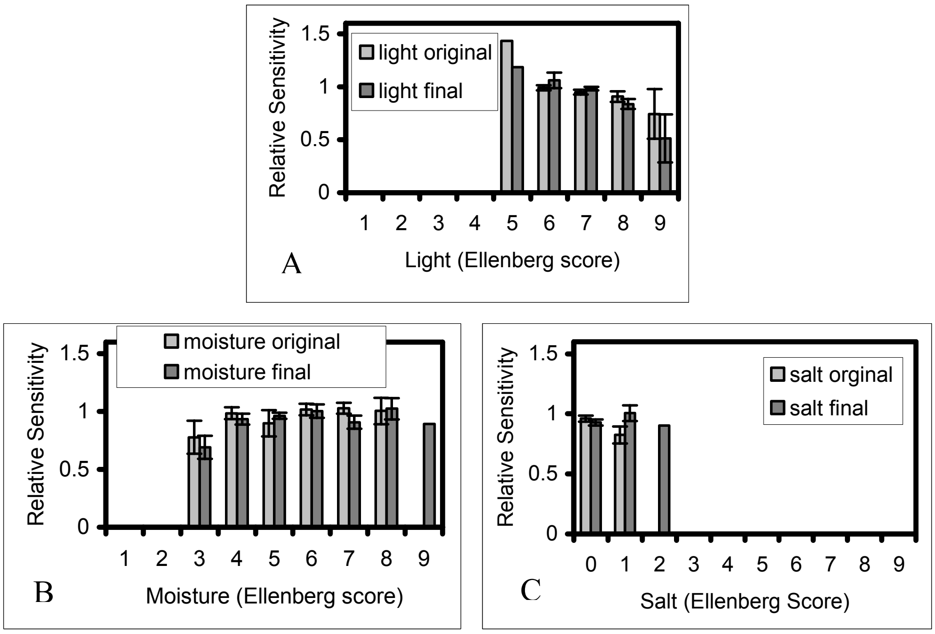


Figure 4.7 Relative sensitivity for Ellenberg habitat scores a) Light. P=0.033 for original, P=0.000 for final; b) Moisture. P=0.182 for Original P=0.009 for Final ; c) Salt. P=0.044 for Original, ns for Final.

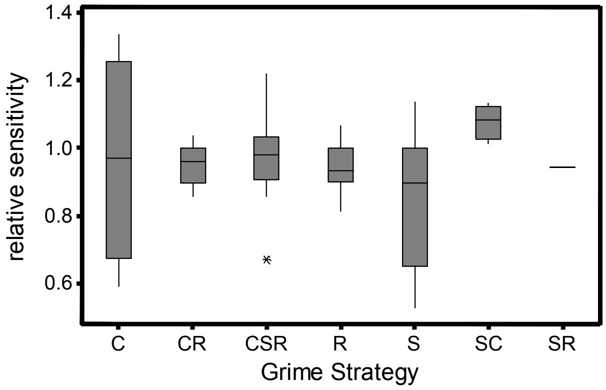


Figure 4.8 Boxplot of the relationship between relative sensitivity to ozone and Grime strategy. Box shows median, upper and lower quartiles, whiskers show adjacent values, asterisks show potential outliers. N = (in order of x-axis category) 4, 5, 20, 8, 10, 4, 1. No significant differences were found.

4.3.5 Predicting ozone-sensitivity to ozone using Ellenberg Indicator values (CEH Bangor)

Of all the potential indicators of ozone sensitivity investigated in the previous section, Ellenberg Indicator values showed the greatest potential for wider application. The principal advantage of predicting sensitivity using Ellenberg Indicators is that values have been assigned for most of the temperate European flora, nearly 3000 species in all (Ellenberg *et al*., 1991). Therefore, if robust relationships between ozone sensitivity and Ellenberg Indicator values can be established, this provides an invaluable starting point from which to predict the sensitivity of the bulk of the species in the European flora. This allows us to move from estimating ozone sensitivity of individual species to that of whole vegetation types based on the responses of the component species.

The database represents quite faithfully the distribution of species within the European flora (Figure 4.9), with the exception of Ellenberg Continentality, some of the lower values for Ellenberg Light, and the higher values for Ellenberg Moisture and Salinity. Continentality was excluded from the subsequent analysis, as the distribution of values was not well represented in the database, and because the original definitions had a strong geographical rather than a climatic basis (Ellenberg *et al*., 1991). All other Indicators were retained for model development.

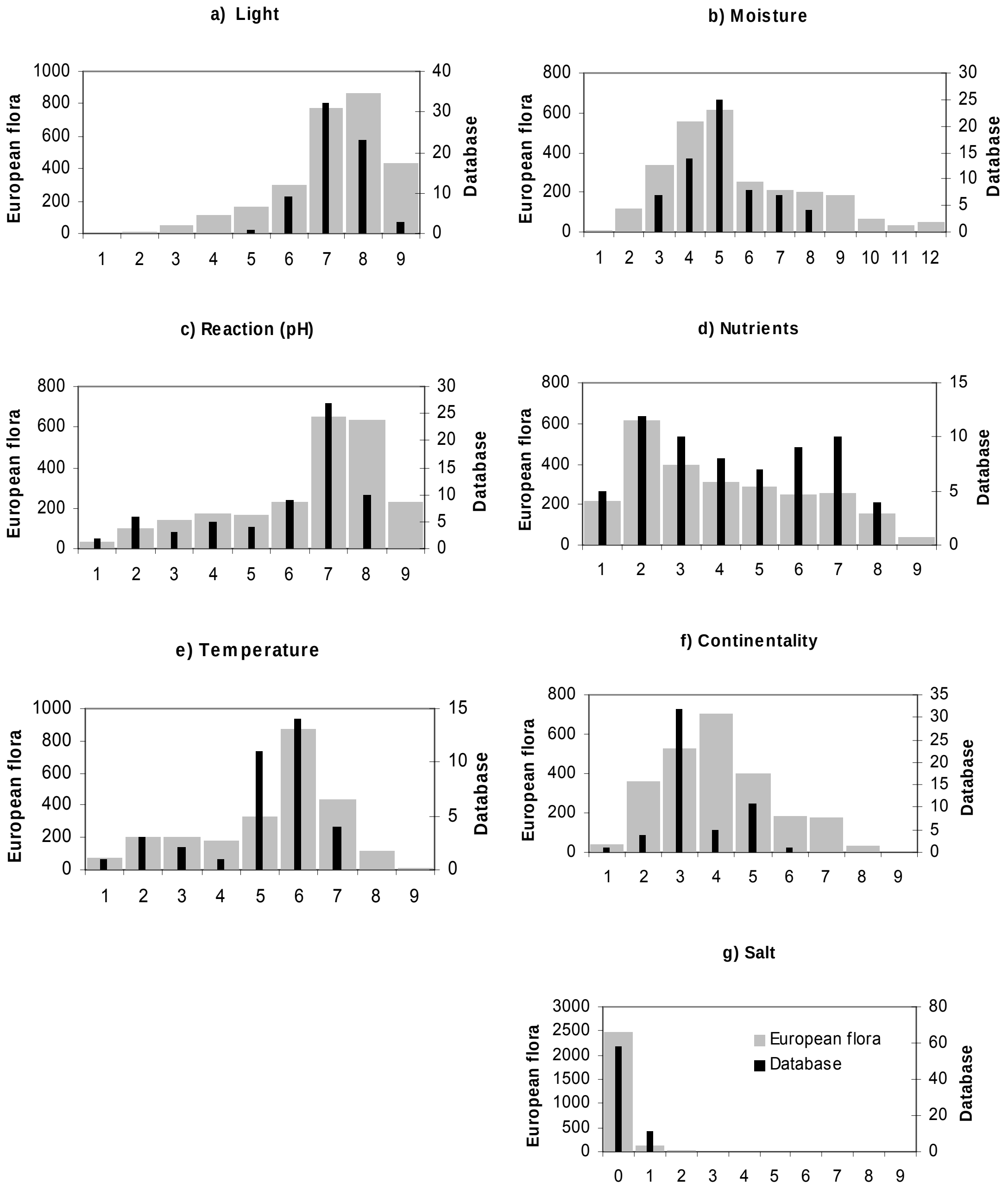


Figure 4.9 Histograms showing distribution of Ellenberg Indicator Values within the database (thin black bars) and within the European flora (thick grey bars). Histograms show a) Light, b) Moisture, c) Reaction (pH), d) Nutrients, e) Temparature, f) Continentality and g) Salt

Some of the species included in OZOVEG were of American or Mediterranean origin, did not have Indicator values assigned and these were excluded. Of the species in the database with Ellenberg Indicators, there were gaps where species had a wide ecological amplitude, denoted as ‘X’ by Ellenberg *et al.* (1991). Omission of these species would have further reduced the available pool of species. Therefore, on the basis that species living in the same habitat are likely to have closer ecological requirements than those in other habitats, the missing Indicator values were estimated through association, by taking the median value in a subset of species with similar combinations of other Indicator values. The principle of allocating an actual value to species previously denoted as having a wide amplitude has been successfully applied in the modification of the Ellenberg Indicator values for the UK flora (Hill *et al.*, 1999; Hill *et al.*, 2000). As a result of these procedures, the number of useable species in the database was 65.

Developing a single species model

As a first step, each Indicator was analysed using regression to determine its ability to predict ozone sensitivity. Analysis of the residuals suggested additional quadratic responses, therefore quadratic terms were also included. Hayes *et al.* (submitted (a)) show that the UK modified Ellenberg values (Hill *et al.*, 1999) proved slightly better predictors of relative sensitivity to ozone than the original un-modified Ellenberg values (Ellenberg *et al.*, 1991). However, the requirement to develop a methodology applicable across much of central and western Europe meant that some optimality within the equations had to be sacrificed, and the original un-modified Ellenberg values were used for developing the model. Further analysis (Table 4.3) showed that the relationship was improved by weighting the contribution of each species to the regression by the R^2 of the empirically-derived response function from the database - see Hayes *et al.* (submitted (a)) for further details. This improved the fit for most Indicators, but particularly so for Light. In the weighted regressions (Table 4.3), Light (L) was highly significant ($p < 0.001$) in explaining ozone sensitivity, with an R^2 of 21.5 %. However, Moisture (F), Salinity (S) and the quadratic Light term (LL) were also significant at $p < 0.05$, and Temperature (T) and the quadratic Moisture term (FF) were significant at $p < 0.01$. Figure 4.10 shows the relationships between RS and each Ellenberg Indicator assessed in the model.

It was likely that a combination of Ellenberg Indicators would give the best predictive power. Therefore, multiple linear regression was used to build the model, taking three approaches: 1) Inclusion of all terms; 2) Inclusion of only those terms which were significant as single factors; 3) Backwards elimination using both approaches 1 and 2 as starting points, and removing the least significant variable at each step. The best equations retaining two terms both contained Light, together with either Salinity (Equation 3a) or Moisture (Equation 3b). The backwards elimination procedure showed that retention of most of the terms gained little in the way of significance. Approaches 1 and 2, and the equations resulting under approach 3, including the equation with Light as a single factor (Equation 3c), were retained for further testing. The terms used and the R^2 and significance for the resulting five equations are shown in Table 4.4.

The regression equations were developed using the whole useable database of 65 species. In order to determine how robust the equations were in predicting ozone sensitivity, the equations were tested using two methods: jacknifing (Efron and Gong, 1983; Tukey, 1958), and a performance test which assessed the risk of misclassifying a species as either sensitive or insensitive under each equation. In the jacknifing routine, written to run in Minitab v14.1, each species was excluded in turn from the database. Then, for each equation being tested, the remaining 64 species were used to develop regression coefficients from which the RS of the omitted species was predicted. This approach was chosen in preference to splitting the database into development and test subsets e.g. (Gatz, 2003; Hargreaves *et al.*, 2005) in order to maximise the pool of available species responses in what is a relatively small predictive dataset. The Mean Absolute Deviation (MAD) and Root Mean Square Error (RMSE) of the jacknifed predictions for each equation are shown in Table 4.4. In the performance test, a threshold value of +/- 0.2 difference from a relative sensitivity of 1 (no change in yield) was used to classify species in the database as particularly sensitive to ozone. The predicted RS values using each approach were classified in the same way and the number of species misclassified under each equation was then calculated (Table 4.4).

Table 4.3 The R^2 and p value for unweighted and weighted regressions on each Ellenberg Indicator and its quadratic term. † Salinity values in the database were either 0 or 1 and were not squared.

	Unweighted regression		Weighted regression	
Ellenberg Indicator	R^2	p	R^2	p
Light (L)	10.2	0.010	21.5	< 0.001
Moisture (F)	7.3	0.030	7.4	0.028
Reaction (pH) (R)	1.3	0.365	0.6	0.552
Nutrients (N)	0.0	0.928	0.1	0.848
Salinity (S) †	5.4	0.063	7.2	0.030
Temperature (T)	1.0	0.421	2.3	0.225
Quadratic terms:				
LL	9.8	0.011	20.5	< 0.001
FF	6.4	0.042	5.9	0.052
RR	1.2	0.365	0.6	0.531
NN	0.1	0.828	0.0	0.929
TT	2.0	0.262	4.2	0.103

Table 4.4 Description of the five equations initially developed from the OZOVEG database, showing: Terms included and the significance of each equation; test results from the jacknifing procedure (MAD = Mean Absolute Deviation and RMSE = Root Mean Square Error of the jacknifed predictions); and test results from the performance test which assessed the risk of misclassifying a species as either sensitive or insensitive.

					Results from jacknifed predictions					No. of spp. mis-classified as:	
Approach	Terms included	R^2	F	p	MAD	RMSE	No. sensitive species predicted (target=11)	% Predicted/ target	Total no. of spp. mis-classified	Sensitive	Insensitive
1) All terms	L F R N S T LL FF RR NN TT	34.9	2.58	0.010	0.195	0.255	13	118.2	14	8	6
2) All terms significant at p<0.05 as single factors	L F S LL	28.9	6.11	< 0.001	0.167	0.216	9	81.8	12	5	7
3a) First equation derived from backwards elimination	L S	25.9	11.38	< 0.001	0.162	0.201	10	90.9	13	6	7
3b) Second equation derived from backwards elimination	L F	23.8	9.67	< 0.001	0.156	0.202	9	81.8	14	6	8
3c) L on its own	L	21.5	18.09	< 0.001	0.155	0.199	4	36.4	11	2	9

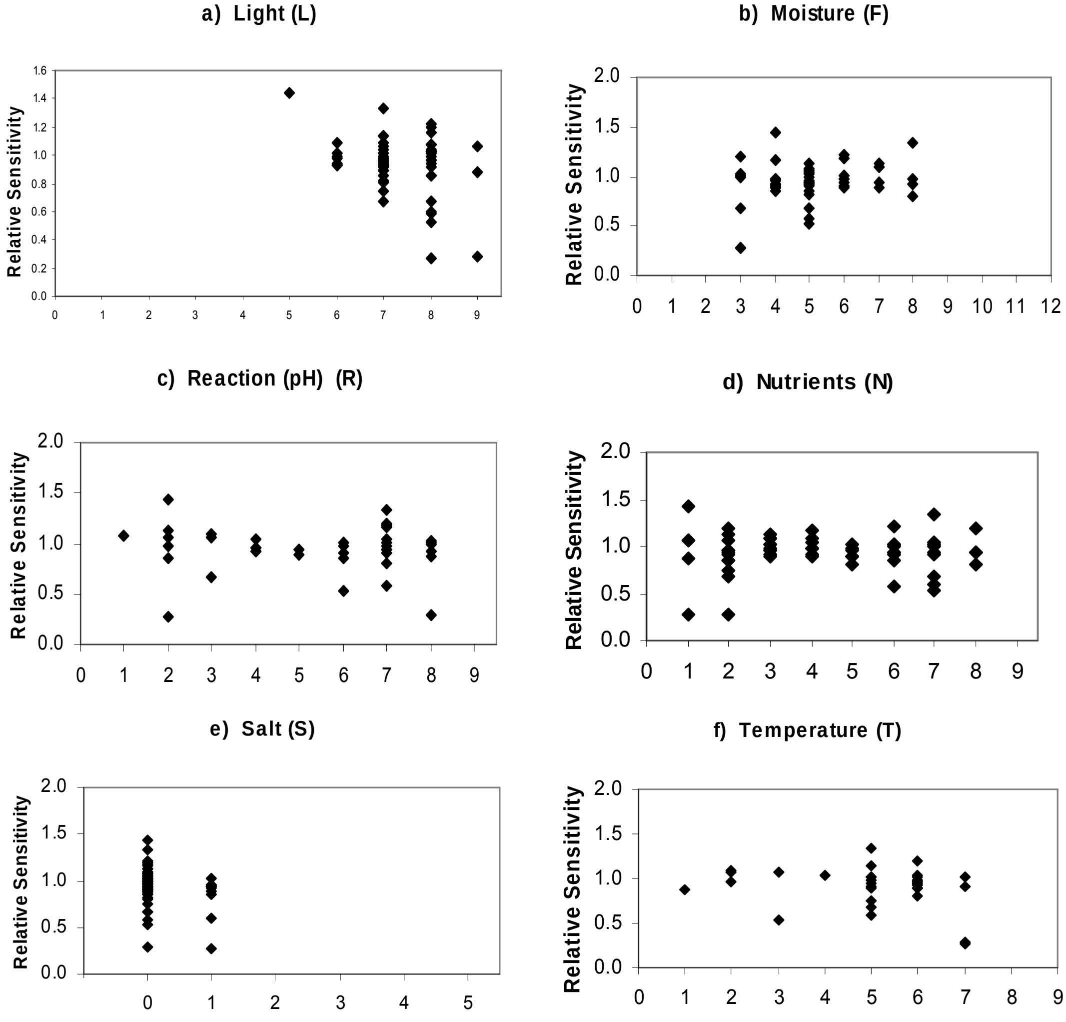


Figure 4.10 Relationship between Relative Sensitivity (RS) to ozone and individual Ellenberg Indicators showing a) Light, b) Moisture, c) Reaction (pH), d) Nutrients, e) Salinity and f) Temperature. Only the lower part of the range is shown for Ellenberg Salinity values.

All equations produced significant regressions (Table 4.4), with equation 1 (all terms) having the highest R^2 of 35%, but being the least significant. The most significant equation was 3c containing just Light on its own. However, this had the lowest R^2 of 21.5%. Of the equations containing 2 terms, equation 3a with Light and Salinity had a higher R^2 and was more significant than equation 3b with Light and Moisture. Equation 2 (only terms significant as single factors) had a higher R^2 than 3a, but was less significant. The equations explained a relatively low proportion of the variation. However, given that there are no clear physiological reasons which explain why Ellenberg Indicator values should be able to predict ozone sensitivity, and the fact that the Indicator values are an ordinal score, this degree of prediction is remarkably good.

The results of the jacknifed predictions show that the prediction error (RMSE) varies from around 20 to 25% overall, but decreases as the equations contain fewer terms. On this basis we might have chosen the equation with the fewest terms. However, the risk of misclassification is an important consideration in selecting the most appropriate equation. Accurate prediction of the response of the most sensitive species is arguably more important than those species which are relatively insensitive to ozone (i.e. with an RS close to 1). In the performance tests, no single equation performed best with respect to the risk of misclassification. Equations 1 and 3a came closest to predicting the actual number of species classified as sensitive. However, this relatively simple measure conceals further differences in the performance of these equations. Looking at the total number of species misclassified, equation 1, which had the highest R^2, misclassified the greatest number of species (14), together with equation 3b. The equation that performed best with regard to the risk of misclassification was equation 3c, containing the single term Light, which only misclassified 11 species. However, this equation seriously underpredicted sensitivity, with a high risk of falsely classifying sensitive species as insensitive.

In order to take the predictive work forward, one equation was chosen. When the observed versus fitted values were plotted (e.g. Figure 4.11), all equations revealed a broadly similar pattern, dictated by the distribution of Ellenberg Light values of the species in the database. Further examination of the fit of the observed values against the residuals suggested that equation 3a was the best predictor for species with the highest and lowest observed RS, and therefore gave the lowest risk of misclassification at this level. This equation (below) was used for further development of the method:

$$RS_p = 1.805 - 0.118 Light - 0.135\sqrt{Salinity} \qquad (3a)$$

Where RS_p is predicted Relative Sensitivity, Light is the Ellenberg Light value and Salinity is the Ellenberg Salinity value for the species being predicted. The square root of S is used to reduce the influence of high Salinity values on predictions of ozone sensitivity. This is because the range for this Indicator extends up to 9 for inundated species of saltmarsh habitats, and predicted ozone effects on species with high Salinity values can not be substantiated based on the coverage of species in the database.

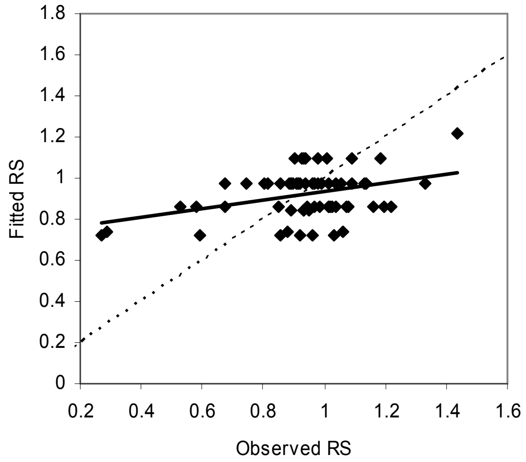


Figure 4.11 Plot of fitted (predicted) relative sensitivity against observed relative sensitivity for Equation 3a applied to all 65 species in the dataset. The dark line shows the line of best fit through the fitted points, the dotted line shows the ideal 1:1 relationship.

The calculated MAD and RMSE for this equation applied to all 65 species in the database are 0.152 and 0.187 respectively. The plot of observed against fitted relative sensitivity for equation 3a above is shown in Figure 4.11. The equation consistently under predicts the true sensitivity of those species at the extremes but the general fit is good. There is considerable scatter in the relationship, which is to be expected when the R^2 is only 25%. However, the relative position of species in the prediction remains similar and the equation accurately identifies the most sensitive species.

Applications of the model at the community level

This far we have described a method which can be used to predict the sensitivity of an individual species to ozone (RS_p), based on its Ellenberg Indicator values for Light and Salinity. An important next step is to be able to apply this to whole communities, and this can be done in two principal ways. Firstly, because it is a regression-based approach, it can be used quantitatively to predict the net change in biomass in a community. Secondly, it can be used to produce a ranking of sensitivity of different vegetation communities.

A list of the dominant species in a community, accounting for as much of the total cover as possible, is required for an estimate of the net change in biomass. RS_p was calculated for each species using Equation 3a above. The difference in RS_p from the theoretical state of no change ($RS_p = 1$) was calculated (i.e. $RS_p - 1$). The net percentage change in biomass in the community, termed the Ozone Response Index (ORI%), was then calculated by averaging the predicted changes in biomass for all species in the community and multiplying by 100 to give a percentage change. The equation is summarised as follows:

$$ORI\% = \frac{\sum_{i=1}^{n} (\mathrm{RS}_{\mathrm{p}_i} - 1)}{\mathrm{n}} \times 100 \qquad (4)$$

Where ORI% is the Ozone Response Index, RS_{pi} is the predicted RS for species *i* and *n* is the number of species utilised in the prediction of biomass change.

Equation 4 above, applied to simple presence/absence data can give a rough estimate of the net predicted change in biomass. However, it assumes equal cover distribution between all species. A more realistic estimate will be achieved by weighting the predicted change in biomass by some measure of the relative abundance of each species. Cover-weighting proceeds as follows: RS_p is obtained for each species and the difference from $RS_p = 1$ calculated as in equation 4 above. This is then multiplied by the percent cover for each species and all values are summed to give a net change. The final value is scaled as a proportion of the total cover available in the community to give the cover-weighted prediction of net change in biomass $ORI\%_{cw}$. The equation is summarised as follows:

$$ORI\%_{cw} = \frac{\sum_{i=1}^{n} \left[(\mathrm{RS}_{\mathrm{p}_i} - 1) \times (\mathrm{cover}_i) \right]}{\sum_{i=1}^{n} (\mathrm{cover}_i)} \times 100 \qquad (5)$$

Where ORI%$_{cw}$ is the cover-weighted Ozone Response Index, RS_{pi} is the predicted RS for species *i*, $cover_i$ is the percentage cover or other measure of abundance of species *i*, and *n* is the number of species utilised in the prediction of biomass change.

As an example, these methods were applied to the only vegetation community on which the techniques can, at present, be tested: the Le Mouret experiment in Switzerland (754 m a.s.l., 46°45’N/7°10’E) (Volk *et al*., 2003). The system in that study was a mid-elevation grassland of low to medium productivity (*c*. 0.9 kg m^{-2} y^{-1}). The community was identified as an *Arrhenatherion elatioris* alliance, confirmed by the presence of *Bromus hordeaceus*, but without a single dominant species. Important subdominants included the grasses *Holcus lanatus*, *Trisetum flavescens*, *Alopecurus pratensis* and *Arrhenatherum elatius*, the non-leguminous forbs *Plantago lanceolata* with *Ranunculus friesianus* and the legume *Trifolium pratense*. A total of 53 species of vascular plants was identified within the plot borders.

Results from five years exposure of ozone at an average AOT40 of 34.0 ppm h against an average background ozone concentration of 8.4 ppm h indicate a net change in biomass of – 23% (Volk *et al*., 2006). For accurate comparison with the ORI% tools, the biomass change at Le Mouret was re-scaled by the actual ozone exposure in each year, which gave a compound biomass change of – 22.3 % over the five years. Using the species presence and abundance data at Le Mouret for each year, the ORI% and ORI%$_{cw}$ were calculated. This gave a compound predicted change in biomass of – 25.1 % for the ORI% method and a compound cover-weighted prediction of – 26.9 % for the ORI%$_{cw}$ method over the five years.

The results for Le Mouret show that the ORI% methods accurately predict both the direction of response and achieve a fairly close prediction of actual change in biomass. It should be noted that the biomass change observed in the field increased over time (Volk *et al*., 2006), whereas the predicted changes using ORI%$_{cw}$ were reasonably similar from year to year as the relative dominance of species in the community did not change markedly and there was no great reduction in species number. This reflects both the cumulative nature of pollutant effects, and the difficulty of using changes in cover to predict changes in biomass. Bearing in mind these provisos, the successful application of the ORI% tools to field data reinforces a major advantage of the ORI% method, which is the ability to quantitatively predict changes in biomass in response to ozone.

Development of a community sensitivity index (CORI).

The second potential application of this model is to predict the sensitivity of a community to ozone, since an estimate of the predicted change in biomass may not show the full picture. For example, as species of high conservation value usually occur at low cover and at low frequency in a community, cover-weighted predictions of change in biomass will not highlight potential damage to these species. Secondly, while many species are negatively affected by ozone, some species are stimulated. Co-occurrence of both positively and negatively affected species in the same community may cancel each other out, leading to a low predicted change in biomass, concealing real ecological changes in community composition.

For these reasons, a separate tool was developed, designed to predict the sensitivity of a range of communities. We named this tool the Community Ozone Response Index (CORI), calculated as follows: A species list for the community was obtained. The RS_p of each species was predicted, and the difference in RS_p from the theoretical state of no change was calculated (i.e. $RS_p - 1$). In order to give greater weight to those species more strongly affected by ozone, and to take account of species which respond both positively and negatively to ozone, the Root Mean Square of ($RS_p - 1$) for all species was calculated. The resulting index was scaled within a range of 0 – 10, using a theoretical maximum value based on the maximum predicted change in biomass of any species in the European flora (69% using equation 3a above). The equation is summarised below:

$$CORI = \sqrt{\frac{\sum_{i=1}^{n} (\mathrm{RS}_{\mathrm{p}_i} - 1)^2}{n}} \times \frac{10}{0.69} \qquad (6)$$

Where CORI is the Community Ozone Response Index, RS_{pi} is the predicted RS for species i and n is the number of species utilised in the prediction of community sensitivity.

To illustrate this application, the technique was applied to 48 grassland and montane communities under the UK National Vegetation Classification (NVC) system (Rodwell, 1992), equivalent to level 4 in the EUNIS hierarchy. This classification was chosen because comprehensive species lists for NVC communities are readily available. The predicted CORI values for these grassland and montane communities ranged from 1.53 to 4.75. In practice, the CORI value for most communities will lie in the lower half of the range as the majority of species in the European flora are not strongly affected by ozone. The five most sensitive and five least sensitive of the 48 tested communities are shown in Table 4.5. The two communities predicted to be most sensitive to ozone were calcareous grasslands (CG2 - *Festuca ovina-Avenula pratensis* and CG3 - *Bromus erectus* grasslands). The two communities predicted to be least sensitive to ozone were also calcareous grasslands (CG8 *Sesleria albicans-Scabiosa columbaria* grassland, and CG9 *Sesleria albicans-Galium sterneri* grassland). This suggests that community sensitivity is strongly driven by the component species, rather than broad community type.

The basic requirement for calculating a CORI value for a community is a list of species and their Ellenberg numbers. The question then arises as to how many species are required to get a reliable estimate of CORI? While researchers working on a particular site may have a full species list, in practice, a comprehensive species list for each community may not be available. The problem of reduced species lists is even more likely to occur if the technique is to be applied as a screening tool for a wide range of communities. With this in mind, we conducted a sensitivity analysis using two of the NVC communities shown in Table 4.5, to determine a minimum number of species required to provide an acceptable estimate of the community sensitivity (CORI).

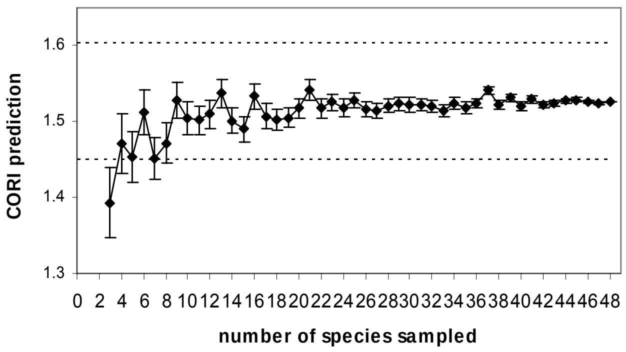
Table 4.5 Prediction of community sensitivity to ozone using the Community Ozone Response Index (CORI) scaled from 0 to 10, applied to 48 UK NVC grassland communities. The five most sensitive and five least sensitive communities are shown.

UK NVC code	NVC community description	CORI (0 - 10)
Most sensitive		
CG2	*Festuca ovina-Avenula pratensis* grassland	4.75
CG3	*Bromus erectus* grassland	4.49
U17	*Luzula sylvatica-Geum rivale* tall-herb community	3.52
U2	*Deschampsia flexuosa* grassland	3.17
U1	*Festuca ovina-Agrostis capillaris-Rumex acetosella* grassland	3.11
Least sensitive		
MG4	*Alopecurus pratensis-Sanguisorba officinalis* grassland	1.86
MG5	*Cynosurus cristatus-Centaurea nigra* grassland	1.86
U12	*Salix herbacea-Racomitrium heterostichum* snow-bed community	1.67
CG8	*Sesleria albicans-Scabiosa columbaria* grassland	1.56
CG9	*Sesleria albicans-Galium sterneri* grassland	1.53

Communities of contrasting species richness were chosen. The species list for the CG9 calcareous grassland contained 48 typical species, while the U17 tall-herb montane community contained 24 typical species (Rodwell, 1992). A repeat-sampling routine was written to run in Minitab v14.1 which took random samples of species from the community and calculated the CORI value. The number of species sampled initially was three, increasing in steps of one until all species were included. For each subset size tested, the population was sampled 200 times, and the average CORI and standard error were calculated (shown in Figure 4.12).

The results suggest that to be reliably within 5% of the CORI value, a sample of 9 species is required. As a relatively low proportion of the European flora is strongly affected by ozone, smaller sample sizes will tend to under-predict sensitivity. Therefore, as the sample size increases, so does the estimate of CORI until it approaches the calculated value. The minimum number of species required for a reliable estimate will increase slightly with the species richness of the community. In addition, variability in the prediction will alter with the community, depending on the sensitivity of the component species. However, this analysis gives an extremely useful indication of the number of species needed for an acceptable estimate of CORI for a community.

a) CG9 - *Sesleria albicans-Galium sterneri* grassland



b) U17 - *Luzula sylvatica-Geum rivale* tall-herb community

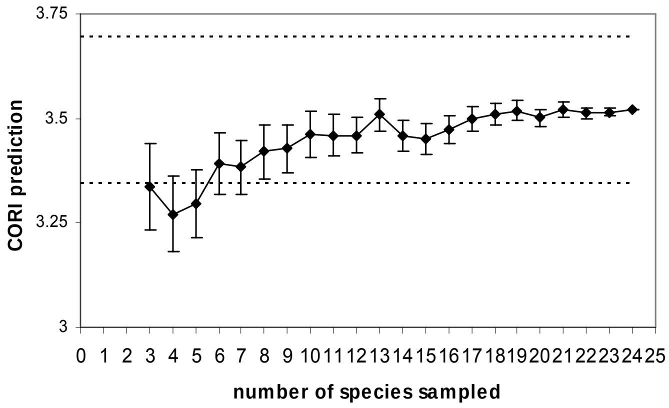


Figure 4.12 Sensitivity analysis illustrating how the CORI prediction varies with increasing sample size in two communities of contrasting species richness, where: a) n = 48 species and b) n = 24 species. Bars show +/- 1 s.e. Dotted horizontal lines show limits of +/- 5% from CORI value calculated using all available species

4.3.6 Identifying ozone-sensitive communities suitable for mapping exceedance of critical levels

(CEH Bangor)

The Ellenberg method described in the above section shows good predictive powers for communities for which species composition and abundance data are available. Initial investigations have shown the suitability for applying the method to UK NVC communities. For wider application within Europe, we have investigated simpler indices that can used to identify ozone-sensitive communities that could be mapped using currently available European land-cover maps. Cinderby *et al.* (in press) recently reported on progress with harmonisation of two European land-cover datasets: the SEI land-cover dataset (Cinderby, 2002) and the European Environment Agency (EEA) Corine land- cover dataset (de Smet and Hettelingh, 2001). For (semi-)natural vegetation, additional data on climatic zone, moisture regime, soil pH and altitude were included with the combined dataset to aid differentiation between related vegetation classes within a EUNIS category to separate spatially communities such as EUNIS F4.1 (Wet heath) and EUNIS F4.2 (Dry heath). The feasibility of mapping those communities identified at EUNIS levels 2, 3 and 4 as ozone sensitive will be described here, with example maps provided. Thus, this part of the study shows how ozone sensitive communities of semi-natural vegetation can be identified based on the responses of individual species, confirms and adds to the choices identified in the Mapping Manual (LRTAP Convention, 2004) as potentially ozone sensitive and indicates factors to be taken into account in mapping the location of these communities within the framework of the EUNIS Hierarchy.

Establishing which EUNIS communities the 83 species in the OZOVEG database are present in was difficult since such information is incomplete for the whole of Europe. Such data does exist, however, for the 69 species in the database that are found in the UK, in the form of the National Vegetation Classification, NVC (Rodwell *et al.*, 1992). Using the UK National Biodiversity Network (NBN) habitats directory (www.nbn.org.uk/habitats), the NVC communities with six or more species sensitive to ozone (either negative ($RS \leq 0.9$) or positive ($RS \geq 1.06$)) were identified and converted into EUNIS code. RS values of 0.9 and 1.06 represented the median values for RS values below and above 1 respectively (Figure 4.13), and were selected as the delimiters for ozone sensitivity. In cases where more than one NVC community was represented by a EUNIS code, values for the indicators described below were averaged, resulting in a dataset representing 54 EUNIS communities at level 4. When needed, these were subsequently averaged to provide mean values for each level 2 habitat, with 19 such habitats represented in the database, and again for level 1 habitats with 7 represented.

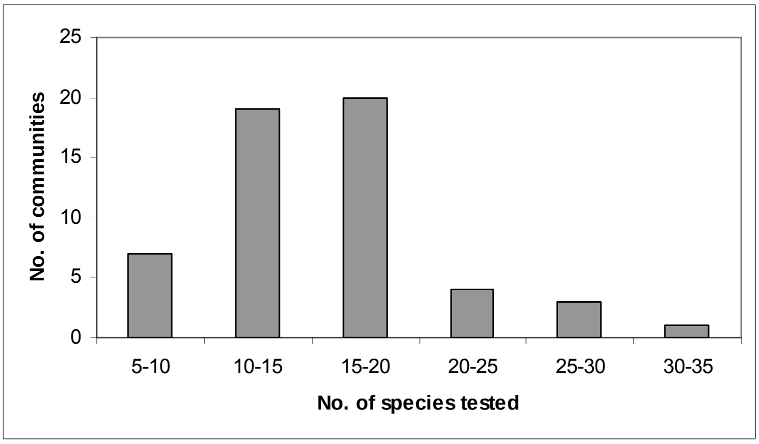


Figure 4.13 Frequency distribution for the number of species tested for ozone sensitivity within EUNIS level 4 communities.

Use of the UK NVC classification system to identify species present in EUNIS communities has introduced a northern-European bias to the predictions presented. Although this bias was in keeping with the central and northern European bias of the RS data, it was not possible to include Mediterranean communities such as the Dehesa grassland (EUNIS E7.3) identified within the Mapping Manual as ozone-sensitive from the work of Gimeno *et al.* (2004a). In many cases, the information for each EUNIS level 4 community was an average of that for several NVC communities making estimates of the number of OS species present per community less reliable. For example, EUNIS E1.72 (Non-Mediterranean dry acid and neutral closed grassland [*Agrostis*] – [*Festuca*] grassland) is represented by the nine NVC communities, CG2-CG10 which range in species number from 85 to 133. However, the benefit of enabling community comparisons outweighed the disadvantages of using NVC to identify species present in EUNIS communities.

For many communities, RS data was only available for a relatively small number of species. The use of 6 or more OS species as a selection criterion reduced the number of communities studied from 100 to 54, but improved the relevance of the predictions made. For the 54 communities studied, the mean number of species per community present in the database was 15.3, with a 1st to 3rd quartile range of 11 to 18.

Several indices have been considered as descriptors of ozone sensitivity for a community. The aim throughout was to use the simplest approach possible to ensure that the methods used could be easily applicable at all geographical scales. Estimating the percentage of ozone-sensitive species (%OS: no of OD+OI as a percentage of no of species from the specified community within the database) meets these criteria. For the 54 level 4 communities, the %OS had a mean of 53.3% and a range of 29.4 to 88.9%. The functions used were by necessity (see introduction) for species grown in individual pots without inter- and often intra-specific competition. Since several studies have shown that ozone impacts on an individual species are influenced by competition (see reviews by Davison and Barnes, 1998, Fuhrer *et al.*, 2003, Bassin *et al.*, in press), there will be species-specific "noise" associated with the calculation of RS when interpreted in relation to communities which introduces uncertainties into predicting community responses.

The %OS method used to identify ozone-sensitive communities appears to be most suitable for application at EUNIS levels 1 and 2 where broad conclusions are required. At EUNIS levels 3 and 4, additional information may be required to aid interpretation. For example, the predicted OS_n ranged from 20 to 104 with a mean of 46.9. This indicator may be of more relevance for species-rich communities, but it has the disadvantage of requiring information on the number of species typically present in a community. Unfortunately, this information is not readily available for many European communities of (semi-)natural vegetation. Where such detail is present together with species names, more sophisticated analysis methods that predict the responses of previously untested species could be used (Section 4.3.5, Jones *et al.*, in press).

The 54 level 4 communities studied represented seven EUNIS level 1 categories (Figure 4.14). By far, the most communities at 23 were representatives of EUNIS code E (Grasslands), with Heathlands, scrub and tundra (EUNIS code F, henceforth described as "Heathlands") and Mires, Bogs and Fens (EUNIS code D) having the next highest representation at eleven and eight level 4 communities each respectively. Less than five communities within the database were representatives of Coastal habitats (EUNIS Code B), Inland un-vegetated or sparsely vegetated habitats (EUNIS Code H) and Regularly or recently cultivated agricultural, horticultural and domestic habitats (EUNIS Code I, henceforth described as "Cultivated land").

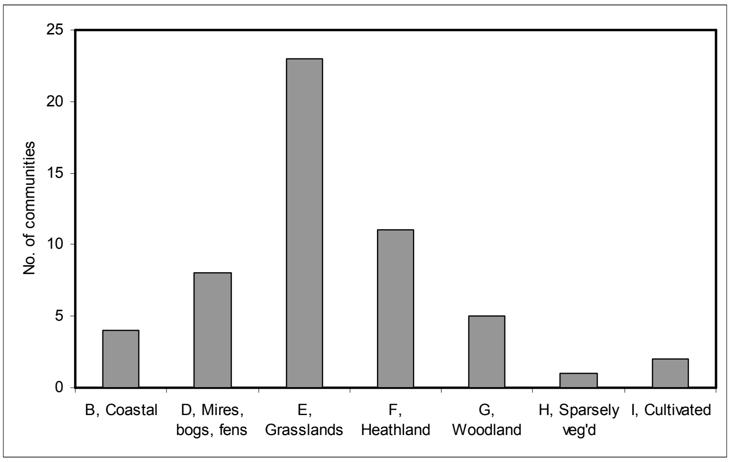


Figure 4.14 The number of level 4 communities in each EUNIS level 1 category (abbreviated names used).

For several habitats at EUNIS level 2 (F9, D1, G3, F2, E4, and D5 in order of decreasing sensitivity, abbreviated names provided in Table 4.6), it was predicted that over two thirds of species present are potentially sensitive to ozone. However, ≤15.0% of the species had been tested from four of these habitats (F9, D1, G3 and D5) representing just 7 to 9 species, and thus these results should be treated with some caution. These communities were omitted from subsequent analysis, along with B3 and I1 since their results were derived from only one and two level 4 communities respectively (Table 4.6). There is greater confidence in the results for the remaining habitats with 20% or more species represented in the database: F4, E1, E3, B1 and E2 in decreasing order of %OS from 51.7% to 30.7%. These habitats were selected for further analysis, together with E4 (19.3% tested, 68.1 %OS) and E5 (19.3% tested, 51.6 %OS).

Of the seven EUNIS level 2 habitats selected for further study, E2 habitats (Mesic grasslands) were the least sensitive to ozone based on %OS. E1, E3 and E5 (Dry grasslands, Seasonally-wet and wet grasslands (abbreviated henceforth to “Wet grasslands”) Woodland fringes respectively) were of similar medium sensitivity, and E4 (Alpine and sub-alpine grasslands) and F4 (Temperate shrub heathland) habitats were the most sensitive (Table 4.6). Within the E4 habitat, E4.32 (Acid alpine and sub-alpine grasslands – Oroboreal acidocline grassland) was the most sensitive with 75.0% of species showing sensitivity to ozone (based on 20.2% of species tested, data not presented). The other two E4 communities, E4.11 (Boreo-alpine acidocline snow-patch grassland and herb habitats) and E4.21 (Oroboreal [*Carex bigelowii*]-[*Racomitrium*] moss heaths) had similar %OS of 62.7 and 66.7% respectively. The four F4 communities had a broader range of %OS (42.9 to 67.2%), with F4.21 (Temperate shrub heathland –Dry heaths –Sub-montane [*Vaccinium] –* [*Calluna*] heaths) being the most sensitive (data not presented).

Table 4.6 Ozone sensitivity at EUNIS level 2 determined from the relative sensitivity of component species.

EUNIS	**Abbreviated name**	**Mean No. of spp. in habitat**	**No. of level 4 comm. included**	**Mean No. of spp. tested**	**No. of OS[1] spp.**	**% OS[2]**
B1	Coastal dunes and sandy shores	80.9	3	16.6	6.7	41.6
B3	Rock cliffs and shores	73.5	1	15.5	6.5	42.0
D1	Raised and blanket bogs	81.5	2	7.5	6.0	80.4
D2	Valley and transition mires	119.0	1	10.0	6.0	60.0
D4	Calcareous mires	98.3	4	10.8	6.3	59.8
D5	Sedge and reed beds	60.0	1	9.0	6.0	66.7
E1	Dry grasslands	91.9	6	20.5	9.8	48.6
E2	Mesic grasslands	78.8	4	25.6	7.9	30.7
E3	Seasonally wet grasslands	79.4	6	15.6	6.8	45.0
E4	Alpine and sub-alpine grasslands	72.7	3	13.6	9.1	68.1
E5	Woodland fringes	101.8	4	17.9	9.4	51.6
F2	Arctic, alpine and sub-alpine scrub	78.9	3	12.8	8.6	72.4
F3	Temperate and Mediterranean – montane Scrub	94.7	3	15.7	7.7	49.8
F4	Temperate shrub heathland	67.9	4	13.4	6.8	51.7
F9	Riverine and fen scrubs	112.0	1	7.0	6.0	85.7
G1	Broadleaved deciduous woodland	126.6	4	11.4	6.4	56.4
G3	Coniferous woodland	70.0	1	8.0	6.0	75.0
H1	Cave systems	99.0	1	17.0	9.0	52.9
I1	Arable land and market gardens	76.0	2	17.0	6.5	38.2

[1] Ozone-sensitive

[2] Percentage of ozone sensitive species within the community

Table 4.7 The ten EUNIS level 4 communities with the highest %OS.

Rank	EUNIS	Name	Mean No.of spp. per comm.	% of species tested per comm.	Predicted % OS[1] within comm.	OS_n (No. of spp predicted to be O_3 sensitive)
1	F2.11	Boreo-alpine acidocline snow-patch [*Salix herbacea*] scrub	43	20.9	88.9	38.2
2	E4.32	Oroboreal grassland	54.7	20.2	75.0	41.0
3	E1.71	[Nardus tricta] swards of non-Mediterranean dry acid and neutral closed grassland	79	20.3	68.8	54.3
4	E3.52	Heath [Juncus] meadows and humid [Nardus stricta] swards	52	21.2	54.5	28.4
5	E5.3	[Pteridium aquilinum] fields of Woodland fringes and clearings and tall forb stands	70	24.3	52.9	37.1
6	F4.25	Ibero-Atlantic [Erica – Ulex – Cistus] heaths	70	21.4	50.5	35.3
7	E1.72	[Agrostis] – [Festuca] grassland of non-Mediterranean dry acid and neutral closed grassland	100	25.1	48.8	48.8
8	E1.92	Perennial open siliceous grassland	120	20.8	48.0	57.6
9	E1.B	Heavy-metal grassland	46	34.8	43.8	20.1
10	F4.12	Southern wet heaths	60	23.3	42.9	25.7

[1] Ozone-sensitive

The highest %OS for a level 4 community was 88.9 for F2.11 (Boreo-alpine acidocline snow-patch [*Salix herbacea*] scrub) which was calculated using data for 20.9% of the species associated with this community (Table 4.7). A further five EUNIS level 4 communities had %OS values of >50% (E4.32, E1.72, E3.52, E5.3 and F4.25 in descending order of %OS, names provided in Table 4.7). For the ten highest ranking level 4 communities presented in Table 4.7, the total number of species commonly found in each community varied from 43 to 120 whilst the % of species tested for ozone sensitivity was on average 23.2% (range 20.2 – 34.8). Applying the %OS to the number of species in each community provided an estimate of the number of ozone sensitive species. Using this criterion, E1.92 (Perennial open siliceous grassland) and E1.71 (Non-Mediterranean dry acid and neutral closed grassland – [*Nardus stricta*] swards) had the highest sensitivity of these ten communities at 57.6 and 54.3 OS species per community respectively (OS_n). Whether %OS or OS_n should be used to describe ozone sensitivity at EUNIS level 4 would depend on the application of the analysis. %OS may be more suitable for comparisons at the European scale where detailed species lists are not available for communities, whilst OS_n provides a more useful indicator at the local scale where conservationists might need to rank the sensitivity of communities based on number of species potentially at risk.

4.3.7 Mapping the location of ozone-sensitive communities
(SEI-York)

The UNECE now recommends the EUNIS classification as the basis for defining critical loads and critical levels for different communities. However, EUNIS is a classification scheme, and not a mapping scheme; no map of EUNIS communities for Europe exists, which makes the application of critical levels and loads based on the EUNIS classification problematic; in the absence of such maps, UNECE is reliant on plant community maps used by individual countries which may be quite inconsistent in their basis. This issue is particularly difficult for semi-natural communities, for which empirical critical loads of nitrogen have been defined for specific EUNIS classes, and for which differentiated ozone risk assessment has been developed in this project. The work reported here is the first attempt to produce a map of important EUNIS categories across Europe; inevitably, due to unforeseen problems and limitations in key datasets, it has not been possible to provide a definitive map of all desired EUNIS categories; nevertheless the work reported below provides an important step in mapping EUNIS classes for air pollution risk assessment.

The identification and mapping of semi-natural habitats across Europe has involved the development of a base map of relevant environmental conditions, including climatic zones, soil moisture and soil pH. This environmental conditions map allows for the refinement of the classification structure for semi-natural vegetation, and particularly for grasslands, beyond the basic level contained in the SEI and EEA land cover maps. This enables the identification of particular EUNIS habitat classes, which are of interest in terms of their sensitivity to ozone and nitrogen deposition and their conservation importance. However, it should be emphasised that it is only possible to map certain EUNIS classes beyond Level II.

The base datasets, and their application in mapping of grassland EUNIS classes, are summarised in Table 4.8.

Table 4.8 Summary of base datasets for mapping.

Name	Source	Resolution/Scale	Derived Classes	Description
Corine Land Cover 2000	European Environment Agency	100m pixels	3.2.1 – Natural Grasslands 2.3.1 – Pastures 2.4.2 – Complex Cultivation Patterns 2.4.3 – Land principally occupied	Identifies the extent of grassland across Europe.
Soil Map of the World	Food and Agriculture Organisation	1:5M	Soil pH and texture for soil associations	Identifies pH to delimit EUNIS classes and texture for use in deriving soil moisture regimes
Soil Water Index from ERS Scatterometer Data	University of Vienna	Interpolated Point Dataset	Soil Water Index	Identifies soil water indexes for use in assessing soil moisture.
Bio-Geographical Zones	European Environment Agency	1:10M	Bio-Geographical Zone	Used to identify climatic zones to delimit EUNIS classes.

The Soil Water Index (SWI) data provide information on the volume of water present in the soil relative to the volume of pores. The SWI is calculated using a two-layer infiltration moduleel to estimate water present in a soil profile of 0-100 cm, using remote sensed information for the moisture content in the soil surface layer, since the electromagnetic waves transmitted by scatterometers penetrate only a few centimetres into the soil surface. In order to assess soil moisture for the whole rooting depth of grasslands soil texture was also assessed. Soil moisture potential release curves were used to determine three classes of grassland soil moisture for the various combinations of texture and water index. In the geographic information system the SWI point data was interpolated to develop a continuous surface of SWI values across Europe. The FAO soil association data was reclassified into five classes of texture, coarse, medium-coarse, medium, medium-fine and fine. In the final groupings, medium-coarse and medium-fine textured associations were assigned medium texture to assess their soil moisture. The two maps were then overlaid and the soil moisture class for the combination of values determined according to the parameters in Table 4.9.

Table 4.9 Summary of classification of wet, mesic and dry grassland areas using soil texture and Soil Water Index data.

Soil texture	**Soil Water Index (%)**		
	Wet	Mesic	Dry
Coarse	> 13	13 to 9	< 9
Medium	> 28	28 to 22	< 22
Fine	> 63	63 to 53	< 53

For coastal habitats and heathland, the appropriate EEA class maps were used, but for grasslands, the EEA data provide only a breakdown of pastures and managed grasslands. The environmental data compiled from a variety of sources has been used to further delimit the natural grasslands identified on the EEA Corine land cover map into EUNIS habitat classes. This classification structure has been adopted as the preferred scheme for European mapping to be used in the assessment of air pollution impacts. Figure 4.15 below summarises the mapping process, using SWI data, climate data, and soils data to distinguish particular grassland communities of interest both in terms of the impacts of ozone and in terms of the impacts of nitrogen deposition.

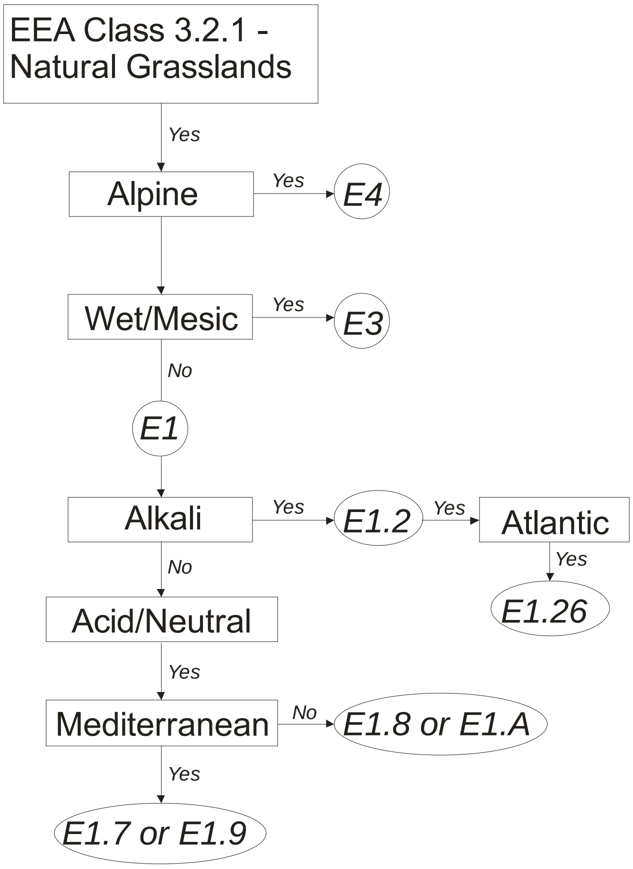


Figure 4.15 Decision tree for mapping of European grasslands by EUNIS classes.

To illustrate the results of the mapping process, Figure 4.16 presents the distribution of major grassland communities and heathlands for an area of northern and western Europe. Since the classification is based on EEA classifications, to ensure consistency with other major EU databases, maps do not include non-EU countries (Switzerland; Norway). These areas will be mapped using the SEI land-cover database.

As is expected, class E2 (mesic grassland) dominates the map visually, reflecting the fact that productive and improved pasture is the dominant grassland type in most areas of Europe. Therefore, in order to improve the visual identification of other EUNIS classes which are of greater conservation interest, E2 has been omitted from Figure 4.16. E3 (wet grassland) is found in cooler, moister areas, such as the Cambrian Mountains in Wales and the Pennines and Cumbria, where it occupies significant areas. E4 (alpine and sub-alpine grassland), as expected is dominant in the montane areas such as the Alps and the Pyrenees, suggesting that the climatic criteria used worked successfully. There is only a very small area of E1 (dry grasslands) shown on this map, although larger areas dominated by E1 are apparent at a full European scale, particularly in Mediterranean regions. It is possible that the criteria for distinguishing dry grasslands was too rigorous, so that communities of northern and western Europe which are classified as dry grassland within the EUNIS scheme are not identified in the map. However, this may also reflect the spatial scale of the underlying scatterometer data used for calculating SWI being at too coarse a resolution, or not identifying water stress in these parts of Europe. The results for heathlands are broadly consistent with expectations. Temperate shrub heath (F4) is shown as dominant areas of moorland in northern and western

Britain, supplemented by small fragments of lowland heath in England (e.g. Dorset and Surrey), the Benelux countries and northern Germany. In contrast, the Mediterranean area shows substantial areas of garrigue and maquis (F5/F6). The identification of F4, rather than F5/6, in north-west Spain and central France needs further confirmation against descriptions of local vegetation.

The map presented in Figure 4.16 can be interpreted in relation to Section 4.3.6. EUNIS category E4 (alpine and sub-alpine grassland) was identified as potentially the most sensitive to ozone based on %OS, with E1 (dry grasslands), E5 (woodland fringes) and F4 (Temperate shrub heath) also identified as ozone –sensitive.

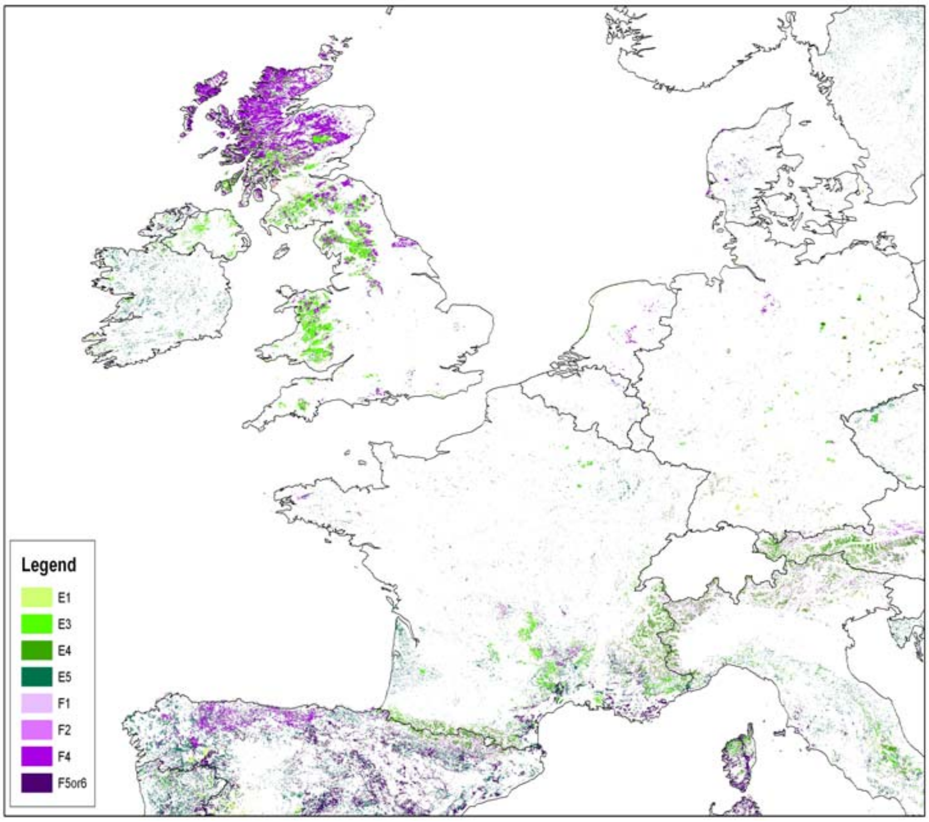


Figure 4.16 Geographical distribution of major EUNIS classes for grassland and heathland across north-west Europe.

4.3.8 *Effects of management, nutrient status, water stress and species composition on ozone flux to grasslands*

(SEI-York)

It had originally been proposed that a database of individual community parameters that relate to seasonal flux would be constructed and used to develop a qualitative risk assessment for different communities. The approach was modified for two reasons:- (a) it proved impossible to obtain relevant data as planned from ICP Vegetation participants or from other reference sources and (b) insights from application of the DO_3SE flux model under Defra contract SPU24 showed that the individual community parameters were not independent risk factors, but were actually interdependent in quite subtle ways. The latter insight is particularly significant, as it demonstrates that the original qualitative risk assessment, based on ranking of independent factors, would be very misleading. For this reason, we have focussed on a model-based analysis of risk, using the grassland types identified as ozone-sensitive using the OZOVEG database, as described in Section 4.3.6. The analysis has concentrated on three typical grid squares in Continental Central Europe (southern Germany) (CCE), Atlantic Central Europe (southern Scotland) (ACE), and Northern Europe (southern Finland) (NE). No assessment was carried out for southern Europe due to limited representation of Mediterranean species in the OZOVEG database and the limited basis for parameterisation of the DO$_3$SE model for species from this area.

Predictions based on a mono-species grassland growth model

In order to assess the effect of community characteristics on the seasonal development of leaf area index, and soil water deficit, we used a grassland growth model (Eatherall *et al.*, 1992; Terry & Woodward, 1994) which has the capacity to simulate morphological and physiological processes of temperate grass growth as a function of changes in solar radiation, fractional day-length, temperature, humidity, rainfall, soil nitrogen and CO_2 concentration. The model can be divided into seven components, as follows: - (1) generation of assimilate (photosynthesis); (2) partitioning of assimilate; (3) canopy expansion (leaf area); (4) respiration of assimilate; (5) ageing and death of tissue; (6) soil moisture and plant water loss (transpiration); (7) soil nitrogen uptake and recycling. Each of the model routines is computed for 1 m^2 of ground area. Leaf area of individual leaves is calculated from a light dependent function for specific leaf area and the amount of assimilate partitioned to leaf growth each day; leaf area index (LAI) is calculated from the running totals of individual leaf areas. Throughout the model a value of $\psi = -1.5$ MPa is taken as the permanent wilting point for the grass crop; feedback from the effect of soil water potential on stomatal resistance (hence transpiration) is modelled by a simple relationship based on field measurements for grasses. The minimum and maximum stomatal resistances were changed from the original grass model for consistency with the values used in the DO$_3$SE model. An optional harvesting routine is also included which simulates the effects of management intervention.

Two forms of nutrient status were modelled. Firstly, the model was run assuming an improved sward with high soil nitrogen (initial soil N content 5 g N m^{-2}) and daily input of nitrate fertiliser of 0.5 g N m^{-2} d^{-1}. In the second simulation, the model was run using low soil nitrogen (initial soil N content 2.5 g N m^{-2}) and no fertiliser additions. Three different soil types were used ranging from coarse (sandy soil), through medium to fine (clay soil). Soil moisture release curves were constructed for the three soil types and the parameters of the algorithms in the grassland model were adjusted so that the characteristics of the curves were comparable to those of the DO$_3$SE model. Two rooting depths of 0.3 m and 0.9 m were simulated in the models as surrogates for shallow and deep soils. Management intervention of simulated ‘cuts’ was introduced for the grassland model, with regular ‘cuts’ in the high nutrient soils once the LAI reached 4. In the low nutrient status runs LAI generally never increased much above 4 in the early season flush of growth, followed by a mid-season dip before peaking in late summer. Therefore, just a single late season cut was introduced for the low nutrient soils to coincide with the last cut for the high nutrient soils for a given region and soil type.

To provide a reference point for the analysis of community-related factors, and to assess the combined impact of nutrient status and management, Figure 4.17 shows annual time-courses of LAI and soil water potential (SWP) for a productive mesic grassland (E2) (left-hand column), with regular hay cuts, and a similar system with no nutrient input and only one end of season hay cut, for the three locations. Productive grassland is characterized by much more rapid canopy growth compared to the low nutrient swards for all three locations. The growth season starts earlier in the warmer climates (CCE) compared to the cooler locations (ACE; NE). The longer growing season for CCE allows for 4 productive grassland 'cuts' compared to only 3 for NE and ACE. An interesting feature for all three locations is the much greater mid-season soil water potentials generated by the low nutrient swards compared to those of productive grasslands. This can be attributed to the fact that the regularly cut swards have much lower leaf area durations because of the regular removal of biomass, and hence reduced transpiration compared with the low nutrient swards. Mid-season SWPs were much greater for CCE and NE than for ACE, which generally has a wetter climate due to the maritime weather conditions.

Figure 4.18 shows annual time courses of canopy stomatal conductance (Gsto) and accumulated stomatal flux by an upper canopy leaf without an accumulation threshold ($AF_{st}0$) at the three locations, using the DO_3SE model parameterised for a productive mesic grassland (E2) (left-hand column), with regular hay cuts, and a similar system with no nutrient input and only one end of season hay cut (right-hand column). The range of values for both parameters at the three locations is comparable, but there are differences in the annual time course of Gsto, which reflect the effect of temperature and soil moisture deficit, partly through their effect on the development of LAI. These differences in the seasonal patterns of Gsto will have implications for modelled ozone flux, partly because of the timing of active growth in relation to periods of high ozone concentrations, and partly because of the influence of soil moisture deficit.

In the fertilised, heavily managed grasslands, temperature and ozone concentrations have the greatest effect on modelled flux, because, as shown in Figure 4.17, significant soil moisture deficits tend not to occur, because of the reduced evapo-transpiration associated with regular removal of biomass. For this reason seasonal $AF_{st}0$ is higher in CCE than in ACE or NE. However, with the unfertilised, low intensity management grasslands, the pattern is changed; although active growth, and Gsto, start earlier in central Europe, the faster growth rate and warmer drier climate lead to an earlier onset of limitation of Gsto by soil moisture deficit.; for example, SWP limitation of Gsto is predicted by about day 200 in CCE, but by day 240 in ACE; Given that the highest ozone concentrations commonly occur in July and August, this is a significant difference. In terms of flux-related community sensitivity, the key conclusion is that mesic productive grasslands may experience a greater ozone flux and impact, but that this effect is lost in cooler, wetter climates.

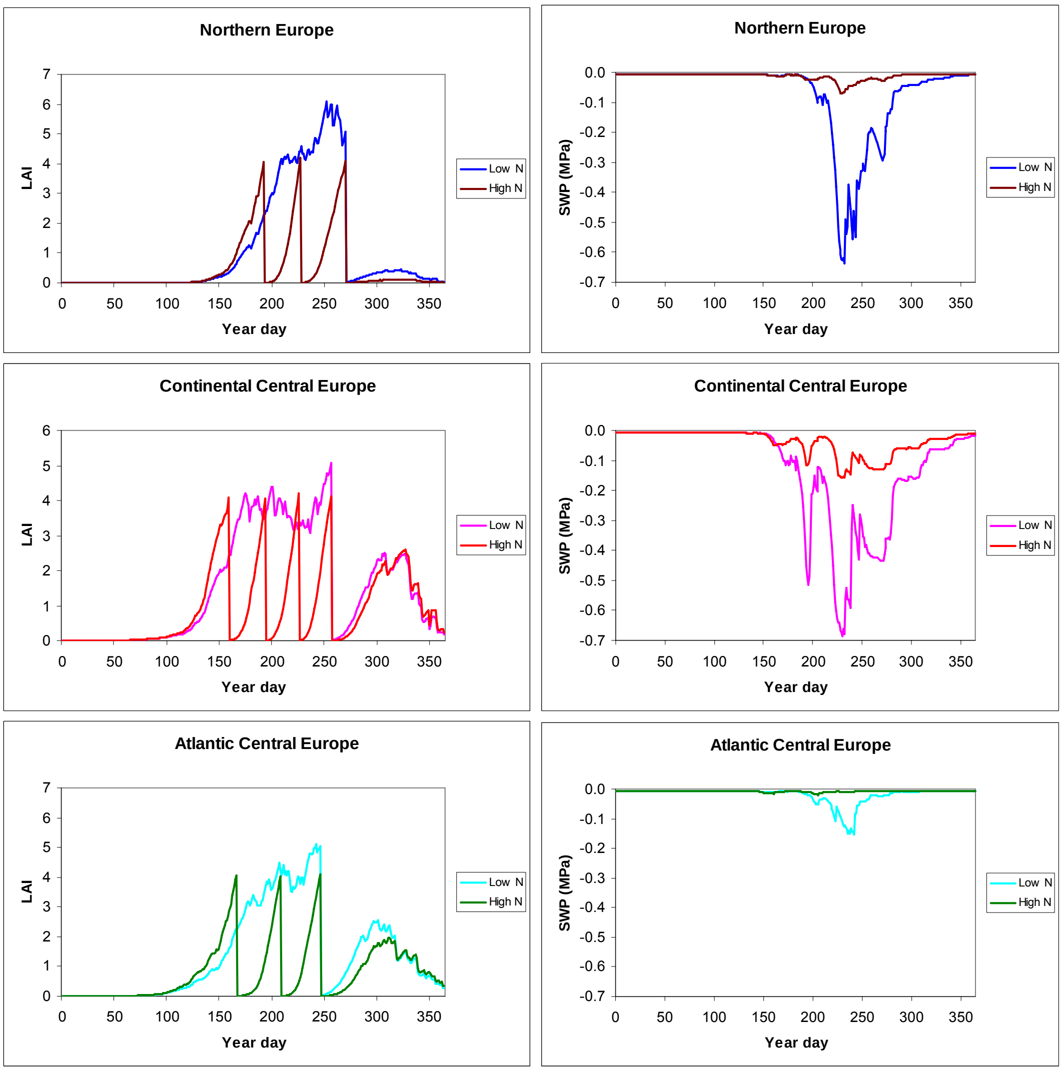


Figure 4.17 Modelled time-course of LAI (left-hand column) and SWP (right-hand column), comparing a productive, actively managed canopy, and an unfertilised low-intensity management at the three locations.

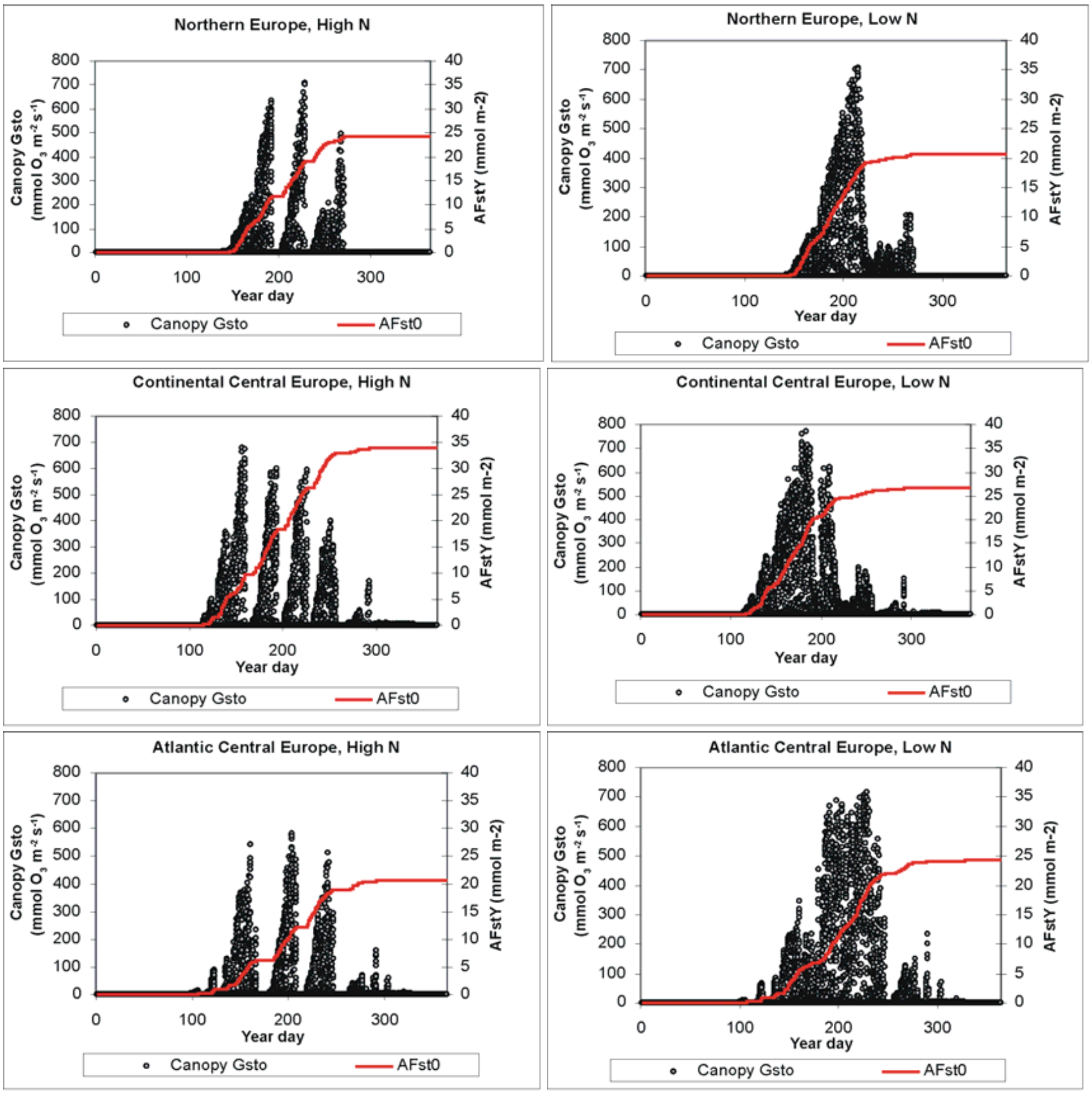
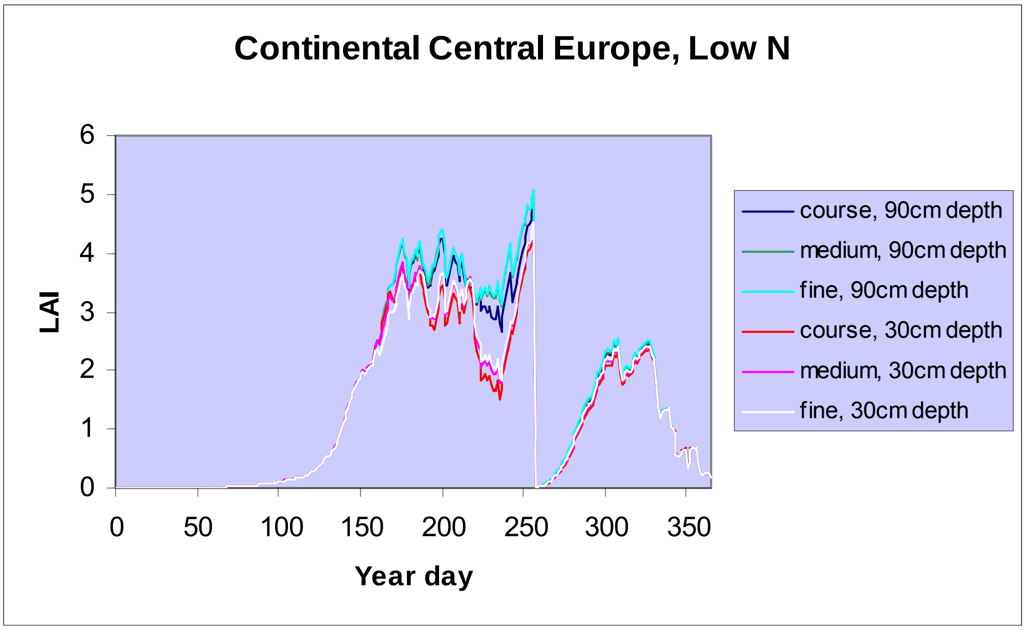


Figure 4.18 Modelled timecourse of Gsto and AF$_{st}$0 for three locations in Europe. The left-hand column shows simulations for productive, intensively managed grassland, and the right-hand column shows simulations for unproductive, low intensity managed grasslands. For further detail, see text.

The results shown in Figures 4.17 and 4.18 assume a medium soil texture and a rooting depth of 90 cm. Many semi-natural grassland communities of high conservation value, however, are often found on fine (organic peaty) soil or coarse (sandy) soils. Furthermore, rooting depths are often confined to surface soil layers, because of the poorly developed soil structure, waterlogging or the presence of bedrock close to the surface. In order to investigate the effects of such factors, Figure 4.19 shows the modelled seasonal time-course of LAI and SWP, for the CCE location only, for a full combination of three soil textures (fine, medium, course) selected for consistency with the soil classes used in mapping (see below) and two rooting depths (30 and 90 cm). The main feature of these simulations is the mid-seasonal difference in peak LAI and SWP for the two rooting depths of the swards. Clearly, the swards growing on the shallow soils generate greater SWPs as they deplete their limited soil moisture supplies more rapidly than those of the deeper soils. A similar, but much less pronounced effect is evident also for the different soil textures for a given rooting depth. In this case the swards growing on the coarser (sandy) soils, deplete their available soil moisture reserves quicker (lower SWP) than the medium and fine soils which have a greater available soil moisture capacity.

Figure 4.20 shows the modelled seasonal timecourse of Gsto and $AF_{st}0$ for these six combinations of rooting depth and soil texture. With a standard rooting depth of 90 cm, there is a clear effect of soil texture; as expected from Figure 4.19 soil moisture limitation of Gsto is greater on the coarse soil, of lower water holding capacity, and hence seasonal $AF_{st}0$ is reduced. However, this effect is not as large as might be expected, largely because of the similar accumulation rates of $AF_{st}0$ in the early part of the growing season, between days 120 and 180. With the restricted rooting depth, a different pattern is observed; because rooting depth is the dominant constraint, there is little effect of soil texture. In terms of flux-related community sensitivity, the key conclusion is that grasslands of thin soils with limited rooting depth may experience a reduced ozone flux and impact.



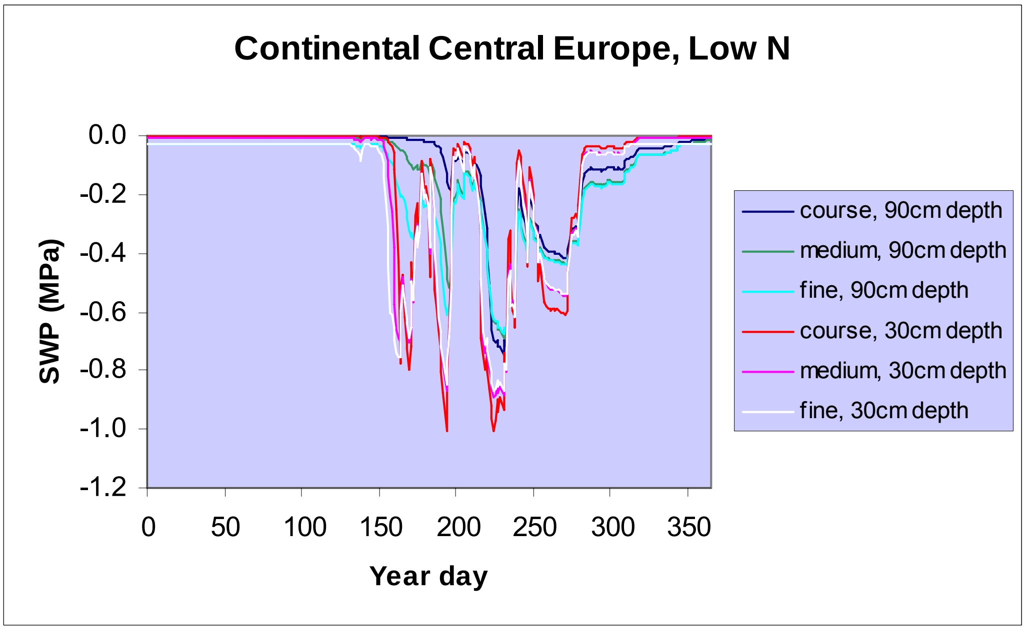


Figure 4.19 Modelled seasonal time-course of LAI and SWP, for the CCE location only, for a full combination of three soil textures (fine, medium, coarse) selected for consistency with the soil classes used in mapping, and for two rooting depths (30 and 90 cm).

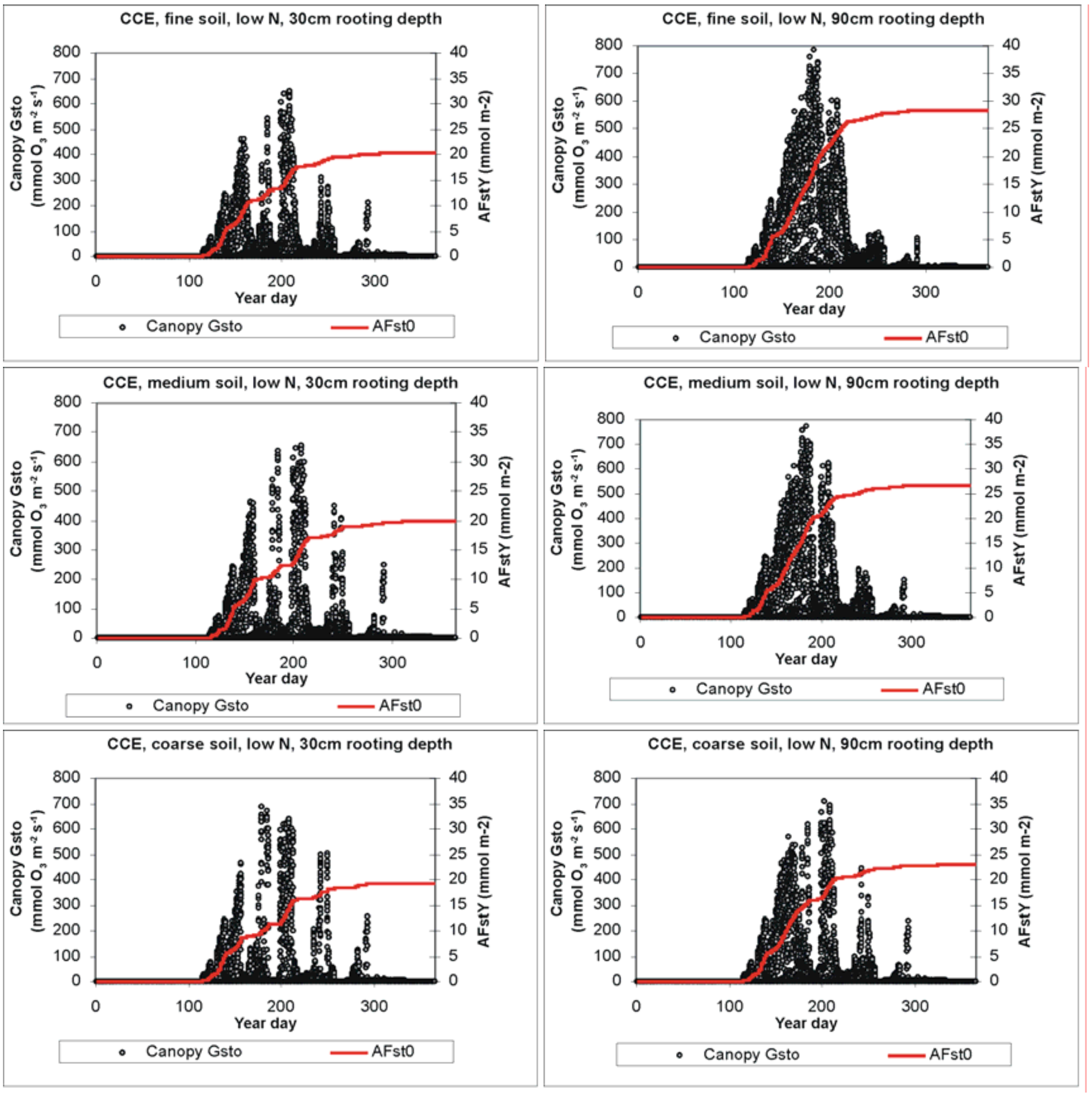


Figure 4.20 Annual time-courses of Gsto and AF$_{st}$0 for Continental Central Europe using the DO$_3$SE model parameterised for different soil textures (fine, medium, coarse) and different rooting depths (30 and 90 cm). A low intensity management with no added N was used.

Alpine grasslands are identified as a class of high sensitivity to ozone, according to the OZOVEG analysis. In order to specifically model some aspects of the changes in flux in such situations, we have modified the previous CCE runs to include (i) a reduction of 50% in g_{max} and (ii) a reduction in mean air temperature of 3°C. The results are shown in Figure 4.21. The grassland model simulations show that the reduction in g_{max} effectively allows the sward to conserve more water over the season, indicated by the slightly smaller peak SWPs attained during the drier periods (dark blue line) compared to the 'business as usual scenario' (light blue line). The 3°C lowering of mean air temperature delays the growing season by about 25 days and the depletion of soil moisture reserves occurs much later in the season indicated by just one significant drop in SWP between days 210 to 250. The overall effect of these factors was to reduce both Gsto and $AF_{st}0$ significantly.

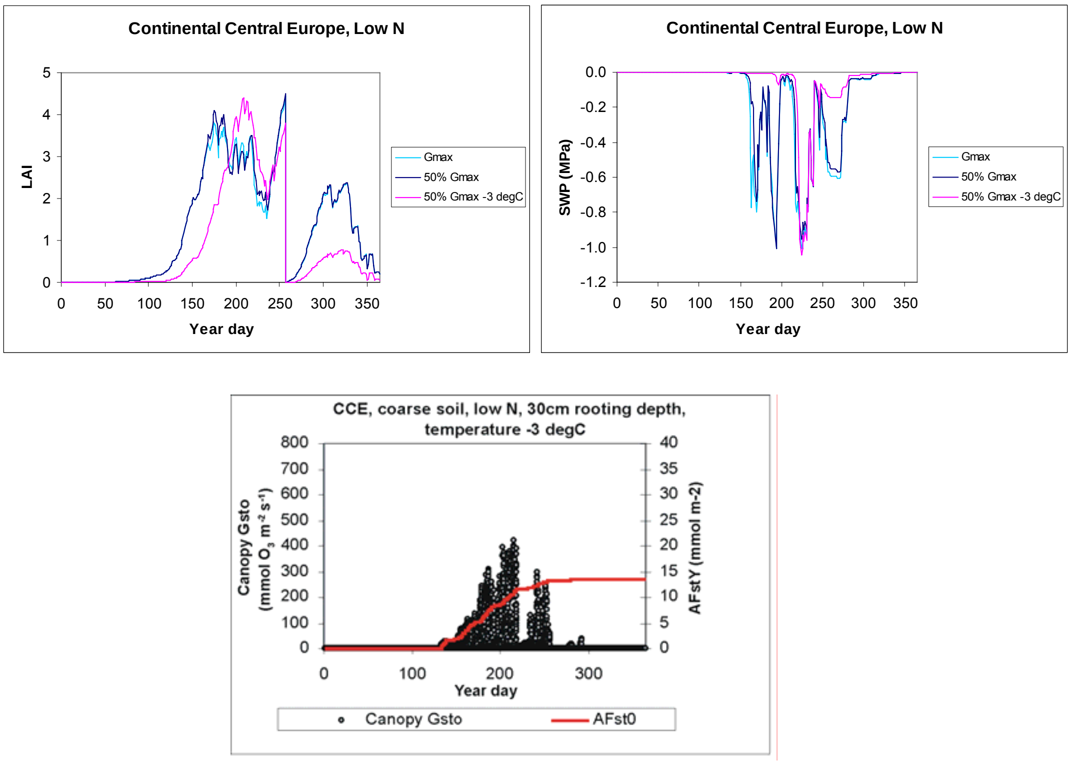


Figure 4.21 Simulation of an 'alpine' grassland scenario. Time courses of LAI and SWP for CCE using the grassland model (top left and right) showing the effect of a 50% reduction in g_{max} (50% Gmax) and the added effect of a 3°C lowering of temperature (50% Gmax – 3 degC). Annual time-courses of Gsto and $AF_{st}0$ for CCE were simulated using the DO$_3$SE model. Both models parameterised for coarse soil texture, 30 cm rooting depth and a low intensity management with no added N.

Table 4.10 presents an attempt to summarise the key conclusions from this assessment, based on the model runs above and other factors, for grassland communities. The sensitivity from the OZOVEG analysis is included, so that the flux-based factors and species-based factors can be compared. A relative ranking on a 1-3 scale has been used from the OZOVEG analysis and the model results described above. It is important to note that differences between EUNIS classes for both the OZOVEG index and the flux index are not large since those included here all have at least six ozone-sensitive species, and hence the 1-3 scale should not be over-interpreted. It is also important to note that the flux simulations are based on EMEP model data for one year – 1997. It would be valuable to compare results for a hot, dry, high ozone summer, such as 2003, to assess if a similar pattern of results was obtained. More detailed model parameterisation is also essential for more robust assessment.

The results do not indicate a consistent pattern between the two indices; i.e. no one community has both a high proportion of sensitive species and a high predicted flux. Refinement of both indices is needed to improve the basis of risk assessment.

Table 4.10 Summary of risk assessment for major EUNIS categories using the OZOVEG and DO_3SE methods. The comments refer to specific factors which influence the flux-based index. 3: relatively sensitive; 2: intermediate; 3: relatively insensitive.

EUNIS code and description	OZOVEG index	Flux index	Comment
E1 Dry grasslands	2	1	Nutrient poor soils with low rooting depth – soil moisture likely to limit flux significantly
E2 Mesic grasslands	1	2	Management regime likely to be dominant factor influencing canopy flux and likelihood of soil water limitation
E3 Seasonally wet grasslands	2	3	Water stress not limited, nutrient limitation may influence canopy growth and flux
E4 Alpine grasslands	3	1	Lower evapotranspiration rates may reduce limitation by soil water, but lower growth rates reduce accumulation period
E5 Woodland fringes	2	Unknown	Community not mapped or modelled and key characteristics will depend on local factors
E7 Dehasa	Unkown	Unknown	High proportion of legumes, so probably not N limited. Seasonal drought significant.

Maximal stomatal conductance of grassland species

As a first step towards developing a multi-species grassland flux model, an analysis of literature sources was carried out at SEI-York to identify values of g_{max} for a range of grassland species. This data has been added to that from solardome experiments conducted by CEH Bangor as part of Defra ozone umbrella contract, increasing the number of species included to 39. The g_{max} values were split into three functional groups, based on the species listed in Table 4.11 with the mean and standard error presented. G_{max} values were calculated as the 95 percentile of the range of conductances measured. The data suggest no clear differences between conductance values for grasses, forbs and legumes. It should be noted that within each functional group, there is considerable species-specific variation in g_{max}, and that the mean values in Table 4.11 may change as more data becomes available.

Table 4.11 Summary of values of g_{max} obtained from secondary sources and experiments conducted at CEH Bangor.

Functional group	**Species represented**	**Mean g_{max}**	**SE**	**n**
Grasses	*Dactylis glomerata, Trisetum flavescens, Bromus erectus, Bromus madritensis, Poa annua, Poa alpina, Holcus lanatus, Lolium perenne, Anthoxanthum odoratum*	358	62.7	9
Forbs	*Plantago lanceolata, Centaurea jacea, Rumex obtusifolius, Knautia arvensis, Bellis perennis Ranunculus friesianus, Campanula rotundifolia, Carex laevigata, Carex panicea Eriophorum angustifolium, Oxalis acetosella Oxyria digyna, Potentilla erecta, Ranunculus acris, Senecio fluviatilis, Viola lutea, Ranunculus repens, Viola tricolour, Papaver rhoeas, Melandrium dioicum, Melandrium album, Leucanthemum segetum Alliaria petiolata, Digitalis purpurea, Verbascum nigrum, Carex bigelowii*	284	31.3	26
Legumes	*Trifolium pratense, Trifolium repens, Medicago minima, Medicago glomerata*	313	93.4	4

4.3.9 Conclusions

The range of sensitivity to ozone, as calculated for the 83 natural vegetation species in this meta-analysis, indicates the wide range in above-ground biomass responses to ozone that may be found in ambient ozone conditions. Approximately one third of the species in this database show above-ground biomass reductions equating to over 10% at 15 ppm.h compared to 3 ppm.h. A further fifteen species showed above-ground biomass stimulations by ozone. The *Fabaceae* family has been identified as particularly sensitive to ozone, but as many families were not sufficiently represented in the database to investigate fully, there may be additional sensitive families. Similarly, the therophyte life-form has been identified as a strategy that is equated with sensitivity to ozone, but several life-forms were also not sufficiently represented to investigate fully. Although a few trends in relative sensitivity to ozone in terms of physiological or ecological conditions have been identified, such as mature leaf nitrogen concentration, in many cases the lack of available data (in particular plant physiology data) has meant that some other relationships may not have been identified by this study. The relationships between relative sensitivity to ozone and physiological and ecological requirements identified so far, such as relationships with Ellenberg light, moisture and salt values, allowed opportunities to model the relative sensitivity to ozone of species that have so far not been tested.

The model using Ellenberg Light and Salinity Indicator values for a species to predict the responses of individual species to ozone had a relatively low R^2 (26 %) and the error in the prediction was fairly high (RMSE = 19 %), but within the range of error reported for similar regression-based applications (Gatz, 2003). This is understandable considering that the underlying data are Ellenberg values which are an ordinal scale. The principle advantage of this model is that it can be applied to any European plant species for which Ellenberg values have been assigned, almost 3000 species and subspecies in all (Ellenberg *et al.*, 1991). There are some species where caution should be exercised when predicting ozone sensitivity with this model, due to poor representation in the underlying database of species with low Light values, high Salinity values or very high Moisture values. These include strongly shade-adapted species, aquatic or periodically submerged plants and halophytic species. A weakness of this model from the European perspective is the limited application to Mediterranean regions due to the low number of southern European species for which Ellenberg numbers have been assigned.

The community level predictions using the CORI model ignore the likely effects of competitive interactions between species. This simplification is because research to date has focussed mainly on individual species responses to ozone, and because modelling of species interactions is complex and requires information at a level of detail not available for most species. While the responses of species mixtures and communities have been assessed in some studies, including the Biostress project, (Ashmore and Ainsworth, 1995; Bender *et al*., in press; Gimeno *et al*., 2004b), there has been only one community-scale ozone fumigation in the field (Volk *et al*., 2003; Volk *et al*., 2006). Thus, there is little empirical evidence of how species interactions are likely to affect community responses to ozone. The biomass changes at Le Mouret (Volk *et al*., 2006) suggest a strong lag in the response to ozone, in common with field-based nitrogen deposition experiments (Carroll *et al*., 2003). Thus, while the CORI tool can predict the direction of change, the timescales over which communities are likely to change are uncertain.

When considering the responses of individual species, this study supports the choices of habitats included in the Mapping Manual (LRTAP Convention, 2004) as potentially ozone-sensitive. These were Dry grasslands (E1), Mesic grasslands (E2) and Seasonally-wet grasslands (E3); Dehesa grasslands (E7.3) could not be validated here because of the use of NVC to estimate EUNIS and the inability to plot response-functions due to the limited number of ozone treatments used in the study by Gimeno *et al*. (2004a). The woodland fringes habitat (E5) identified for possible use in the Mapping Manual has also been shown to be appropriate for inclusion. This study has shown that E4 (Alpine and subalpine grasslands) and F4 (Temperate shrub heathland) could also be included in the Mapping Manual bearing in mind that these communities show a high proportion of species stimulated by ozone. It is feasible to map the potential location of these communities using the harmonised SEI land-cover dataset (Cinderby, 2002) and European Environment Agency (EEA) Corine land-cover dataset (de Smet and Hettelingh, 2001) described in Cinderby *et al*. (in press) and a first example is presented here. At EUNIS levels 3 and 4, predictions of the number of ozone-sensitive species per community may provide a better indicator of relative community sensitivity than the %OS. However, this necessitates a reasonably accurate estimate of the number of species present per community. Since mapping procedures are more difficult at levels 3 and 4, it may not currently be possible to map the communities identified as the most sensitive if suitable community-specific information is not currently available.

The analysis presented here has confirmed that it is appropriate for Grasslands (EUNIS category E) to be identified within the Mapping Manual (LRTAP Convention, 2004) as the generic vegetation type that is most sensitive to ozone since Grassland habitats had twice as many communities with six or more ozone-sensitive species than any other vegetation type. In addition, the analysis has justified the choice of grassland types E1, E2 and E3 as being appropriate for application of the critical level for (semi-)natural vegetation. The E5 category, suggested in the Mapping Manual as probably sensitive to ozone has also been shown to be appropriate for inclusion. This analysis has also shown that E4 (Alpine and sub-alpine grassland) and F4 (Temperate shrub heathland) had the highest potential sensitivity to ozone and should also be considered for inclusion in the Mapping Manual.

A linked growth and flux model was used to identify the effect of nutrient stress, water stress and active management in modifying ozone flux and impacts. These factors were shown to interact in determining flux. Mesic, wet, heavily managed communities are likely to experience greater ozone flux than the communities of nutrient poor, and climate or water limited, habitats that are typically of greatest conservation value.

The first pan-European map was produced of EUNIS categories, which are used within LRTAP Convention to define critical loads and levels in national data submissions. Further assessment of the accuracy of this map is required; inconsistencies may reflect uncertainty in the classification criteria but also errors in the underlying databases.

Three communities were identified as being likely to be sensitive to both ozone and nitrogen deposition:- dry grasslands, alpine and sub-alpine grassland and temperate shrub heathland. Of these, synergistic effects of the two pollutants at current European effects are most likely to occur in the two grassland communities.

4.3.10 Further work

Although considerable progress has been made towards identifying and mapping the location of ozone-sensitive communities of semi-natural vegetation, significant uncertainties remain with the methods developed. In the next contract, it is proposed to:

1. Further develop the OZOVEG database (CEH Bangor) with the aim of quantifying the modifying influence of competition on the relative sensitivity of species according to their role in communities e.g. for functional groups (e.g legumes, forbs and grasses) and for species of different positions in the canopy (e.g. low growing, tall).
2. Further develop the methods for predicting ozone sensitive communities by incorporating other factors within the Ellenberg models (dependant on data availability) such as maximum canopy height, relative growth rate, phenology, a numerical score for plant form/growth habit, use of Ellenberg light values as a surrogate for position in the canopy (i.e. high values for canopy forming species, low values for low in the canopy) and an empirically derived index for ozone response in competition compared to grown individually (see above development of OZOVEG database).
3. Consider the modifying influence of nitrogen deposition by including nitrogen as a factor in the Ellenberg model.
4. Applying the Ellenberg predictive modelling approach to European communities (CEH Bangor). During years 1 and 2, a database will be developed that lists the species present in EUNIS level 3 and 4 communities that belong to the broad classes identified at EUNIS level 2 as potentially ozone sensitive (Mills *et al*., in press). In year 3, the European Ellenberg model will be applied to these EUNIS level 3 and 4 communities to predict relative sensitivity.
5. Refining the methods for mapping the location of ozone-sensitive communities initiated within this contract.
6. Conducting a flux-based risk assessment for managed pasture, which takes into the account the complex structure of grassland ecosystems.

5. WORK PACKAGE 4: HEAVY METALS

5.1 Introduction

Concern over the accumulation of heavy metals in ecosystems, and their impacts on the environment and human health, increased during the 1980s and 1990s. The LRTAP Convention responded to this concern by establishing a Task Force on Heavy Metals (and persistent organic pollutants) under the Working Group on Abatement Techniques. In 1998, the first Protocol for the control of emissions of heavy metals was adopted and signed by 36 parties to the Convention. The Protocol stated that "an effects-based approach should integrate information for formulating future optimised control strategies taking account of economics and technological factors". Cadmium, lead and mercury emissions were targeted by the Protocol.

The ICP Vegetation is addressing a short-fall of data on heavy metal deposition to vegetation in two ways. Firstly, the clover clones used in the 2000, 2002 and 2004 ozone experiments have been analysed for lead, cadmium, copper, arsenic and nickel concentration. This work was conducted by Dr Ludwig De Temmerman (Belgium) as a contribution in kind. Comparison of the heavy metal concentration in white clover with the bulk deposition measured at these experimental sites is allowing a method for determining the level of deposition at any site to be developed (Mills *et al*., 2005). Secondly, the ICP Vegetation has taken over the coordination of a well-established programme that monitors the deposition of heavy metals to mosses. The programme, originally established in 1980 as a joint Danish-Swedish initiative, involves the collection of mosses and analysis of their heavy metal concentration at five-year intervals; it included over 6,500 samples of mosses taken from 29 European countries in the 2000/2001 survey (Buse *et al*., 2003; Harmens *et al*., 2004a). The ICP Vegetation is currently coordinating the European heavy metals in mosses survey 2005/6.

The European metals in mosses survey provides data on concentrations of ten heavy metals (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, V, Zn) in naturally growing mosses (Buse *et al*., 2003; Harmens *et al*., 2004a). The technique of moss analysis provides a surrogate, time-integrated measure of the spatial patterns of heavy metal deposition from the atmosphere to terrestrial systems. It is easier and cheaper than conventional precipitation analysis as it avoids the need for deploying large numbers of precipitation collectors with an associated long-term programme of routine sample collection and analysis. The higher trace element concentrations in mosses compared to rain water makes analysis more straightforward and less prone to contamination. Although the moss concentration data does not provide a direct quantitative measurement of deposition, this information can be derived by using one of several regression approaches relating the results from moss surveys to precipitation monitoring data (Berg and Steinnes, 1997; Berg *et al*., 2003). The results of the 2000/2001 European moss survey were published in 2003 (Buse *et al*., 2003). Preparation of data from that and previous surveys for trend analysis is described in Chapter 8 (Option 3).

5.2 Further reporting of results of the 2000/1 European moss survey (Objective 8)

Some aspects of the reporting of the results from the 2000/1 survey fall within the remit of the general coordination of the ICP Vegetation and are covered under Objective 1 (Section

2.2.2 and 2.2.3). Dr Harry Harmens was invited as a key-note speaker at the 3rd International Workshop on Biomonitoring of Atmospheric Pollution (September 2003, Bled, Slovenia) and published a paper in a special issue of the Journal of Atmospheric Chemistry (Harmens *et al.*, 2004a). In 2003 the EMEP 50 x 50 km grid maps in the 'Heavy Metals in European Mosses: 2000/2001 survey' were updated with data received from Iceland.

5.3 The 2005/6 European moss survey (Objective 8)

Preparations for the 2005/6 moss survey started in year 1, and in year 2 of the contract the Manual for collecting and analysis of moss samples was reviewed and revised by the Coordination Centre and the participants in the moss survey (Harmens *et al.*, 2005a). All participants agreed at the ICP Vegetation Task Force Meeting in 2004 that it was very important for quality assurance purposes to include certified moss reference material during the analysis of the heavy metal concentration in the moss samples. Prof. Eero Kubin (Finland) kindly agreed to distribute certified moss reference material (Steinnes *et al.*, 1997) prepared at his Institute to participants at reduced costs. As part of the variation of this contract, agreed in December 2004, the Coordination Centre for ICP Vegetation has provided reference samples to those participants that could not afford to purchase the standards through lack of financial support from their individual countries.

Table 5.1 Participating countries in the 2005/6 moss survey. Countries indicated in italics have yet to secure funding, (N) indicates that the nitrogen concentration in mosses will be determined in addition to the heavy metal concentrations.

Austria (N)	Lithuania
Belarus	*Netherlands*
Belgium (N)	Norway
Bosnia and Herzegovina	Poland (N)
Bulgaria (N)	Portugal
Czech Republic (N)	Romania
Denmark (Faroe Islands)	Russian Federation (N)
Estonia (N)	Serbia and Montenegro
Finland (N)	Slovakia (N)
France (N)	Slovenia (N)
FYR of Macedonia	Spain (N)
Germany (N)	Sweden
Greece	Switzerland (N)
Iceland	Turkey (N)
Italy (N)	Ukraine
Latvia (N)	United Kingdom (N)

In addition to developing and distributing the Manual, the Coordination Centre at CEH Bangor has also been assisting participants to gain funding for the 2005/6 survey by writing letters of support to funding bodies. Participants from 32 countries contribute to the 2005/6 moss survey (with the Netherlands awaiting approval of funding) and will sample mosses from over 7,000 sites across Europe (Table 5.1). Of these, 18 countries will also analyse the nitrogen concentration in mosses at ca. 3,200 sites (see Chapter 7). As in previous years, some countries have kindly offered to assist those without financial support by conducting the analysis of the moss samples on their behalf.

5.4 Heavy metal concentration in clover

As a contribution in kind to the ICP Vegetation, Dr Ludwig De Temmerman (Belgium) determined the heavy metal concentration in clover used in the ozone biomonitoring experiment in 2004. Twelve participants grew the NC-R (ozone-resistant) biotype of white clover at their site using a standard soil mixture distributed by Dr De Temmerman. Harvested foliage was analysed for cadmium (Cd), copper (Cu) and lead (Pb). Data from 2004 were combined with data from previous years and the heavy metal bulk deposition rates were plotted against the heavy metal concentrations in clover (Figure 5.1).

Significant linear correlations were found between the heavy metal bulk deposition rates and the heavy metal concentrations in white clover. Further statistical analyses were conducted to determine the 'normal' background concentrations and pollution thresholds of heavy metals in white clover. The background concentrations were 0.065 µg Cd g^{-1} dry matter (DM), 3.50 µg Cu g^{-1} DM and 0.15 µg Pb g^{-1} DM and the pollution thresholds were 4.15 µg Cu g^{-1} DM and 0.36 µg Pb g^{-1} DM, respectively. For cadmium only a preliminary pollution threshold of 0.155 µg g^{-1} DM could be established as the calculation of this threshold was based on three data points only. Up to a bulk deposition rate of 2.5 µg Cd m^{-2} day^{-1} the cadmium concentration in clover did not differ significantly from the background concentration. For copper the background concentration and the pollution threshold are not far apart. At about half of the participating sites in Europe the pollution thresholds for copper and lead were exceeded at some time during the growth period. In general, the concentration of lead and copper in white clover was above the pollution threshold at three sites in Dunkerque (France) and at Tervuren (Belgium), and at Athens and Volos (Greece) for lead only. The preliminary pollution threshold for cadmium was only exceeded in the centre of Dunkerque (France). The heavy metal concentration in clover was below the pollution threshold for all metals in Thessaloniki (Greece), Pisa (Italy) and Bangor (UK). In conclusion, the white clover biomonitoring network is working well for the trace elements lead and copper, but more data from polluted sites are needed to establish the pollution threshold for cadmium. The use of a standard substrate low in cadmium is required to prevent confounding effects of the uptake of cadmium by the roots from the substrate. Based on the outcome of this work Dr Ludwig De Temmerman, in close collaboration with Dr Harry Harmens, produced a review on heavy metal deposition and the potential contamination of crops (Harmens *et al.*, 2005b, Chapter 3).

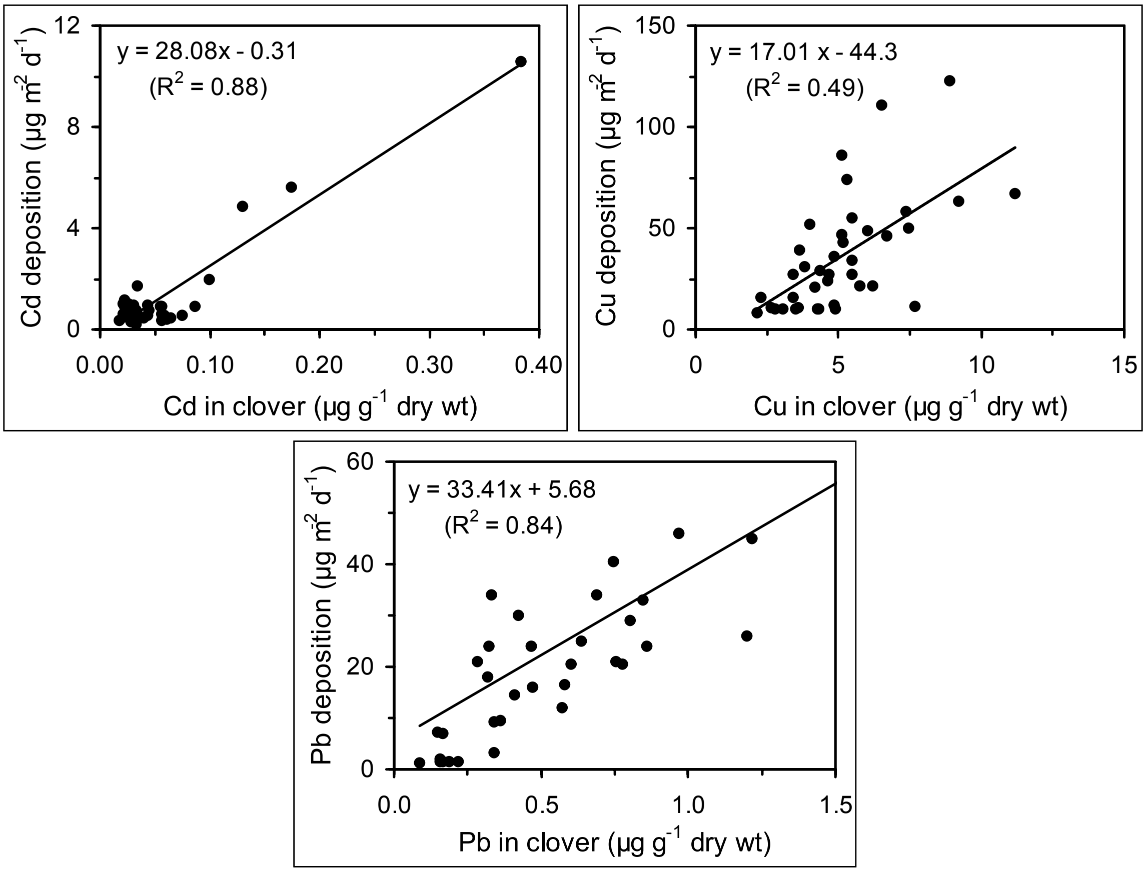


Figure 5.1 Heavy metal bulk deposition rates plotted against heavy metal concentrations in white clover for the metals cadmium (Cd), copper (Cu) and lead (Pb).

6. Option 1: Development of a framework for probabilistic assessment of the economic losses caused by ozone damage to crops in Europe

Note: A report of the work conducted for this objective was submitted to Defra in January 2006. We provide here the Executive Summary and refer the reader to the full report for further details (Holland et al., 2006).

6.1 Background and objectives

Ozone is present at elevated concentrations in the lower atmosphere through reactions involving oxides of nitrogen (NO_x) and volatile organic compounds (VOCs). It has long been recognised as causing losses in crop productivity and changes in the quality of agricultural products. There is now a strong demand from policy makers for the quantification of ozone damages to be fed into cost-benefit analysis of emission control strategies.

The analysis presented in this report investigates the use of both concentration-based (AOT40) and flux-based ($AF_{st}6$) methods to assess the uncertainties in quantifying the ozone-induced loss of production for (largely) arable crops in Europe. The flux-based method is preferred on the grounds that it estimates yield loss against received dose of ozone, rather than against simple exposure to ambient levels. However, the flux-based method can so far only be applied to wheat and potato, and so is not suitable for providing a comprehensive assessment of crop damage involving a wide range of crops. Parallel use of the two methods was intended to improve understanding of their reliability relative to one another.

The objectives of this study were:

1. To describe the uncertainties present in AOT40-based modelling.
2. To examine the difference between estimates of yield loss made using AOT40-based and flux-based functions.
3. To consider how AOT40- and flux-based methods can be combined in the future to enable a reasonably complete estimation of ozone impacts on crop yield in Europe that maximises use of the research that has been carried out in this field.
4. To provide European estimates of the range of expected yield losses under a range of scenarios, taking account of uncertainties where possible.
5. To identify areas for further refinement of the crop loss model.

6.2 Analysis using concentration-based methods

Using concentration-based methods, this study has quantified a range for ozone-induced losses for 23 crops in 47 countries in Europe of €4.4 to 9.3 billion/year, around a best estimate of €6.7 billion/year for year 2000 emissions (Table 6.1). The @RISK package has been used to quantify the combined impact of the uncertainties that affect the analysis. Results for a series of scenarios considered in the EU's recent CAFE (Clean Air For Europe) Programme for 2020, by when all current legislation should be fully in place, are also shown in the table. The core estimate represents losses equal to 2% of arable agricultural production in Europe. These estimates do not account for damage via visible injury, changes in crop quality, or interactions with pests.

Table 6.1 Core estimates of total damage to the crops considered across the 47 countries considered in the analysis, with 90% confidence interval. Units: €billion/year.

Scenario	Core	90% confidence interval
2000	6.7	4.5 – 9.3
2020 baseline	4.5	3.0 - 6.3
D_23 low (CAFE programme scenario)	3.9	2.6 - 5.4
D_23 mid (CAFE programme scenario)	3.7	2.4 - 5.2
D_23 high (CAFE programme scenario)	3.6	2.4 - 5.1
Maximum Feasible Reduction according to the RAINS model	1.7	1.1 - 2.3
EU's Thematic Strategy on Air Pollution	3.9	2.6 - 5.5

The @RISK analysis shows that the largest sources of uncertainty in the concentration-based estimates presented in Table (i) are, in order of decreasing importance:

- Response function for vegetables
- Variation in ozone concentration with height
- Crop yield estimates
- Response function for potato

6.3 Analysis using flux-based methods

The use of flux-based methods, that take account of dose received by sensitive plant tissues rather than simply ambient ozone concentration, is strongly preferred from a theoretical perspective, but is not yet possible for crops other than wheat and potato. Results based on the use of flux-based methods for five grid cells representing each of five European climate zones indicates both increases and decreases in flux-based yield loss estimates relative to concentration-based estimates, depending on climatic zone. Additional analysis is required to ensure that the findings on the bias by climatic region relative to concentration-based yield loss estimates are truly representative of the different climate zones, before reaching any firm conclusions on a possible additional factor to be incorporated into the concentration-based analysis to approximate flux.

The analysis performed in this report identifies a number of other issues relative to the use of the flux-based methods that need further assessment:

a) That the increase and decrease in yield loss estimates made in different locations using flux- compared with concentration-based methods truly represent effects found under field conditions and are not an artefact of extrapolation of experimental dose-response relationships to field conditions.

b) That the flux modelling for wheat and potato can provide information on the role dose-modifiers may play in altering crop losses for other crop species estimated using concentration-based functions.

c) That formulation and parameterisation of the stomatal flux model is appropriate for different climate regions. Perhaps the most important issues are to ensure that g_{max} and the flux accumulation period are identified correctly, and that the method to estimate the extent and influence of soil water potential (SWP) on stomatal conductance (g_s) provides realistic values.

6.4 Policy implications of overall conclusions

In view of the need to investigate a number of factors relating to the flux-based methods in more depth, it may be considered premature to recommend a protocol for adjustment of the concentration-based results using flux estimates at this time. However, although there are clear differences within climate zones, the results presented here show limited evidence for a systematic difference in the results generated by the two methods at the European level.

In the course of this work an alternative, top-down approach was developed at the University of Reading, though so far this method has been applied only for wheat grown in the UK. This generated roughly 50% lower estimates of damage than those given by either of the methods used here, though given the limited nature of the analysis it is not clear whether the same would be found if the method were applied to other crops, to other countries, or to other years. However, given that all three methods give results of the same order of magnitude, it seems likely that the overall level of damage from direct effects of ozone on yield of (largely) arable crops is in the order of a few €billion across Europe each year.

The methods for cost-benefit analysis of air quality policy used at the end of the 1990s in the development of the Gothenburg Protocol to the Convention on Long-range Transboundary Air Pollution gave an indication of the likely robustness of results, but did not take this through to a quantitative assessment of the impacts of uncertainty on the balance of costs and benefits. However, the analysis reported here and in part for the CAFE programme, demonstrates how quantified uncertainties can be factored into the analysis to describe the probability of benefits exceeding costs.

It is recommended that work in the development of these methods, including the work done at the University of Reading, should be continued. Adding impetus to this recommendation is the possibility for a major increase in background ozone concentrations as a consequence of global warming and increasing ozone precursor emissions (e.g. in Asia) over the next decades.

7. OPTION 2: INCORPORATION OF NITROGEN WITHIN THE ICP VEGETATION

7.1 Introduction

Nutrient nitrogen has been incorporated into remit of the ICP Vegetation by exploiting the existing heavy metals in mosses sampling network. Earlier studies have indicated that there is a clear relationship between moss %N in mosses and N deposition (e.g. Pitcairn *et al.*, 1995). The aim of the ICP Vegetation was to analyse moss samples already collected from selected countries for their nitrogen concentration, since these are carpet-forming mosses that obtain most of their nutrients directly from precipitation and dry deposition.

7.2 Total N concentration in mosses collected between 1972 and 2001

Individual countries that have had long involvement in the European heavy metals in moss survey were asked if they could provide the Coordination Centre with samples from 20 sites per survey year for analysis of the total N concentration, using CHN elemental analysis. Certified apple leaf reference material was analysed at the same time for quality assurance purposes. Those countries that have sent moss samples and the years represented are shown in Table 7.1; data rather than moss samples were received from Germany. There were significant differences between countries regarding the total N concentration in mosses, with higher N concentrations in mosses collected in Germany than in Scandinavian countries and values for Spain being intermediate (except in the 1970s), but no significant temporal trends within countries were observed (Figure 7.1). The Scandinavian countries also provided the Coordination Centre with N deposition data. For Norway for the years 1977, 1990 and 2000 good correlations were observed between the N deposition rates (reduced, oxidised and total N) and the total N concentration in mosses. For Sweden for the year 2000 good correlations were observed too between the N deposition rates (reduced, oxidised and total N) and the total N concentration in mosses, but for Finland for the years 1990 and 2000 the correlations were weaker, though still significant (Figure 7.2; Table 7.2). These results indicate that mosses can potentially be used as biomonitors of atmospheric N deposition.

Table 7.1 Moss samples analysed for total N concentration: countries, species and years sampled.

Country	Moss	Years sampled		
Norway	*Hylocomium splendens*	1977	1990	2000
Sweden	*Pleurozium schreberi*	1980	1990	2000
Finland	*Pleurozium schreberi.*		1990	2000
Germany	*Pleurozium schreberi, Hypnum cupressiforme, Scleropodium purum*		1995	2000
Spain	*Hylocomium splendens*	1972&1981	1995	2001

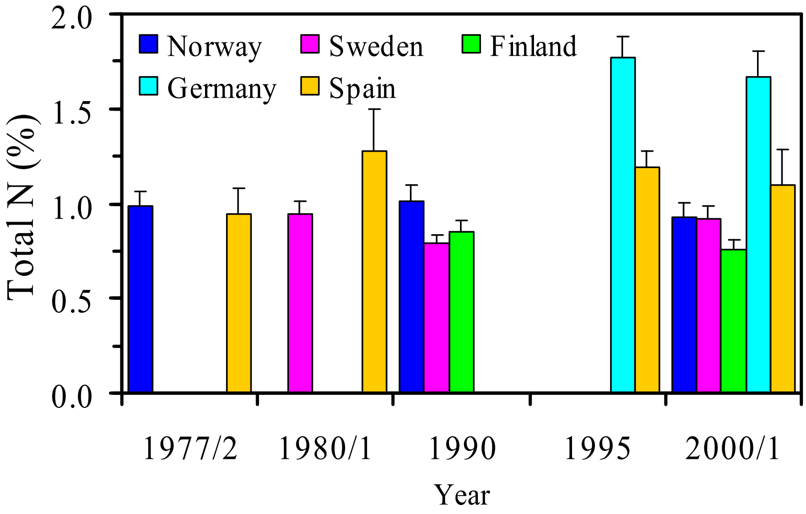


Figure 7.1 Total nitrogen concentration in mosses per country per sampling year. Values are mean + one standard error.

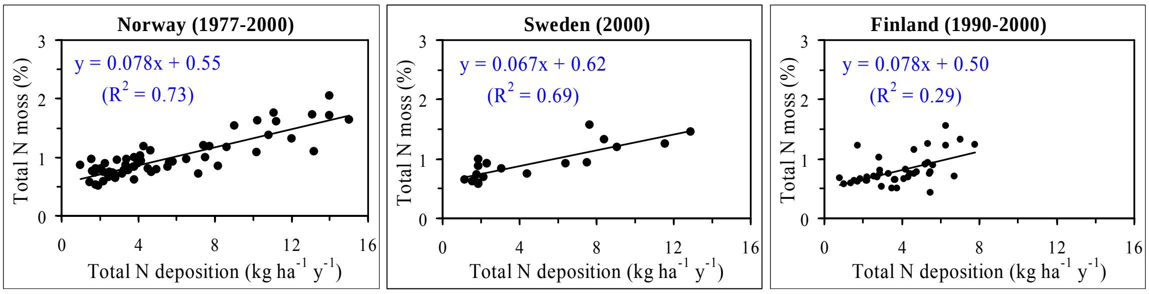


Figure 7.2 Total nitrogen concentration in mosses plotted against total nitrogen deposition rates at selected sites and years in Norway, Sweden and Finland.

Table 7.2 Regression equations and coefficients of determination (R^2) for the total nitrogen concentration in mosses (%) plotted against oxidised, reduced and total nitrogen deposition rates (kg N ha^{-1} y^{-1}) at selected sites and years in Norway, Sweden and Finland.

Country	Years pooled	N species	Regression equation	R^2
Norway	1977, 1990, 2000	$N_{oxidised}$	y = 0.143x + 0.59	0.74
		$N_{reduced}$	y = 0.160x + 0.53	0.67
		N_{total}	y = 0.078x + 0.55	0.73
Sweden	2000	$N_{oxidised}$	y = 0.129x + 0.60	0.66
		$N_{reduced}$	y = 0.136x + 0.64	0.70
		N_{total}	y = 0.067x + 0.62	0.69
Finland	1990, 2000	$N_{oxidised}$	y = 0.128x + 0.48	0.32
		$N_{reduced}$	y = 0.184x + 0.55	0.21
		N_{total}	y = 0.078x + 0.50	0.29

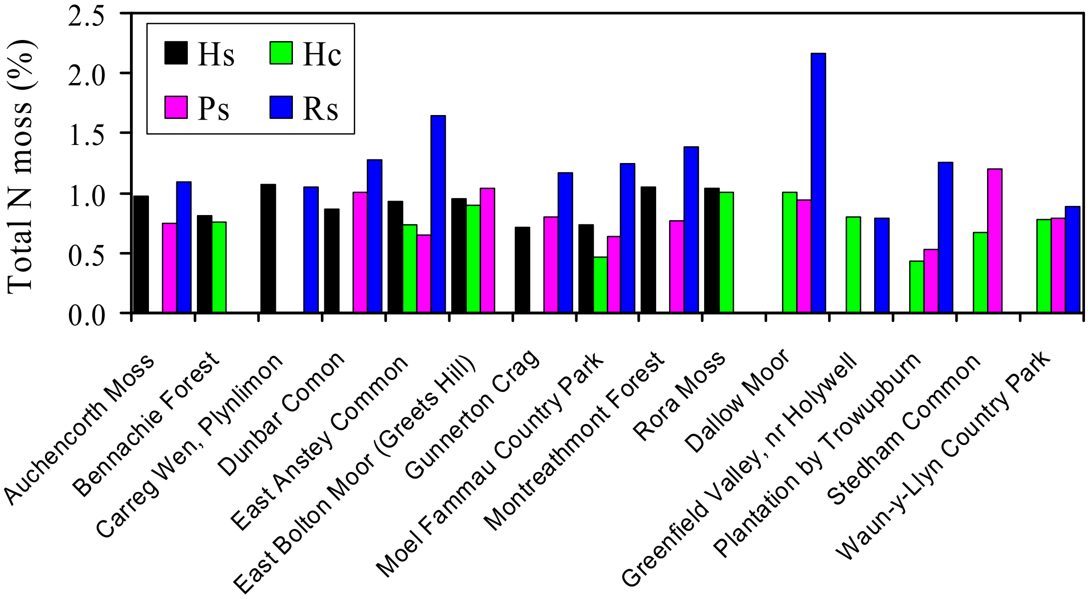


Figure 7.3 Total nitrogen concentration in different moss species sampled at sites across the UK in 2000. Hs = *Hylocomium splendens*, Hc = *Hypnum cupressiforme*, Ps = *Pleurozium schreberi* and Rs = *Rhytidiadelphus squarrosus*.

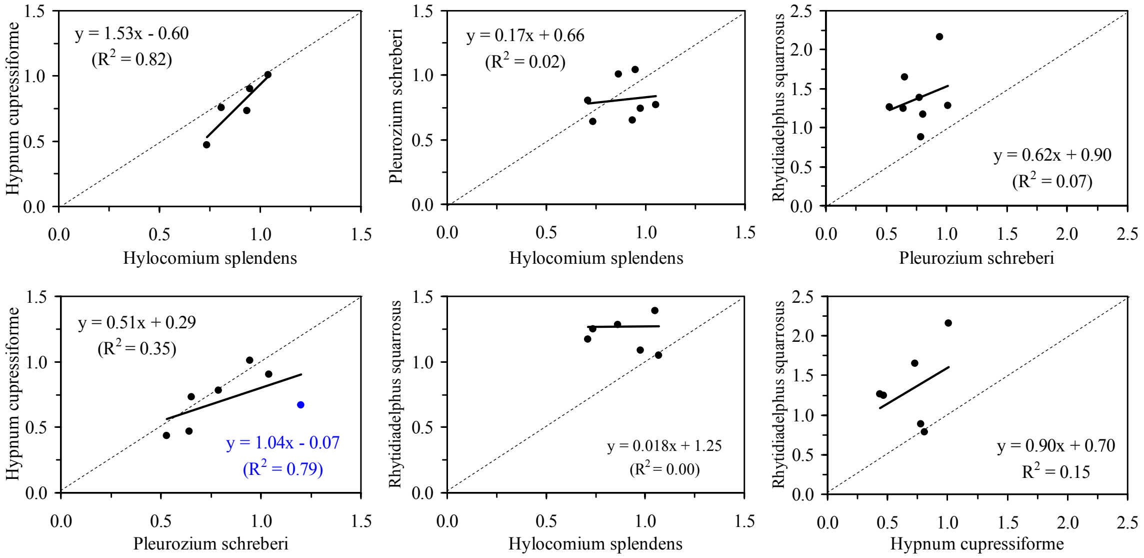


Figure 7.4 Relationships between the total nitrogen concentration in different moss species sampled at sites across the UK in 2000. The blue correlation equation indicates the relationship between *H. cupressiforme* and *P. schreberi* when the blue outlier is excluded.

In the pilot study regarding the N concentration in mosses collected between 1972 and 2000 (see above), different moss species were used to establish the relationship between the total N concentration in mosses and atmospheric N deposition rates. To establish whether accumulation rates of N in mosses are species-specific, moss samples from the UK 2000 survey were sorted for the last three year's growth. In 2000 several moss species were sampled at the same sites in the UK to allow interspecies-calibration for heavy metals (Ashmore *et al.*, 2002). For a selection of those sites, moss samples were analysed in 2005 for total N concentration for the purpose of interspecies calibration.

In general, the total N concentration in *Rhytidiadelphus squarrosus* was higher than that in the other species (Figures 7.3 and 7.4) and the correlation between the N concentration in *R. squarrosus* and the other species was very weak. Good correlations were found between the total N concentration in *Hypnum cupressiforme* and *Hylocomium splendens*, and *H. cupressiforme* and *Pleurozium schreberi* (when excluding one outlier). These results indicate that species-specific N accumulation in mosses could potentially confound the use of mosses as biomonitors of atmospheric N deposition across Europe when using different moss species within and between countries. However, the range of total N concentration in the UK mosses and the number of samples used in this pilot study was rather limited, possibly contributing to a relatively high scatter and weak correlations between the total N concentration in different species. More data points over a wider range of total N concentration in mosses are needed to establish firmer interspecies calibration equations.

7.3 Total N concentration in herbarium moss samples (1829 – 2004)

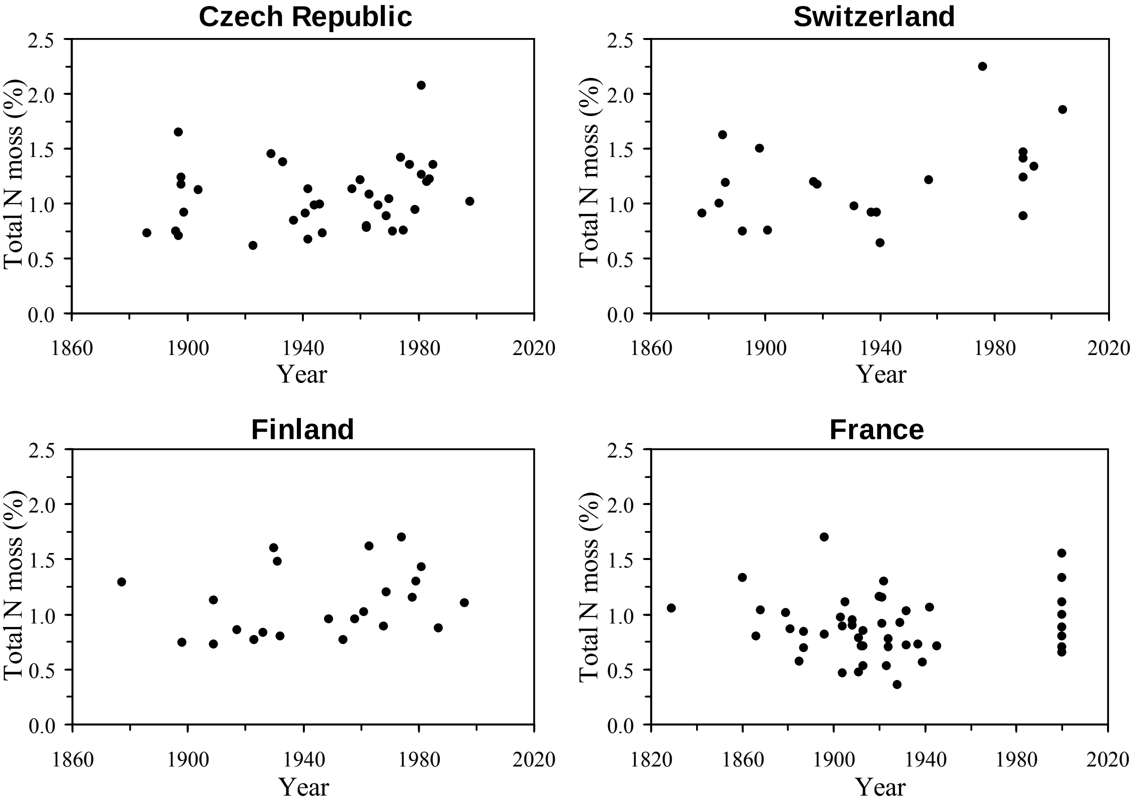


Figure 7.5 Total nitrogen concentration in herbarium moss samples from four different European countries. Moss species: Czech Republic and Finland – *Pleurozium schreberi*, Switzerland – *Hylocomium splendens*, France - *Pleurozium schreberi* and *Hylocomium splendens*.

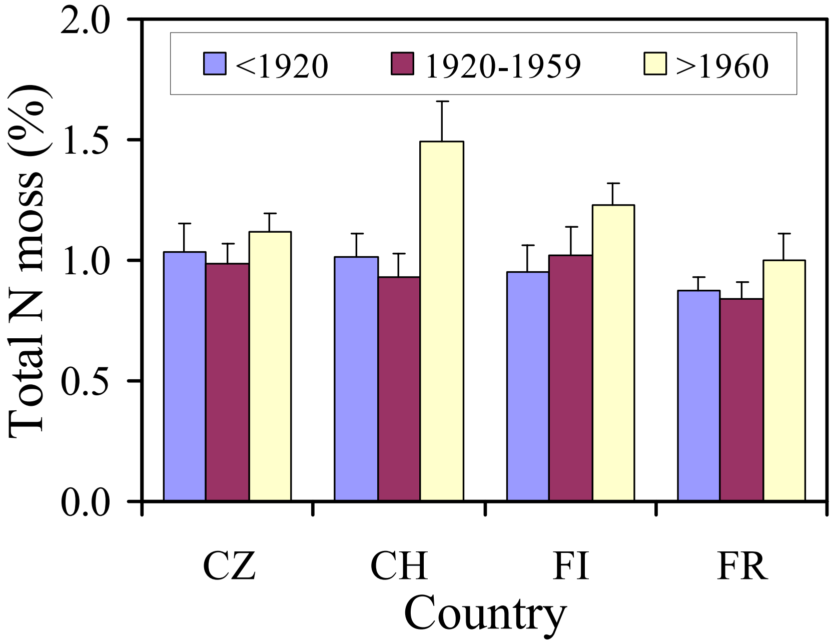


Figure 7.6 Historic trends of total nitrogen concentration in herbarium moss samples from four European countries for three different time periods. Moss species: Czech Republic (CZ) and Finland (FI) – *Pleurozium schreberi*, Switzerland (CH) – *Hylocomium splendens*, France (FR) – *Pleurozium schreberi* and *Hylocomium splendens*. The values are means + one standard error.

Participants of the European moss survey were asked by SEI-York to identify and contact appropriate herbaria in their own countries to obtain a small number of samples of mosses, preferably *Hylocomium splendens* or *Pleurozium schreberi*. The provisional target was to collect 30 samples from each of five countries where samples from previous European moss surveys are available. Ideally, time series of six dates for five different sites in each country were requested. However, in reality the best that the participants could do was to send samples from several regions in their country and from whatever year samples were available in the herbaria. Historic moss samples were received from the Czech Republic, Finland, France and Switzerland.

The historic data show a lot of scatter (Figure 7.5) and when all pooled together, there was a trend towards an increase in the total N concentration in mosses of ca. 0.2% over a century, with the increase being slightly higher in Switzerland and virtually no change with time in France. When the data were grouped into different time periods, there was no significant change in the total N concentration in mosses up to 1960. However, beyond 1960 there was overall a significant ($P < 0.001$) increase in the total N concentration in mosses compared to earlier years (Figure 7.6), which was due to a significant increase for Switzerland ($P = 0.028$), as the increases in the other countries were not significant ($P > 0.1$). Up to 1920 the average total N concentration in the mosses was 0.96%, between 1920 and 1960 it was 0.93% and beyond 1960 it was 1.18%. There was a significant country effect on the total N concentration in the mosses, with the average values being 0.89, 1.06, 1.10 and 1.20 for France, the Czech Republic, Finland and Switzerland, respectively. There was no significant difference between the total N concentration in *H. splendens* and *P. schreberi*.

In the near future, we intend to compare the results of the herbarium mosses with the modelled historic N deposition by EMEP/MSC-West. This comparison should give an indication whether herbarium moss samples can be used to indicate historic trends in N deposition across Europe, as was previously shown for the UK (Pitcairn *et al.*, 1995). Although EMEP/MSC-West has now modelled historic N emission data, modelling and reporting on historic N deposition is currently in its final stage.

8. OPTION 3: SPATIAL AND TEMPORAL TRENDS IN THE HEAVY METAL CONCENTRATION IN MOSSES

8.1 Introduction

Just prior to the start of this contract, the entire heavy metals in mosses dataset was transferred to the Coordination Centre at CEH Bangor. Spanning surveys conducted between 1970 and 2000, the dataset contains a vast amount of information on both the spatial and temporal trends in the heavy metal concentration in mosses in Europe. However, the format of the data was not standardised between countries or survey years, with much of the data only present as ASCII code or in obsolete versions of Excel. During 2004/5, the aim was to complete the standardisation of the data and during 2004/5, to initiate analysis of the temporal trends in selected European countries with analysis to be completed in 2005/6.

8.2 Data standardisation

All data have now been standardised according to the format used for the 2000/2001 European heavy metals in mosses survey. Data are in excel spreadsheets and coordinates for the sampling sites have been standardised with longitude and latitude in decimal degrees (Buse *et al*., 2003). Any zeros were deleted and values below the quantification limit were set at the quantification limit. For quality assurance purposes, the data in the excel spreadsheets were cross checked with the data received from the participants and with the minimum, maximum and calculated median value per metal per country presented in the appendixes of the original reports (Rühling, 1994; Rühling and Steinnes, 1998).

8.3 Temporal trends in the heavy metal concentration in mosses in selected European countries

Statistical analysis of temporal trends were conducted for five selected European countries: Norway, Slovakia, Sweden, Switzerland and the United Kingdom. Two statistical analyses were performed. Firstly, trends in heavy metal concentrations were compared between countries at the sampling years of 1990, 1995 and 2000. Analysis was by General Linear Model (GLM) ANOVA of heavy metal geometric means with year specified as a covariate and country specified as a fixed factor. The analysis was weighted by the number of samples from each country at each of the sampling years. Pair wise comparisons between countries were conducted with the Tukey test. Secondly, heavy metal concentrations for each sampling year were compared for each country individually, with data for Sweden and Norway going back to 1970 and 1977, respectively. Analysis was by GLM ANOVA with year specified as a fixed factor and pair wise comparisons were conducted with the Tukey test.

The temporal trends in the cadmium (Cd), lead (Pb) and mercury (Hg) concentrations in mosses were compared with deposition data reported by EMEP/MSC-East for the five selected European countries (EMEP 2004, 2005). In 2005, EMEP/MSC-East performed new calculations of depositions of Pb, Cd and Hg for 1990 - 2003 with 50 km resolution (EMEP, 2005) according to adjusted emission scenarios. The new deposition data are not based on official emission data submitted by Parties to the LRTAP Convention Secretariat, so they should be considered as preliminary. According to the adjusted emission scenarios for Pb and Cd, the emission of these metals in some European countries was 2-3 times (or even higher) than the officially submitted emission data. However, the new EMEP model results are now much more in agreement with measurement data than previous model results. In the new ICP Vegetation contract we envisage to receive the new deposition data from EMEP/MSC-East with the aim to compare the temporal trends for Cd, Pb and Hg across Europe with the temporal trends in the concentrations in mosses, including the comparison of EMEP deposition and moss maps between 1990 and 2000.

8.3.1 Temporal and spatial trends in the heavy metal concentration in mosses: comparisons between selected countries

For detailed information on the sources of heavy metals in each country we refer to the reports of the individual surveys (Rühling, 1994; Rühling and Steinnes, 1998; Buse *et al*., 2003). These reports also discuss in more detail the spatial trends observed across Europe, showing that in all surveys there was a general trend of higher heavy metal concentrations in eastern parts compared with other parts of Europe. The results presented below are based on geometric mean values.

Arsenic

Not all five selected countries had determined the arsenic concentration in mosses in every survey year. Overall, the arsenic concentration in mosses was not significantly different between the five countries ($p = 0.055$). However, Tukey pair-wise comparisons showed that over the three sampling years, the arsenic concentration in mosses was significantly higher in Slovakia compared with Norway ($p = 0.046$) or Sweden ($p = 0.038$), which was due to the high arsenic concentration in mosses in Slovakia in 2000, as arsenic was not determined in Slovakia in previous years (Figure 8.1). With time the arsenic concentration decreased in Switzerland (1990 compared with later years) and the UK, where it decreased 61% between 1995 and 2000. In Norway, the arsenic concentration decreased with time, except between 1977 and 1985, and in Sweden, it decreased significantly between 1985 and 1995 with no changes between other sequential years (Figure 8.2).

Cadmium

Overall, the cadmium concentration in mosses was significantly different for the five countries ($p = 0.001$), due to the high levels in Slovakia. Over the three sampling years, the cadmium concentration in mosses was significantly higher in Slovakia than in the other countries ($p < 0.005$). In all countries the cadmium concentration in mosses was decreased in 2000 compared to 1990 (Figure 8.1). In the UK, the cadmium concentration in mosses was slightly higher in 1995 than 1990, but had decreased by 41% in 2000, whereas in Switzerland the cadmium concentration declined with time. In Norway, the cadmium concentration decreased with time, except between 1977 – 1985 and 1990 – 1995; the geometric mean was reduced by 46% in 2000 compared with 1977 (Figure 8.2). In Sweden, the cadmium concentration decreased considerably with time, except between 1995 – 2000; the geometric mean was reduced by 76% in 2000 compared with 1970.

Chromium

Overall, the chromium concentration in mosses was significantly different for the five countries ($p = 0.011$), due to the high levels in Slovakia. Over the three sampling years, the chromium concentration in mosses was significantly higher in Slovakia than in the other countries ($p < 0.02$). The chromium concentration in mosses increased in the UK (155%) and Slovakia (266%) between 1990 and 1995, but it decreased in Sweden (45%) and Switzerland (71%) between 1990 and 1995 and in Norway (32%) and Slovakia (49%) between 1995 and

2000 (Figure 8.1). In Norway, the chromium concentration decreased significantly between 1985 – 1990 and 1995 – 2000, whereas in Sweden, it decreased significantly between 1970 – 1975, remained similar in later years with another decline between 1990 – 1995 (Figure 8.2).

Copper

Overall, the copper concentration in mosses was significantly different for the five countries ($p = 0.001$), again due to the high levels in Slovakia. Over the three sampling years, the copper concentration in mosses was significantly higher in Slovakia than in the other countries ($p < 0.005$). In most countries, the copper concentration in mosses did not change much with time, with slight increases, no changes or slight decreases being observed (Figure 8.1). Nevertheless, the copper concentration in mosses in the UK decreased by 31% between 1990 and 2000 and in Slovakia by ca. 50% in 2000 compared to earlier years. In Norway, the copper concentration declined between 1977 – 1985 and 1995 – 2000, with no changes between 1985 – 1995 (Figure 8.2). In Sweden, copper showed a similar trend as chromium.

Iron

As for many other metals, the iron concentration in mosses was significantly different for the five countries ($p = 0.000$) due to the high levels in Slovakia. Over the three sampling years, the iron concentration in mosses was significantly higher in Slovakia than in the other countries ($p = 0.000$). In the UK, the iron concentration in mosses increased by 215% between 1990 and 1995, but was not determined in 2000 (Figure 8.1). In Switzerland and Slovakia, the iron concentration hardly changed with time. In Norway, the iron concentration showed increases, no changes and decreases with time, whereas in Sweden, it dropped steadily over the years with a slight increase in 2000 (Figure 8.2).

Lead

Overall, the lead concentration in mosses was significantly different for the five countries ($p = 0.000$) due to the high levels in Slovakia. Over the three sampling years, the lead concentration in mosses was significantly higher in Slovakia than in the other countries ($p = 0.001$ or lower). Lead was the only metal that had decreased significantly ($p = 0.001$) between 1990 and 2000 in all five countries, although an increase (24%) was observed in Slovakia between 1995 and 2000 after it had decreased (48%) between 1990 and 1995 (Figure 8.1). After an increase between 1990 and 1995 (23%), the lead concentration in mosses decreased between 1995 and 2000 (56%) in the UK. In Norway and Sweden, the lead concentration declined significantly with time, with a reduction in the geometric mean between the first and last year of the survey of 84 and 92%, respectively (Figure 8.2).

Mercury

There was limited data available for the mercury concentration in mosses, resulting in no significant country effect ($p = 0.23$). Once again, high mercury concentrations were found in mosses in Slovakia, where it increased between 1995 and 2000 by 86% (Figure 8.1). In Norway, Sweden (except between 1985 – 1995) and Switzerland the mercury concentration in mosses decreased with time (Figures 8.1, 8.2) and the drop in the geometric mean of the mercury concentration between the first and last year of the survey was 24, 92 and 33%, respectively.

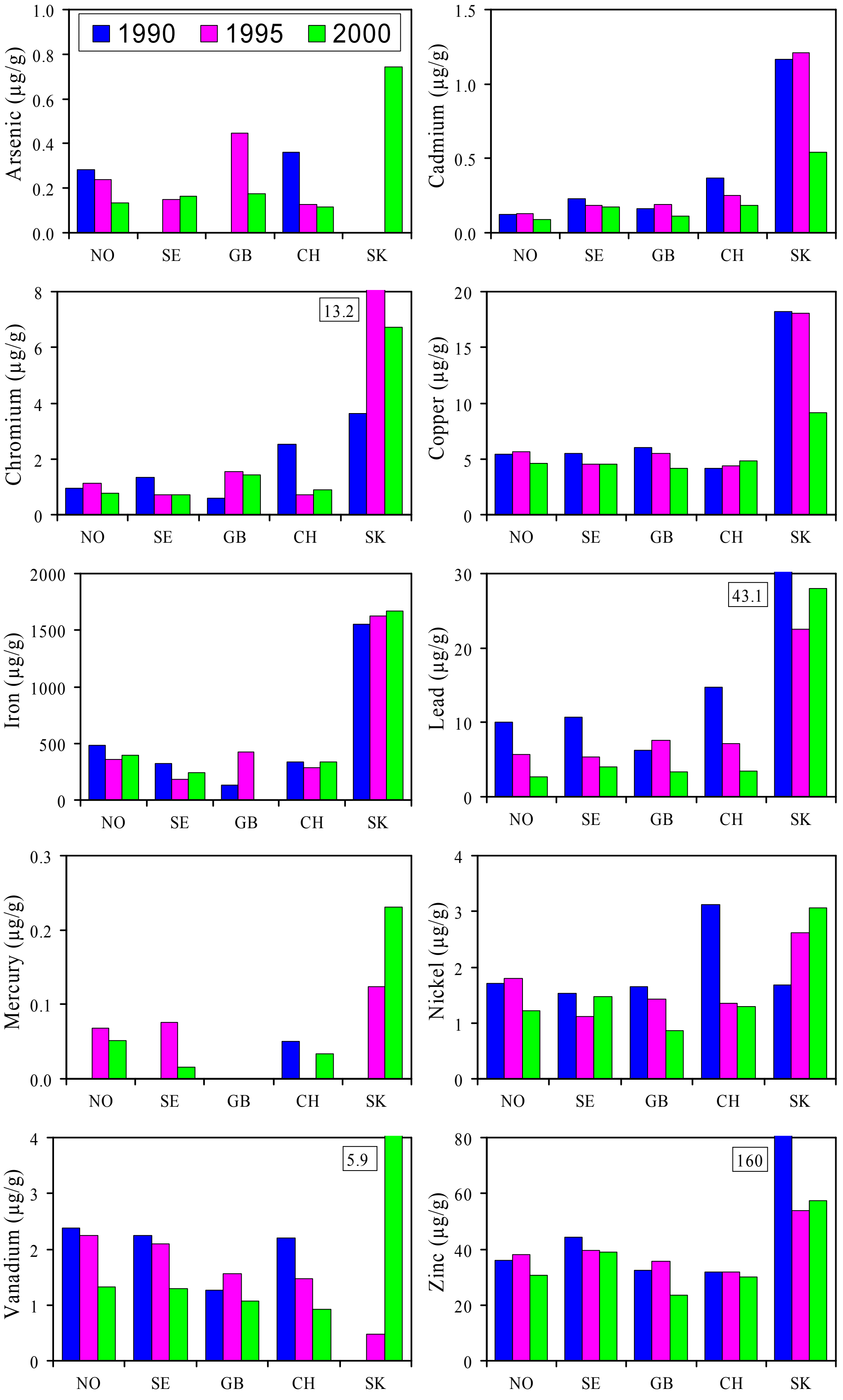


Figure 8.1 Geometric mean concentration of heavy metals in mosses in Norway (NO), Sweden (SE), United Kingdom (GB), Switzerland (CH) and Slovakia (SK) between 1990 and 2000

Nickel, vanadium and zinc

There were no significant country effects on the concentration of nickel (p = 0.18), vanadium (p = 0.47) and zinc (p = 0.84) in mosses. These metals showed either increases, no changes or decreases with time (Figures 8.1, 8.2). The vanadium concentration in mosses generally decreased between 1990 and 2000, except in Slovakia. In the UK, the nickel, vanadium and zinc concentration in mosses decreased between 1990 and 2000 by 48, 16 and 28%, respectively (although a slight increase was observed for vanadium and zinc between 1995 and 2000). In Switzerland, the nickel concentration declined significantly between 1990 – 1995 (no change between 1995 – 2000), but no changes in the zinc concentration were observed with time. In Norway, the concentration of vanadium showed a steady decline with time, although not always significant between years, whilst the concentration of nickel and zinc only declined significantly between 1995 – 2000. In Sweden, these metals showed a steady decline with time for most years.

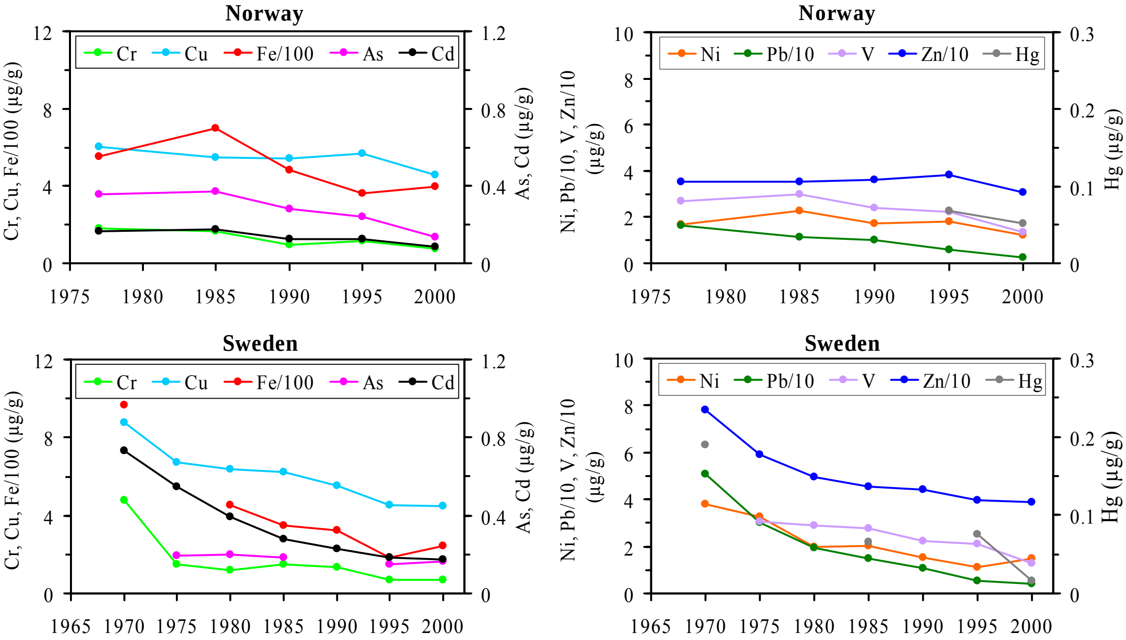


Figure 8.2 Geometric mean concentration of heavy metals in mosses in Norway and Sweden between 1977 - 2000 and 1970 – 2000, respectively.

In summary, for most heavy metals their concentration in mosses was higher in Slovakia than in the other countries. In Norway and Sweden, most metals showed a decline with time, although some more than others. This could indicate that long-range transboundary heavy metal pollution to those countries decreased with time as Norway and Sweden do not have many local pollution sources of heavy metals (Rühling, 1994; Rühling and Steinnes, 1998; Buse *et al*., 2003). In the other countries, only arsenic, cadmium and lead concentrations in mosses showed a consistent decline between 1990 – 2000. For lead the decline was most consistent in all countries, resulting in a significant decrease with time for all five countries.

8.3.2 Temporal trends in cadmium, lead and mercury concentration in mosses: comparisons with modelled EMEP deposition data.

It should be noted that the comparisons below are based on geometric mean values of the lead and cadmium concentrations in mosses. The EMEP deposition data are based on official emission data submitted by Parties to the LRTAP Convention Secretariat before 2005 (EMEP, 2004) and are not based on the new calculations of depositions of cadmium and lead using adjusted emission scenarios (EMEP, 2005). For details regarding the differences between the official emission scenarios and the adjusted emission scenarios, see EMEP (2005). The old modelled EMEP deposition data, based on official emission data, are less in agreement with measurement data than the new modelled deposition data, based on adjusted emission scenarios (EMEP, 2005). However, this might not necessarily affect the temporal trends in modelled deposition as reported by EMEP (2004). The modelled EMEP heavy metal deposition data contain high uncertainties due to the high uncertainties with emission data, which the model uses as input data.

Cadmium

In Norway, the cadmium concentration in mosses was generally lower than in other countries. Between 1977 – 2000 the cadmium concentration decreased 1.9 times (1.4 times between 1990 – 2000), which is similar to the 1.3 times decrease in modelled total deposition between 1980 – 2000 (EMEP, 2004). In Sweden, the cadmium concentration in mosses declined 4.2 times between 1970 – 2000 and 2.3 times between 1980 – 2000. The latter decline is of a similar order of magnitude as the 1.5 times decrease in modelled total cadmium deposition. In the UK and Slovakia, the cadmium concentration in mosses decreased 1.5 and 2.1 times between 1990 – 2000, respectively, which is in agreement with the 2 and 2.5 times decrease in the modelled cadmium deposition between 1980 – 2000, respectively. In Switzerland, the decline in cadmium concentration in mosses between 1990 – 2000 (2 times) appears to be higher than the decline in modelled cadmium deposition between 1980 – 2000 (1.6 times).

Lead

In Norway, the lead concentration in mosses decreased 6.1 times between 1977 – 2000, which is of a similar order of magnitude as the reported 5 times decline in modelled total lead deposition (EMEP, 2004). In Sweden, the lead concentration in mosses decreased 4.8 times between 1980 – 2000, which again is similar to the 5.7 times decrease in modelled total lead deposition. For the UK, Switzerland and Slovakia the decrease in lead concentration in mosses between 1990 – 2000 (1.9, 4.2 and 1.5 times, respectively) was lower than the decrease in modelled total lead deposition between 1980 – 2000 (17.5, 7.2 and 5.7, respectively). However, when taken into account that on average over Europe modelled lead deposition declined ca. 6.5 times between 1980 – 2000 and only ca. 2.5 times between 1990 – 2000, the observed decline in the lead concentration in mosses between 1990 – 2000 in Switzerland and Slovakia is in reasonable agreement with the reported decline in modelled total lead deposition. For the UK, the decline (ca. 2 – 3 times) in the measured lead concentration in air and precipitation between 1990 – 2000 (EMEP, 2005) seems to be better in agreement with the decline in the lead concentration in mosses.

Mercury

For mercury there was not enough moss data available to determine reliable trends. In the majority of European countries modelled total mercury depositions decreased 1.2 – 2.5 times between 1990 – 2000 (EMEP, 2004). The decline for Norway, Sweden, Switzerland, Slovakia and the UK were ca. 1.1, 1.2, 1.9, 2.1 and 2.3, times, respectively.

In summary, the magnitude of decline in modelled lead and cadmium deposition determined by EMEP is generally in agreement with the decline in lead and cadmium concentration in mosses observed for the five countries. Not enough moss data was available to determine reliable trends for mercury.

8.3.3 Relationship between the lead concentration in mosses and modelled EMEP lead deposition

In 2004, the ICP Vegetation Coordination Centre sent data from the European heavy metals in mosses survey 2000/1 to EMEP/MSC-East to establish the performance of the EMEP heavy metal deposition model. Since the last three years of moss growth was selected for the determination of the heavy metal concentration in mosses (Buse *et al*., 2003), EMEP/MSC-East compared the lead concentration in mosses with the modelled total accumulated deposition of lead for the years 1997 – 1999 (EMEP, 2005). A significant positive correlation coefficient (R = 0.56) indicated that that EMEP model managed to mimic the spatial pattern of lead pollution levels for the whole of Europe (Figure 8.3a). The correlation coefficient is not as high as normally obtained when the model is verified with concentrations in precipitation measured at the EMEP network. However, it should be noted that the lead concentrations in mosses were not only determined in areas with background levels of lead pollution, but also in relatively polluted areas (Buse *et al*., 2003). In addition, the concentration of metals in mosses is affected by factors such as proximity to the sea, relative contribution of wet and dry deposition, contamination by soil dust, in particular in dry areas etc. (Berg and Steinnes, 1997). Therefore, the correlation between modelled lead deposition and its concentration in mosses can vary from one part of Europe to another. As a result, country-specific correlation coefficients were observed. For example, the correlation coefficient was 0.81 for Norway and 0.41 for Germany (EMEP, 2005). The lower correlation coefficient in Germany might be due to the uncertain spatial distribution of anthropogenic lead emissions in this country. For the UK, the correlation coefficient was 0.52 using the old modelled lead deposition data without the adjusted emission scenarios (see above).

When a comparison was performed between lead concentrations in mosses and modelled total lead deposition for selected grid cells in Scandinavia where EMEP monitoring stations are situated, i.e. a comparison was performed at locations representative for the EMEP task (modelling long-range transboundary air pollution), a very high correlation of 0.91 was found (Figure 8.3b). Scandinavian emissions are relatively low and lead pollution levels are mainly caused by long-range transport (and possibly by natural emissions and re-emissions). The high correlation indicates that the EMEP model simulates atmospheric transport well. The ICP Vegetation Coordination Centre will continue the fruitful collaboration with EMEP/MSC-East to determine the performance of the new, adjusted EMEP model (EMEP, 2005) and to establish the correlation between the heavy metal concentration in mosses and modelled heavy metal deposition for other metals than lead. In addition, confounding factors will be analysed in more detail.

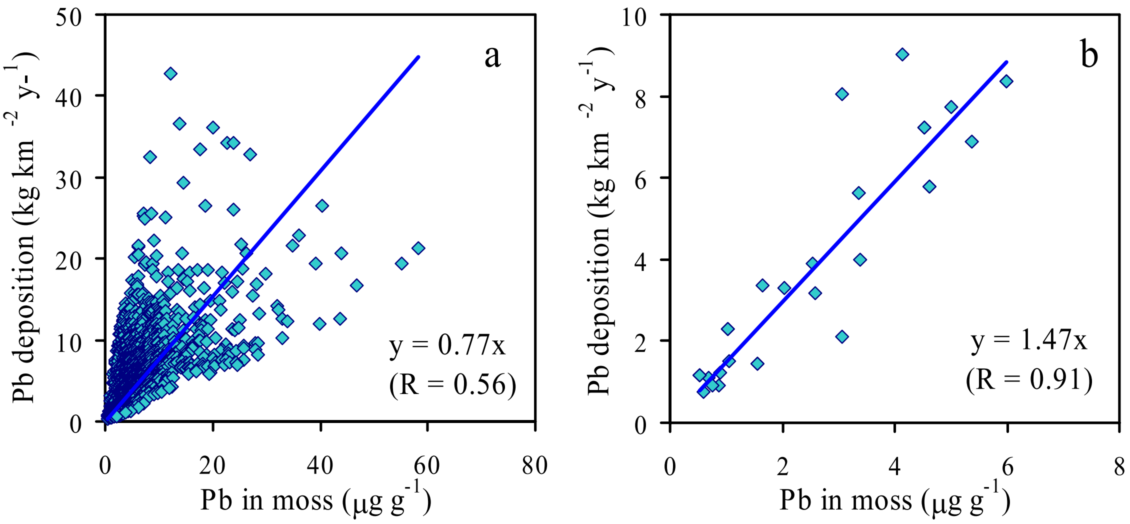


Figure 8.3 Modelled total depositions of lead versus measured lead concentrations in mosses accumulated over 1997 – 1999 (a) across Europe and (b) at sites with background levels of lead pollution in Scandinavia (Norway, Sweden, Finland). Modified after EMEP (2005).

9. OPTION 4: TO PREPARE A REVIEW OF THE IMPACTS OF POLLUTANT MIXTURES ON VEGETATION

9.1 Introduction

Nutrient availability has been identified as an important factor in the ozone sensitivity of semi-natural vegetation (Bassin *et al*., in press; Davison & Barnes, 1998). The nutrient demand *per se* of a species does not necessarily confer particular benefit/disadvantage to that species in terms of ozone response. For example, Ellenberg N values showed no correlation with ozone sensitivity (Hayes *et al*., submitted (a); Jones *et al*., in press; see section 4.3.4). However, the response of species to ozone may be modified by a number of factors, and these may be summarised to identify particular communities or groups of species which may be jointly at risk of high ozone and N deposition.

The *Risk* of any adverse effect on a system is usually described as a combination of *Sensitivity* x *Exposure*. Thus, the mechanisms by which N deposition may alter responses to ozone can be separated broadly into those which affect the *Sensitivity* to ozone (e.g. uptake, detoxification) and those which affect the *Exposure* to ozone (e.g. geographical location, phenology, plant form). These will have further impacts on ecological processes such as litter decomposition, mineralisation and competition.

This chapter gives an introduction to the effects of atmospheric nitrogen (N) on semi-natural vegetation systems, followed by a discussion on the mechanisms by which N and ozone may interact. Evidence from the limited literature available, and from unpublished biomonitoring experiments conducted by the ICP Vegetation programme participants, is used to present some of the observed interactions in semi-natural vegetation communities or species. Some of the communities likely to be at joint risk of N and ozone are identified, and maps showing their geographical occurrence are presented. Lastly, a summary of policy implications is drawn up to advise how N deposition may moderate ozone response in semi-natural vegetation.

9.2 Effects of N deposition on semi-natural plant communities

Nitrogen deposition alters natural and semi-natural plant communities through two principal effects: eutrophication and acidification. As N is often the primary limiting nutrient in oligotrophic systems, atmospheric deposition of N will alleviate this limitation, causing shifts in the competitive balance, leading to invasion or spread of more nitrophilous species and competitive exclusion of low growing or less vigorous species. There may also be direct toxicity effects on species adapted to particularly low levels of nutrient input. Secondly, N is an acidifying pollutant, and has taken over from sulphur as the principal acidifying component of acid rain in the UK (RGAR, 1997). Thus both eutrophication and acidification may alter the available ecological niche resulting in shifts in community composition.

The potential impacts of N deposition on semi-natural communities have been shown by both observational and experimental studies. In Europe, N has been shown to have deleterious impacts on species diversity and composition in a wide range of semi-natural communities

(Achermann & Bobbink, 2003; Bobbink *et al*., 1998; Jones *et al*., 2004; Stevens *et al*., 2004). This usually leads to increased dominance of graminoids in habitats such as heathlands (Aerts & Berendse, 1988; Aerts *et al*., 1990), forest understorey (Strengbom *et al*., 2003), acid and calcareous grasslands (Carroll *et al*., 2003) and montane moss heaths (Jones *et al*., 2002; Pearce & van der Wal, 2002). However, some studies also show an increase in forbs (Willis & Yemm, 1961; Wilson *et al*., 1995). Shifts in community composition are frequently triggered by separate environmental drivers, including pathogens (Heil, 1983; Strengbom *et al*., 2002), climatic events such as frost or drought (Aerts & Bobbink, 1999), and human induced disturbance such as grazing or other management regimes. The direction of change in a community depends, to a degree, on the component species and on edaphic factors such as soil wetness (Aerts & Berendse, 1988; Aerts *et al*., 1990).

N deposition affects partitioning of resources in plants. With adequate nutrient supply to the roots, many species allocate a greater proportion of photosynthate to above ground growth to maximise light capture, with a resulting decrease in root:shoot ratios (Tisdale *et al*., 1993). Nitrogen deposition also has impacts on the soil. Through its acidifying influence, N deposition can cause a decrease in base saturation and a consequent decrease in the pH of the soil and soil waters (Brady & Weil, 1999). N deposition can also affect the soil biota, causing shifts in bacterial and fungal community composition. Studies using Phospho-Lipid Fatty Acids (PLFAs) to estimate micro-organism biomass have shown lower fungal biomass and lower fungal:bacterial ratios in improved pastures, suggesting that eutrophication is detrimental to fungal activity (Bardgett *et al*., 1996; Bardgett *et al*., 1999; Grayston *et al*., 2001).

Many of the environmental receptors described above are also affected by ozone. Thus, there is considerable potential for effects on semi-natural vegetation communities, through interactions between ozone and nitrogen deposition and these are discussed below.

9.3 Potential causes of N x ozone interactions

The mechanisms by which N deposition may alter responses to ozone can be separated broadly into those which affect the *Sensitivity* to ozone (e.g. uptake, detoxification) and those which affect the *Exposure* to ozone (e.g. geographical location, phenology, plant form). These are discussed below, however, some of these mechanisms will have an impact on both sensitivity and exposure.

9.3.1 Ways in which N deposition may alter sensitivity to ozone (i.e. the amount of ozone getting into the plant)

Uptake

Nutrient availability has the potential to modify ozone uptake through altered physiological and morphological parameters such as Specific Leaf Area, stomatal density, stomatal control and water use efficiency e.g. (Andersen, 2003; Franzaring *et al*., 1999). Low chlorophyll levels can restrict stomatal opening capacity and this degree of control can be almost equal to that exerted by solar radiation or vapour pressure deficit (Matsumoto *et al*., 2005). Evidence suggests that black oak trees (*Quercus kelloggii*) subject to a high N treatment showed poor water use efficiency and poor stomatal control in full sun in mid-summer, in comparison to control trees. On the other hand, stomatal control was better in the shade in the high N trees

(Grulke *et al*., 2005). The net effect on stomatal uptake of ozone is unclear and may depend on the balance of leaves in sun or shade and the ozone profile within a forest canopy.

Detoxification and repair

Detoxification of ozone and repair of ozone damage both carry a high metabolic cost. Plants manufacture a range of compounds to assist with these processes including phenolics and lignin for damage repair, and antioxidants which scavenge free radicals (Andersen, 2003; Bassin *et al*., submitted). The metabolic costs of detoxification are generally higher than damage repair (Herms & Mattson, 1991; Tingey & Anderson, 1991). Availability of N has the potential to alter plant responses by indirectly altering these detoxification and repair processes. Thus, increased availability of N allows greater manufacture of the chemicals required for detoxification and may be one mechanism by which N can alleviate ozone toxicity. This was suggested by Whitfield *et al.* (1998) for *Plantago major*. The converse argument is that excess N may stimulate plant growth at the expense of manufacturing secondary metabolites, and it is suggested that, across a range of species and genotypes, a higher Relative Growth Rate (RGR) correlates well with ozone sensitivity (Danielsson *et al*., 1999; Reiling & Davison, 1992).

9.3.2 Ways in which N deposition may alter **exposure** *to ozone*

Geographical

Geographical factors affecting the exposure to ozone also contribute to risk. For example, many of the habitats which are particularly sensitive to N deposition occur in the uplands which also experience higher ozone concentrations. The communities which are most sensitive to atmospheric N deposition include tundra vegetation and alpine/sub-alpine habitats (Achermann & Bobbink, 2003), see also Table 9.1. Ozone exposure in northern boreal regions is relatively low. However, the montane habitats tend to receive higher background ozone concentrations due to the importance of long-range transport at altitude (Auvray & Bey, 2005; Wang *et al*., 2003). These same long range transport processes are responsible for transport of oxidised N, and N deposition is usually increased in montane areas due to higher rainfall and to seeder-feeder scavenger effects of water droplets falling through cloud (Fowler *et al*., 1995). Other habitats, while not inherently over-sensitive to N deposition may, by their geographical location, be at risk from both elevated N and ozone concentrations. Areas of semi-natural vegetation in lowland landscapes often survive as isolated fragments surrounded by intensive agriculture. This is particularly true for patches of lowland heath, some semi-natural grasslands, and lowland fen or mire communities. Since sources of ammonia are primarily agricultural in origin, these fragments are particularly at risk from high ammonia emissions resulting from volatilisation of slurry or fertiliser application, or emissions from intensive poultry- or animal-rearing units. As ozone concentrations are generally higher in rural areas, there exists the clear potential for interacting effects on these communities.

Thus, risks associated with N deposition can occur both in N-sensitive habitats subject to relatively low N loads, but also to more N-tolerant habitats subject to very high N loads from point sources. Vegetation change may be particularly acute where this risk coincides with high ozone exposure. Therefore, co-occurrence both on a regional and on a local scale of N deposition and high ozone concentrations, combined with the location of N-sensitive and ozone-sensitive communities leads to a highly spatially variable matrix of risk.

Phenological

Phenology may affect ozone exposure in a number of different ways by controlling the timing of important biological processes relative to seasonal peaks in ozone concentrations. In temperate latitudes, these peak ozone concentrations occur in spring and early summer. Weightings of ozone response by phenological stage have been calculated for a few species, including one semi-natural species – *Plantago major* (Soja *et al*., 1999). However, application to a wide range of semi-natural vegetation species has yet to be attempted. Furthermore, nitrogen availability is known to affect phenology in many species and any interaction with ozone will depend on the nature and the timing of key growth stages.

Timing of the growing season may account for differential sensitivity between species. For example, growth over winter is important for *Festuca ovina*, whereas *Arrhenatherum elatius* exhibits rapid growth in spring and early summer (Rorison *et al*., 1983), thus *F. ovina* would have lower exposure to ozone. Where nitrogen availability alters the growing season, this may affect exposure to ozone. Bud break in *Vaccinium myrtillus* typically occurs in early spring, but occurs up to 2 weeks earlier when exposed to high N (Jones & Ashenden, 2001). By bringing forward this period of high sensitivity relative to peak ozone concentrations, exposure may be reduced. Shoots of *Calluna vulgaris* commence growing earlier under elevated N, which renders them more sensitive to frost damage (Caporn *et al*., 1994; Power *et al*., 1998), but may also reduce their exposure to ozone.

Species are particularly sensitive to ozone at the seedling stage (Lyons & Barnes, 1998). Thus, species emerging as seedlings during spring and early summer have a higher exposure to ozone than autumn or winter germinating species. Nitrogen deposition may promote faster development of seedlings, which may decrease their exposure by facilitating growth before peak ozone concentrations or may increase sensitivity due to high RGR at this stage, as suggested by Bassin *et al*. (in press). The precise dynamics will depend partly on the species' inherent sensitivity, and on the timing of seedling emergence relative to peak ozone concentrations.

Other stages of the growth cycle are also associated with high sensitivity to ozone. The initiation of reproductive material has been shown to be important in some crop species (Vandermeiren *et al*., 1995). *Phaseolus vulgaris* was more sensitive during pod fill (Tingey *et al*., 2002) and wheat is particularly sensitive around the time of anthesis, with significant negative effects on grain production (Gelang *et al*., 2001; McKee & Long, 2001; Sild *et al*., 2002). These effects on crop plants relate to ozone disruption of internal carbohydrate translocation and have consequences for reproductive success of semi-natural vegetation species. Enhanced nitrogen supply may bring forward the time of reproduction in some species or may delay it in others. The nature of the interactive effects with ozone will depend on the growth strategy of the species, which is discussed below, and the timing of reproduction relative to peak ozone concentrations.

Ozone often accelerates leaf turnover and senescence (Karnosky *et al*., 1996) and, by affecting internal nutrient translocation, leads to leaf litter with a higher N content. The implications of higher tissue N on nutrient cycling are discussed below. However, the timing of senescence and leaf fall also has implications for nutrient cycling, since the rate of litter decomposition is controlled to a large extent by climate-related factors such as temperature and soil-moisture, which alter seasonally.

Life strategy

Nutrient availability in some species controls whether they behave as biennials or annuals, and therefore may affect exposure to peak ozone episodes. Similarly, whether or not a species flowers and sets seed depends both on nutrient availability and on life strategy. For example, ruderal species *sensu* Grime (1973) often accelerate the life cycle in response to environmental stress (including nutrient stress), whereas stress-tolerant perennials will limit or reduce flowering until conditions are more favourable. Thus, N deposition may modify a species exposure to ozone depending on its life strategy.

Growth form

Plant growth form affects exposure to ozone by determining the size of plants and their position within canopy. In semi-natural communities where N is the limiting nutrient, elevated N deposition leads to enhanced growth of most vascular species. Taller plants with larger leaves consequently have a higher exposure to ozone. However, the precise exposure of an individual plant is modified by its relation to other individuals in the community and this is discussed in more detail in the section on competition below.

9.3.3 Potential impacts on ecological processes

Resource partitioning and its ecological effects

Both ozone and N deposition have been known to reduce root:shoot ratios. While N deposition usually increases above-ground biomass, the root biomass does not increase accordingly and the net effect is a reduction in the root:shoot ratio (Tisdale *et al*., 1993). This is because increased N availability leads to retention of carbohydrate in photosynthetic organs and a down-regulation of root growth (Wingler *et al*., 1994). A number of studies, mostly on trees have also shown that ozone reduces root growth relative to shoot growth. Eatough Jones *et al*. (2004) showed reduced root growth in pines experiencing high levels of ozone. This is due to increased resource allocation to stems and branches and reduced carbon allocation to roots (Rennenberg *et al*., 1996; Spence *et al*., 1990). Effects on root growth can be rapid due to the sudden decrease in carbohydrate production caused by ozone damage to leaves (Andersen, 2003). The combined effects of high N and ozone exposure have been shown in gradient studies in the San Bernardino mountains, California, where root growth relative to total biomass was reduced in the most polluted areas (Grulke & Balduman, 1999; Takemoto *et al*., 2001). In non-tree species, experimental manipulations of both ozone and N reduced the root:shoot ratio in *Trifolium subterraneum* (Sanz *et al*., 2005).

Alteration of root:shoot ratios has implications for individual species and community composition. Reduced root biomass may reduce the ability to withstand extreme climatic conditions such as drought or storms. A reduction in root growth in species with mycorrhizal associations may result in less root area for mycorrhizal attachment, and the reduced carbohydrate supply to roots will affect other symbionts dependent on that energy source, such as N-fixing bacteria. Reductions in root exudates will have further implications for soil microflora (Andersen, 2003) as bacterial activity is often carbon limited. In the long-term, this may have consequences for soil mineralisation processes.

Leaf chemistry and its ecological effects

Related to the effects on root:shoot ratios, ozone also affects translocation of nutrients within above-ground plant tissues. Ozone frequently causes premature senescence (Karnosky *et al*., 1996; Miller *et al*., 1999). The N content in these senesced leaves is often elevated as internal N re-allocation within the plant is not complete (Findlay & Jones, 1990). Elevated tissue N in living tissue and in litter can also be a consequence of elevated N deposition (Hicks *et al.*, 2000; Pitcairn *et al.*, 1995). Higher N content of litter can have varying effects on rates of decomposition and nutrient cycling. In general, litter with a higher N content, and therefore a lower C:N ratio decomposes faster, and decomposition rates have been correlated with %N content (positive), and with lignin content, C:N ratio and lignin:N ratio (negative) (O'Neill & Norby, 1996). Ozone can increase the content of lignin and recalcitrant phenolic compounds (Kim *et al.*, 1998; Saleem *et al.*, 2001) and in species with high contents of these compounds such as conifers, the net effect of increased litter N content is to reduce rates of decomposition (Berg *et al.*, 1998). Conversely, in species with rapidly decomposable litter, increased N contents lead to faster decomposition (Fog, 1988) and will speed up N mineralisation rates, with consequences for community succession in oligotrophic habitats (Berendse *et al.*, 1994). Increased rates of leaf turnover as a result of ozone exposure will lead to increased litter fall, and the quantity of litter has as great an effect on nutrient cycling as the chemical quality of the litter (Korner & Arnone, 1992).

Competition

Competition between individuals, species and populations is the ultimate determinant of community composition and integrates the effects of all other environmental drivers. However, competitive processes themselves may alter exposure to ozone by altering the composition and structure of the plant community. Excess atmospheric N deposition in an N-limited system usually leads to increased dominance of faster growing species, often leading to substantial changes in species composition. The classic example in temperate Europe is the conversion of ericaceous heathlands to grassland communities (Aerts, 1989; Aerts *et al.*, 1990; Heil & Diemont, 1983). Changing dominance may increase the exposure of different species, or of other genotypes with differing ozone sensitivity (McDonald *et al.*, 2002) in that community. The dominance of one species usually leads to reduced exposure in other species as they are relegated to a subordinate position within the canopy. Thus, competition will alter the exposure to ozone of individual species. However, it may also alter the sensitivity of the community as a whole. Competitive exclusion, whether as a result of N deposition or ozone (Bobbink, 1998; Stevens *et al.*, 2004), results in the loss of species which each have a particular sensitivity to ozone. Thus, by altering community composition, the outcome of competition may be to fundamentally alter the balance of sensitivity of the whole community to ozone.

9.3.4 Other modifiers of nitrogen x ozone interactions

Herbivory

Nitrogen compounds are important in plant chemical defences against herbivory (Pate, 1983). However, high tissue N content as a result of excess N supply can encourage herbivory, and was a trigger for large-scale canopy damage in *Calluna* moorland, which was subsequently colonised by grasses (Heil, 1983). Ozone exposure can either increase herbivory (Holton *et al.*, 2003; Kopper & Lindroth, 2003a) or decrease herbivory (Kopper & Lindroth, 2003b). The effects may be plant, insect and genotype specific (Jondrup *et al.*, 2002), and are compounded by the combined effects of ozone operating on both insect behaviour and on plant chemistry. Increased herbivory appears to relate to reductions in secondary defence compounds, while decreased herbivory usually relates to poorer nutritive quality of the plant material under elevated ozone. There is the potential for major impacts on community composition where levels of both N deposition and ozone are high, although specific outcomes are not predictable at present.

Disease

Incidence of pathogenic organisms, or the susceptibility of a host to disease can be increased by N deposition. In Scandinavian forests, elevated N leads to greater incidence of the parasitic fungus *Valdensia heterodoxa* on the shrub *Vaccinium myrtillus*, leading to reduced abundance of this shrub species (Nordin *et al*., 1998; Strengbom *et al*., 2002). Ozone exposure has been shown to increase sensitivity of crop species to disease (Gimeno *et al*., 1999). However, the incidence of disease on semi-natural vegetation in relation to ozone is not well studied. Weakened plants are likely to have fewer resources available for detoxification or repair and would be more at risk from ozone, leading to greater effects on community composition in sensitive communities subject to high N deposition.

Management

Management of semi-natural vegetation generally takes the form of cutting/mowing, grazing, burning and agricultural improvement. The last category may involve severe habitat modification including drainage, ploughing, or fertilisation and will not be considered here as this usually leads to loss of semi-natural vegetation. However, the former three categories are often used to maintain semi-natural vegetation in a favourable status for a range of ‘Ecosystem Services’ including ‘biodiversity’ and agricultural production. All involve alterations in canopy structure ranging from burning (least frequent, perhaps once every 15 – 20 years in heathland), to grazing (can be continuous with year-round grazing). Thus, both the exposure and, potentially, the sensitivity to ozone will be altered by management.

It is possible that sensitivity will be altered due to differences in the capacity for detoxification and avoidance in young shoot material, compared with the older material that has been removed by management. While some data support this idea (see section on *Centaura jacea* experiments below), this has not yet been studied in detail. Exposure is altered in numerous ways. Biomass removal leads to a lower canopy, a shorter diffusion path within the canopy and more equitable exposure of all species to ozone, together with reduced evapo-transpiration losses and higher soil moisture. In grasslands, the net consequence is predicted to be a higher ozone flux to the most intensively managed (by grazing/mowing) habitats in mesic habitats, although not in cooler or wetter areas. This is discussed in more detail in the flux modelling work under Objective 7 (this report). The difference between mowing and grazing, with respect to any interactions with ozone is that mowing progressively depletes the soil nitrogen store (Jones, 2005; Olff *et al*., 1994), whereas nitrogen losses under grazing are low (Frissel, 1978; Perkins, 1978). This has implications for long-term soil transformation processes and plant community development, *via* some of the factors discussed above. The timing of any management activity has clear implications with respect to the period of peak ozone episodes.

Limitation of other resources

Soil moisture limitation leads to closure of stomata and hence a reduction in ozone uptake. Similarly, limitation of other resources such as soil phosphorus may also modify responses to ozone and potential interactions with N deposition. Calcareous grasslands are frequently P limited (Carroll *et al*., 2003; Morecroft *et al*., 1994), and this limits any growth response to N deposition. This may prevent some of the N modifications of ozone exposure allied to increases in fast growing competitive species. However, N deposition under conditions of P limitation may still lead to competitive shifts in community composition as species with improved P capture efficiency or those with mycorrhizal associations are favoured, as discussed in Jones (2005). Furthermore, increases in tissue N content may still occur, and there remains the possibility of some N x ozone interactions with respect to mineralisation and other soil processes.

9.4 Summary of evidence from the literature on nitrogen x ozone interactions

As discussed above, there are many potential mechanisms by which N x ozone interactions may arise. To date, the body of knowledge on ozone effects is large, but the study of interactive effects with other factors is relatively small. This is particularly the case for interactions with nitrogen supply. Specific examples from the literature are listed below according to the nature of the interactive response. Many of these data are from forest systems and include both experimental work under controlled conditions and results from field survey data and gradient studies. Much of the latter work comes from the San Bernardino mountains area in California, USA where there is high exposure to both ozone and N deposition. However, variation in environmental factors other than just N and ozone concentrations should also be borne in mind with these data.

9.4.1 Examples where elevated N increases ozone toxicity

In forest systems, ponderosa pine and Jeffrey pine trees in the San Bernardino Mountains exposed to high levels of air pollution (including both ozone and atmospheric N deposition) showed complex responses to experimental N amendment with respect to bark beetle activity and tree mortality. Nitrogen amendment did not affect ozone injury. At the low air pollution site, N amendment increased both beetle activity and tree mortality. However, at the high air pollution site, N amendment increased tree mortality (non-significant), but appeared to reduce bark beetle activity (Eatough Jones *et al*., 2004). This paradox may relate to the proportion of remaining trees at the high pollution site which were susceptible to bark beetle attack, rather than direct or indirect treatment effects on bark beetle activity. This emphasises the potential importance of nitrogen deposition in altering the susceptibility of ozone-exposed plants to external factors such as herbivory.

Neural network modelling by Mills *et al*. (2000) suggested that ozone toxicity in *Trifolium repens*, as defined by the biomass ratio of sensitive clones relative to resistant clones, was higher close to sources of NO. However, this depends on whether interpretation focuses on the absolute biomass ratio, or on the slope and direction of the fitted response curve. If interpretation focuses on absolute biomass then the data could be interpreted differently such that elevated N moderates harmful ozone toxicity at low levels of ozone, but that this beneficial effect is lost at high ozone concentrations. There is also the issue in this study of co-occurrence of other pollutants (e.g. SO_2, NH_3, heavy metals) at urban sites with high NO concentrations.

In the annual legume species *Trifolium subterraneum*, elevated N increased root biomass in the zero ozone treatment, but not in the filtered air or added-ozone treatments (Sanz *et al*., 2005). Although there was no significant interaction, this resulted in the highest shoot:root ratios in the high N + ozone treatment, with potential implications for increased susceptibility to environmental perturbations such as drought, as discussed in the section on resource partitioning above.

9.4.2 Examples where elevated N moderates harmful ozone toxicity

In trees, experimental N additions to pine trees exposed to both high N deposition and high ozone resulted in faster radial growth compared to control trees with no N addition. However, at the low air pollution site, N amendment did not affect radial growth rates (Eatough Jones *et al*., 2004). Thus it appears that N may counteract some of the effects of ozone toxicity perhaps by supplying extra resources for detoxification or repair. Similarly, black oak (*Quercus kelloggii*) showed greater carbon sequestration and greater growth in N amended trees (Grulke & Balduman, 1999; Grulke *et al*., 2005) in a high air pollution climate. In *Picea abies* saplings, N generally had no interactive effects with ozone, with the exception of root starch concentrations where high N treatments appeared to alleviate the negative effects of ozone (Thomas *et al*., 2005).

Semi-natural species shows similar results to those observed in forest trees. In grasslands, high N inputs generally increase above ground biomass, providing they are not P limited. Results presented in Bassin *et al*. (2005), appear to suggest that higher levels of N (+ 25 kg N ha^{-1} yr^{-1} on top of an unknown background deposition) reduce the negative effects of ozone on whole-community biomass production for an alpine grassland. Effects on species groups differed in response to the ozone and N treatments. In the ambient ozone treatment, sedges replaced grasses with elevated N but, at high ozone levels, grasses maintained their cover with elevated N and sedges tended to replace forbs and legumes. Sedges may have been less competitive under high ozone conditions as they had reduced leaf size (Bassin *et al*., 2005). Work on another semi-natural species, *Plantago major*, showed that plants with adequate nutrients were less sensitive to ozone than nutrient-stressed plants, despite higher stomatal conductance and higher ozone flux (Whitfield *et al*., 1998).

There was a significant N x ozone interaction on the seed weight of *Trifolium striatum* (Bermejo *et al*., 2005) where, in non-filtered air, nitrogen fertilisation (at levels much higher than those found in natural deposition) could ameliorate ozone toxicity, but this did not extend to the treatment with added ozone, where seed-weights were uniformly low in all N treatments. In *Trifolium subterraneum*, high N supply reduced senescence in the high ozone treatment, but the interaction was not significant (Sanz *et al*., 2005).

9.4.3 Examples where no clear interaction was shown

Responses to N and ozone are generally complex, with some parameters showing an interaction or different trend in high N versus low N treatments, while other parameters are unaffected. For example, in trees, N amendment did not affect visible ozone injury at either highly polluted or less polluted locations, but differences in bark beetle attack and tree mortality were observed (discussed above) (Eatough Jones *et al*., 2004).

In semi-natural vegetation, there were no interactions on root and shoot biomass of *Trifolium striatum*, although seed weight was affected (see also above) (Bermejo *et al*., 2005). In the legume *Trifolium subterraneum*, N treatment did not alter visible injury response to ozone treatment, or above ground biomass. However, effects on senescence and root biomass were observed (discussed above) (Sanz *et al*., 2005).

Other experiments have shown no interactions between N and ozone. Nitrogen supply did not alter gas exchange rates in the legumes *Trifolium striatum, Trifolium subterraneum* or *Trifolium cheleri* (Alonso, R., unpublished data presented at ICP Workshop, Caernarfon, UK, Jan 2006). There was no significant nutrient x ozone interaction in the biomass of *Arrhenatherum elatius, Bromopsis erecta* or *Lathyrus pratensis* in a calcareous grassland study (Thwaites, 1997).

9.5 Results from the ICP Vegetation N *x* ozone experiments with *Centaurea jacea*

Partners from several countries in the ICP Vegetation undertook experiments with sensitive and resistant clones of *Centaurea jacea* as biomonitors of ambient ozone conditions. In addition, some participants also undertook nitrogen interaction experiments as part of this, with a range of N treatments in factorial design with different biotypes of *C. jacea*. Nitrogen treatments usually comprised: Control (0 kg N ha^{-1} yr^{-1}), Low (30 kg N ha^{-1} yr^{-1}) and High (80 kg N ha^{-1} yr^{-1}), and were conducted in 2002, 2003 and 2004. However, there were variations in the treatment level applied at some sites and different interpretations of the treatment duration with some partners applying the entire N load over the relatively short duration of the experiment resulting in much higher annual N load equivalents. Partners measured a range of parameters including visible ozone injury and insect herbivory, with harvests or biomass removal at intervals throughout the exposure. Variability in the range of parameters measured, the variations in applying the N treatments, and differences in reporting the data (some only reported means +/- standard deviation, rather than raw data) meant that rigorous statistical analysis was impossible. However, some tentative conclusions can be drawn from the results. The most useful data were provided by participants from Slovenia; Ebro Delta, Spain; Trier, Germany and Ascot (Imperial College), UK.

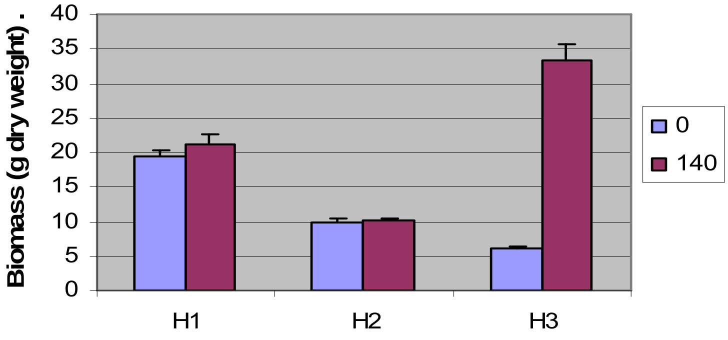
Nitrogen increased the biomass of *C. jacea* at roughly half of the sites for which there were adequate data, with highly significant increases at Ebro Delta in Spain which used much higher annual loads. The degree of biomass increase due to N did not appear to relate to level of ambient ozone exposure. Elevated N resulted in lower ozone injury at some sites in some years. By and large there were few significant differences in N response between the two biotypes (ozone sensitive and resistant). Some seasonal differences in herbivory and growth occurred when plants were grown in two batches in the same year, which appeared to be independent of AOT40. This suggests that seasonal effects and phenology play a strong part in the growth of this species independent of the timing of peak ozone exposure. Plotting of growth responses at all sites against AOT40 did not reveal clear dose-response relationships to ozone, nor did the response to N differ according to ozone exposure.

The inconsistent effects of N in relation to growth and ozone injury between sites appear to relate to whether plants were nutrient stressed or not. By and large, nitrogen-stressed plants tended to show greater ozone damage, while plants receiving N in excess of requirements grew larger and showed ameliorative effects of N on ozone injury. This was borne out by N effects on both growth (increased growth) and ozone injury (reduced injury) only becoming apparent in one experiment after successive biomass removal which may have contributed to N limitation in the soil (Figure 9.1). Results from two experiments suggest that very young, tender shoot material may be more at risk of ozone injury in plants receiving high N, while ozone injury later in the season was reduced by elevated N in one of the experiments (significant at two timepoints only) (Figure 9.2).

9.6 Communities likely to be most at risk from both N deposition and ozone

The wide range of ecological niches in time and space mean that, throughout the growing season, different species will be germinating, flowering or setting seed. Since the response of species to ozone appears to be highly species-specific (Jones *et al.*, in press), with only broad generalisations possible across families and Raunkiaier life form (Hayes *et al.*, submitted (a)), it is difficult to tell at this stage whether co-occurrence of high ozone and N deposition will make communities more or less stable. Bassin *et al.* (in press) argue that more productive habitats are likely to be more sensitive to ozone, and flux modelling shows that intensively managed habitats may receive a higher ozone flux (see Section 4.3.8). This suggests that the grazed, mesotrophic grasslands are likely to be most sensitive to ozone. However, a higher ozone flux does not necessarily equate to greater ozone impact (Whitfield *et al.* 1998), and detoxification mechanisms are an important modifier of the degree of ozone damage (Musselman *et al.* 2006). Legumes are known to be particularly sensitive to both N deposition and ozone, and communities with a high proportion of legumes may be particularly at risk. These may include many semi-improved pastures and may also include early successional communities on poor soils, such as occur in sand dunes.

a) Ebro Delta 2002 -Biomass



b) Ebro Delta 2002 -Ozone injury

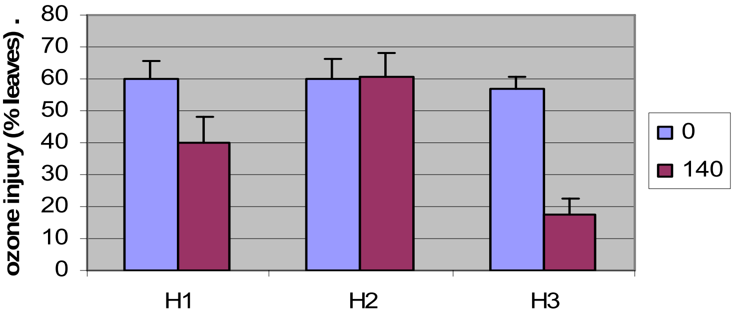


Figure 9.1 Data showing effects ambient ozone concentrations on *Centaurea jacea* at Ebro Delta, Spain, 2002 at successive harvests (H1…H3), for a) biomass and b) ozone-induced leaf injury. N treatments were 0 and 140 kg N ha^{-1} yr^{-1} (in addition to ambient N deposition rates). Mean ozone exposure per day was highest in first growth period, decreasing successively through the season.

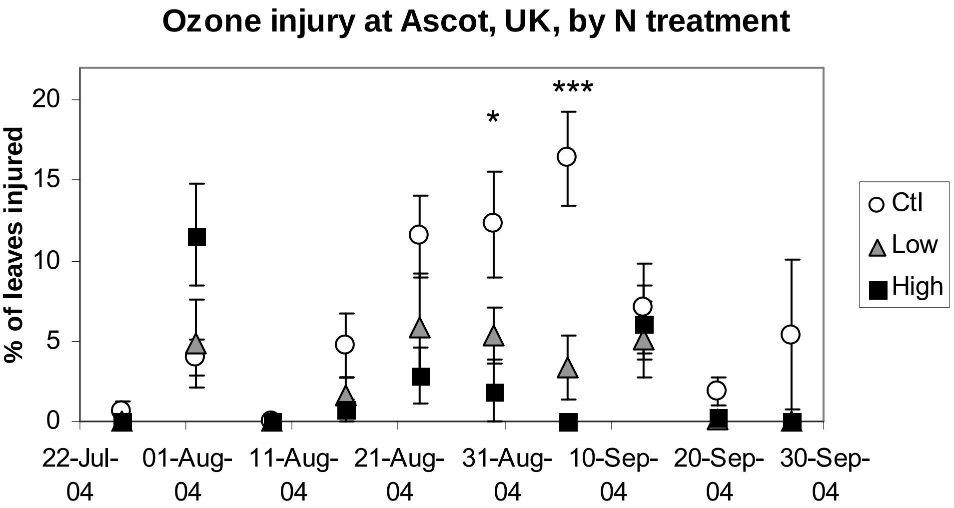


Figure 9.2 Ozone injury on rosette leaves of *Centaurea jacea*, by harvest date at Ascot, UK for 2004, split by nitrogen treatment. Nitrogen treatments were 0, 30, 80 kg N ha^{-1} yr^{-1}, respectively. Asterisks show significant N effects at time points. Ascot is a relatively low ambient ozone site (cumulative AOT40 in 2003 was 1178 ppb h).

With respect to co-sensitivity with nitrogen deposition, in the short term, the bulk of the literature suggests that N may alleviate some of the ozone toxicity effects up to a certain level of ozone exposure. However, in the long term, the consequences of synergistic N and ozone effects on ecosystem processes may be to make communities less stable, and more prone to stochastic environmental stresses including climatic, herbivory, increased pathogens and human-induced events. These are likely to impact most heavily on oligotrophic communities, ultimately leading to invasion of more competitive species and a loss in species of conservation interest. Identification and mapping of the community types predicted to be most at risk from combined ozone and N deposition is described in the next section.

9.7 Mapping communities sensitive to ozone and nitrogen deposition

In order to assess communities which are at high risk of impacts of both ozone and nitrogen deposition, the community ozone sensitivity index described in Section 4.3.5 was combined with the empirical critical loads ranges recommended in the Berne workshop (Achermann & Bobbink, 2003). Both approaches use the EUNIS categories, but there are a number of problems with overlapping the two datasets, because they do not use the same level of detail in defining relevant communities within EUNIS. The ozone sensitivity indices generally relate to Level 2 EUNIS categories, reflecting the fact that broad community types have been identified as sensitive. In contrast, the empirical critical loads of nitrogen generally relate to Level 3 EUNIS categories, i.e. they define sensitive communities to a greater level of specificity. This is because data is only available for specific Level 3 communities, and because there is often a large variation in nutrient status, and hence sensitivity to nitrogen deposition, within the Level 2 categories. It is also important to note that critical loads of nitrogen are only assigned to what are considered sensitive communities, and therefore there is a need to form a value judgement as to whether other communities have not been assigned a critical load because they are insensitive or because there is no relevant information. Table 9.1 summarises how the information on community sensitivity has been combined.

Table 9.1 Table of EUNIS communities[1] and their sensitivity to ozone and nitrogen

EUNIS code and description	**OZOVEG index**	**N critical load range (kg ha^{-1} yr^{-1})**	**Comment**
B1 Coastal dunes and sandy shores	Low	10-20	N critical load only applies to B1.3, B1.4, B1.5
E1 Dry grasslands	Moderate	10-20 (acid/neutral) 15-25 (calcareous)	Only supported by data for E1.7 and E1.26
E2 Mesic grasslands	Low	High	Most pastures will have N addition and these fertile systems would be expected to be N limited
E3 Seasonally wet grasslands	Moderate	Mainly high	Critical load only defined for specific classes of E3.5 which are oligotrpohic and don't have a wide distribution
E4 Alpine and sub-alpine grasslands	High	10-15	Applies to E4.3 and E4.4, which are alpine/subalpine
E5 Woodland fringes	Moderate	Unknown	
E7 Dehasa	Unknown	Unknown, but high?	High proportion of legumes, so likely not highly N limited
F4 Temperate shrub heathland	Moderate	10-20	Applies to F4 categories that dominate the class

[1] All communities considered for ozone sensitivity have an average of six or more ozone-reponsive species (see Section 4.3.6 for further details).

In the case of coastal habitats, the N critical load applies to the most important sub-communities botanically, and all are likely to be nutrient limited; hence the critical load is assigned to the Level 2 category. In the case of grasslands, a number of different arguments have been applied. For E1 and E4 communities, which would generally be expected to be of low nutrient status, the N critical load is applied to the Level 2 community, but it should be noted that empirical data to support the critical load is only available for a few sub-communities. For mesic grasslands and dehasa, there is no critical load of N available but there are reasons to expect that these communities will not be N limited. In contrast, woodland fringe communities are very diverse and no valued judgement can be made on a generic level of sensitivity to, and hence critical load for, N deposition. Seasonally wet grasslands only have a critical load defined for a particular oligotrophic sub-community, and in general may not be N limited. Finally, all the dominant F4 sub-communities have a low critical load and the community as a whole is known to be nutrient limited.

Communities that may be sensitive to ozone and to nitrogen deposition were defined as those that: (i) had a moderate or high ozone sensitivity index (Mills et al., in press), and (ii) had a N critical load with a range encompassing 10 kg ha yr^{-1}. On this basis, three communities can be identified as being most likely to be sensitive to both ozone and nitrogen deposition. These are:-

E1 Dry grasslands
E4 Alpine and sub-alpine grasslands
F4 Temperate shrub heathland

However, the inclusion of heathland in these three classes is based on a high proportion of species showing a positive response to ozone.

Figure 9.3 presents a map of these three EUNIS classes, developed using the methods described in Section 4.3.7. Effectively, these are the data presented in Figure 4.16, with only these three specific communities identified. In order to evaluate qualitatively whether these communities are likely to be at risk from impacts of both nitrogen deposition and ozone, two further maps are presented, both of which are based on recent modelling work undertaken by EMEP. The first of these (Figure 9.4) shows the distribution of exceedance of critical loads of nutrient nitrogen for non-forest ecosystems across Europe, while the second map (Figure 9.5) shows the distribution of modelled AOT40 at 1 m height over a fixed (May-July) 3 month period. It is important to note that both of these maps are based on pan-European modelling using generic land-cover categories and they may not provide reliable estimates of N deposition or ozone exposure to specific EUNIS classes in specific countries.

It is now possible to assess the risk of impacts to the three communities.

Dry grasslands

As noted in Section 4.3.7, the area of dry grassland (E1) identified by the mapping procedure and the soil moisture index used was relatively small. The only significant area outside the Mediterranean zone was in southern Germany, an area where both AOT40 values and exceedance of nutrient critical loads are relatively high. The main effect of N deposition on dry grasslands has been identified as an increase in tall competitive grassland species with a loss of diversity and biomass in characteristic forb species. It is interesting to note that a recent experiment on interactions between nutrient application and ozone on a calcareous grassland community in the UK suggested that ozone favoured faster growing grasses compared with slower growing grass species characteristic of lower nutrient environments, hence partly negating the conservation benefit of treatments to reduce nutrient status. Hence, in this community, both pollutants might have similar adverse effects, and the potential synergies between them need further investigation.

Alpine and sub-alpine grasslands

These communities, within the mapping domain, cover the Pyrenees and Alps primarily. Exceedance of both nutrient N critical loads and ozone critical levels are shown in these two areas. There is some evidence that each pollutant might have similar effects on community composition:- a recent ozone free-air exposure experiment in Switzerland has shown an effect of ozone in decreasing the biomass of legumes and forbs relative to that of grasses, while a general effect of increased N deposition is an increased dominance by tall grass species. However, it is important to note that the critical load of nitrogen for alpine grasslands is based on expert judgement, and there is very little empirical evidence. Hence, more detailed assessment of the nature of mechanisms by which ozone and N deposition might interact to cause changes in species composition in these communities is needed. At least one major field experiment to assess such interactions is now underway in Switzerland.

Temperate shrub heathland

This EUNIS class covers substantial areas of the mapped region, and the risk of combined impacts is variable within this. For the large areas of moorland within the UK, exceedance of nutrient N critical loads and the ozone critical level of 3000 ppb.h is either zero or small. Hence the risk of combined impacts from the two pollutants is relatively small. However, for the smaller isolated areas of lowland heath in the UK, northern Germany, Denmark, and the

Benelux countries, the risk of combined impacts of the two pollutants is greater. As noted in section 4.3.7, the split of heathland between classes F4 and F5/6 in Spain and France needs further assessment, but there are currently no relevant studies of the response of these more Mediterranean communities to either N deposition or ozone. The main impact of nitrogen deposition of concern in these communities is a switch from domination by ericaeous shrubs to domination by acid grassland species. However, the initial effect of nitrogen deposition is to increase the above ground growth or shrub species, which leads to subsequent increased stress sensitivity. If an above-ground stimulation of growth by ozone were to contribute further to these secondary effects, there is a potential for synergistic effects. However, these seem less likely than for the two grassland communities.

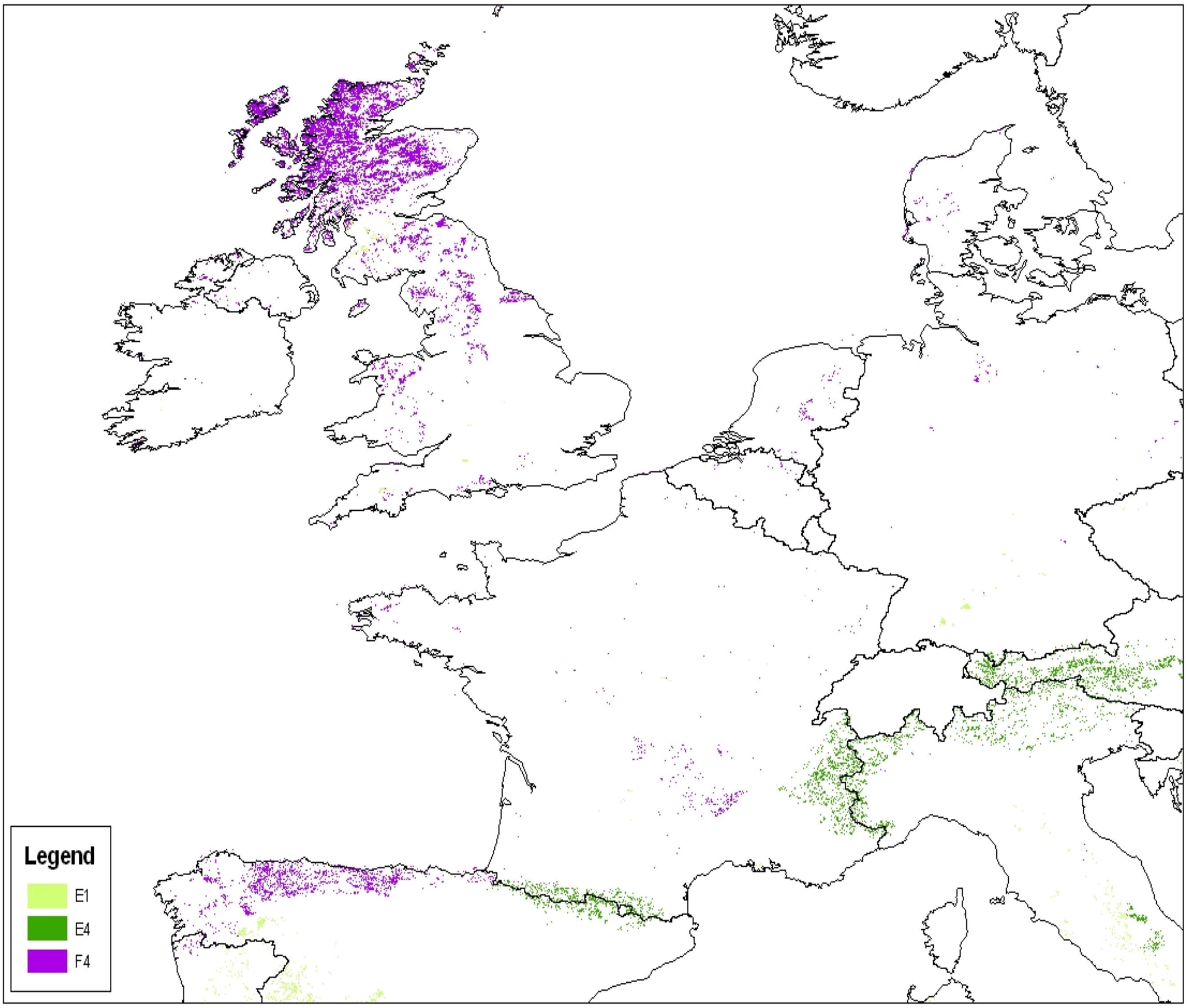


Figure 9.3 Distribution of dry grassland (E1), alpine and subalpine grassland (E4) and temperate heathland (F4) in north-west Europe.

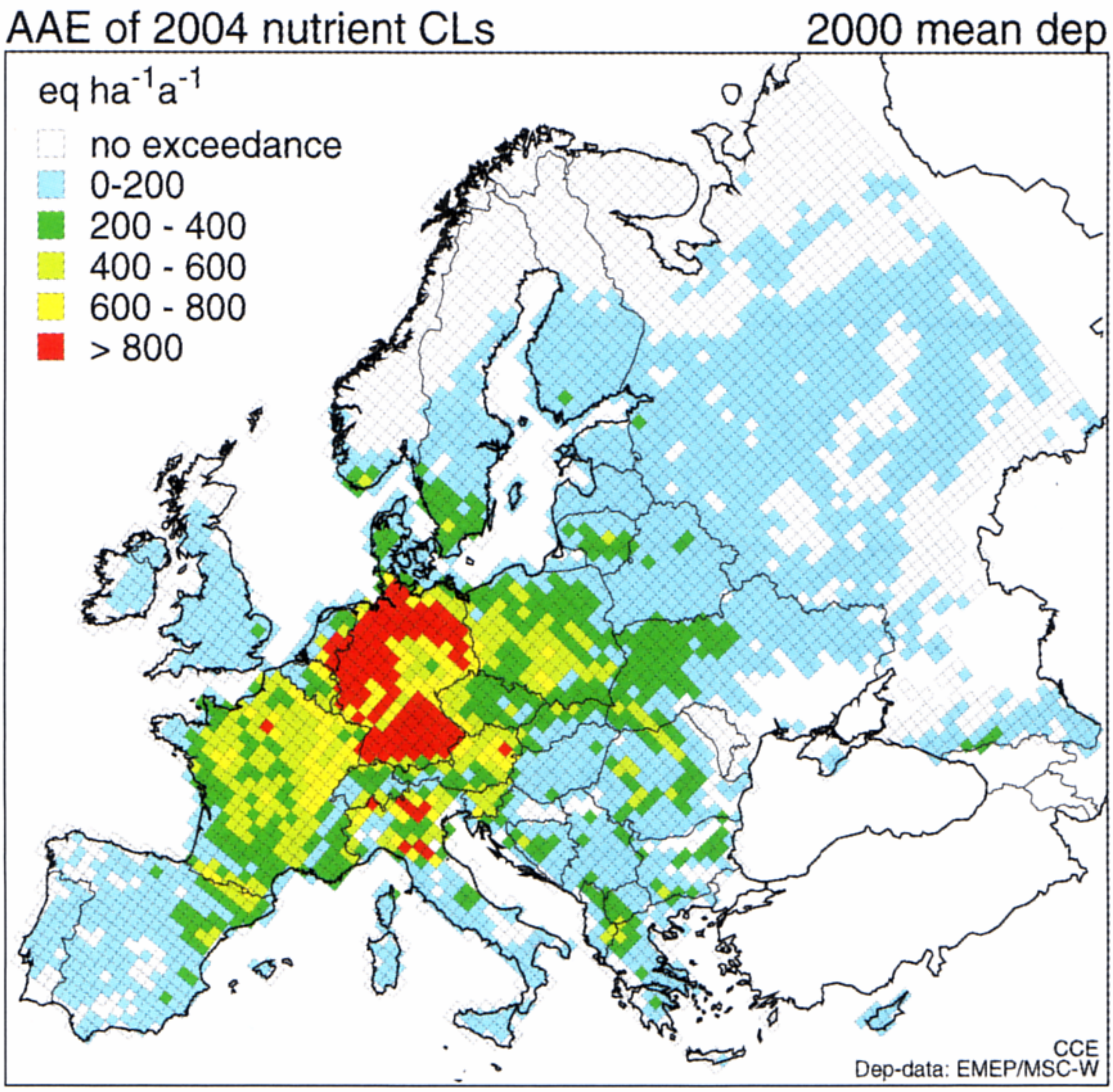


Figure 9.4 Geographical distribution of exceedance of critical loads of nutrient nitrogen, based on modelled deposition to non-forest ecosystems for 2000.

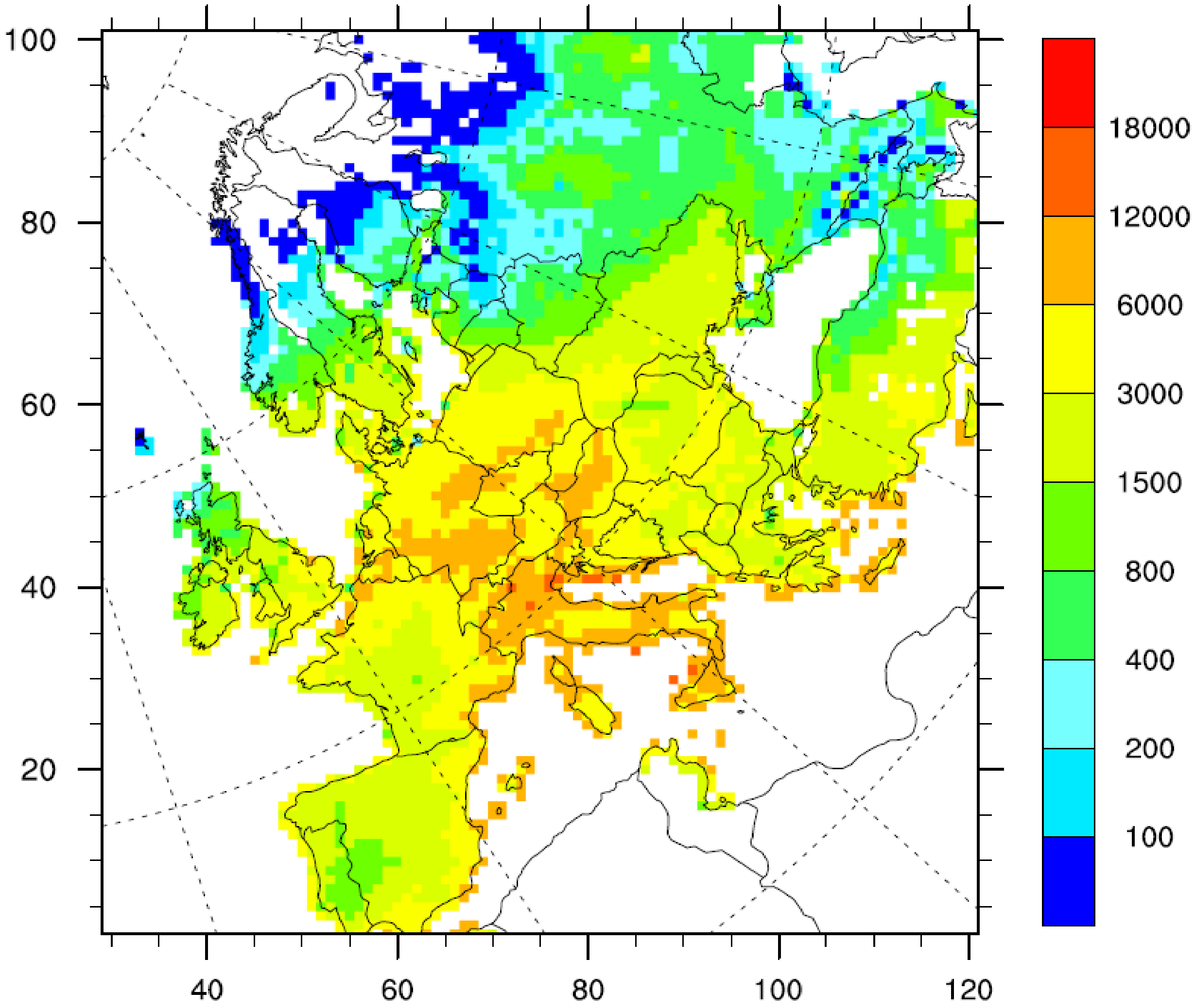


Figure 9.5 Distribution of modelled AOT40 at 1m height for the 3 month period May-July, based on data for 1997.

9.8 Conclusion

This report has shown that nitrogen may alter responses to ozone in many ways. The risk of ozone impact is defined as the combination of sensitivity to ozone and exposure to ozone. Nitrogen can affect the sensitivity of species to ozone by altering uptake *via* physiological and morphological parameters and by altering the capacity for detoxification and repair. There are numerous ways in which N may alter exposure to ozone. These include geographical exposure; life strategy; growth form; and the timing of key events through the year such as seedling emergence, the main period of vegetative growth, flowering and seed set. Changes in resource partitioning and in tissue chemistry due to both N and ozone may have indirect effects on wider components of the ecosystem such as mycorrhizal and symbiotic relationships, soil microbial activity and rates of litter decomposition and N mineralisation. Other effects may be mediated by external factors such as herbivory, disease, management and limitation of other resources such as soil phosphorus, while competitive interactions may affect individual plant exposure and community sensitivity as a whole.

Results from experiments with *Centaurea jacea* suggest that nitrogen-limited plants are unable to allocate sufficient resources to ozone detoxification and cell repair. Elevated levels of N can reduce ozone injury but can also increase injury in very young fresh shoot material.

The evidence above suggests that ozone and nitrogen can have both synergistic and antagonistic effects on species and ecosystem processes, and that they may interact in unpredictable ways to affect plant communities. Although productive, intensively managed grasslands may receive the highest ozone fluxes, high N deposition is likely to ameliorate many adverse effects of ozone. Consequently, it is oligotrophic communities which are predicted to be at greatest risk from the combination of high ozone and high N deposition.

Three EUNIS communities have been identified which are potentially at risk of exposure to both elevated nitrogen and ozone. These are: E1 - Dry grasslands; E4 - Alpine and sub-alpine grasslands; and F4 - Temperate shrub heathland. Geographical co-occurrence of both pollutants is greatest in southern Germany and parts of northern Italy. Co-exposure is most likely to affect E1 and E4 grasslands.

9.9 Policy implications

- In productive species/environments, a high N supply usually ameliorates moderate adverse ozone effects, and it is nitrogen-limitation which exacerbates ozone damage.
- In species and communities adapted to low levels of N, the combined effect of high N and ozone often act together to reduce resilience to external factors such as insect damage and drought, and to alter ecosystem processes such as litter decomposition and soil N processing. Therefore, these communities are considered most at risk from a combination of high ozone and high N deposition. Unfortunately, some semi-natural vegetation types, such as montane communities, occur in geographical areas where exposure to both of these pollutants is high.
- Communities identified as potentially sensitive to both ozone and nitrogen are: E1 - Dry grasslands; E4 - Alpine and sub-alpine grasslands; and F4 - Temperate shrub heathland.
- Co-exposure to nitrogen and ozone is predicted to be highest for E1 and E4 grasslands in Southern Germany and northern Italy.
- More research is needed to address the short term and long term interaction effects of nitrogen and ozone, both through process-oriented studies of short duration, and longer term manipulation experiments in the field at the community scale.

10. VARIATION TO THE CONTRACT: REVIEW OF THE INFLUENCE OF CLIMATE CHANGE ON THE IMPACTS OF OZONE ON VEGETATION

Note: A report of the work conducted under this variation was submitted to Defra in September 2005. We provide here the Executive Summary and refer to the reader to the full report for further details (Harmens and Mills, 2005).

10.1 Background and aims

Many studies have been conducted on the impacts of ozone (O_3) pollution on vegetation, ranging from effects at the cellular level to predicting impacts on a regional and international scale. However, it is becoming increasingly important when predicting future impacts of O_3 to consider O_3 effects within the context of global climate change. This review focuses on the interactive impacts of enhanced O_3 exposure and other climate change factors, in particular warming and atmospheric CO_2-enrichment, on terrestrial ecosystems. Whereas the emphasis is on the results from large scale ecosystems studies, comparisons with controlled environment chamber studies are included as often these studies are the only available source of data. This review primarily considers the impacts on crops and trees and focuses on above-ground vegetation responses. The review provides a cross-policy area document and the aims are:

- To assess the published literature on the influence of climate change on the response of vegetation to O_3;
- To summarise implications for policy makers.

The report contains a brief review of background information followed by consideration of O_3 flux and detoxification in a changing climate, impacts of climate change on the whole plant response to O_3 and consequences for pests and diseases, and policy implications.

Current European levels of tropospheric O_3 have been shown to cause damage to crops, trees and (semi-)natural vegetation. Effects-based research has resulted in the establishment of critical levels of O_3 for vegetation. Historically, critical levels of O_3 for vegetation were based on the concentration of O_3 in the atmosphere, but it has long been recognised that plant responses to O_3 are more closely related to the internal O_3 dose in the leaf modelled as the instantaneous flux of O_3 through the stomata, than the ambient O_3 concentration. Recently, stomatal flux-based critical levels for O_3 were defined for selected crop species and provisionally for trees. These take into account the varying influences of temperature, water vapour pressure deficit (VPD), light, soil water potential (SWP), atmospheric O_3 concentration and plant development (phenology) on O_3 uptake. Climate change factors such as elevated CO_2, temperature and precipitation will affect the flux of O_3 into leaves via direct or indirect impacts on stomatal conductance (g_s), VPD, SWP and phenology.

10.2 Impacts of global change on the flux of ozone

The overall impact of warming on the canopy flux of O_3 is difficult to predict and will depend on the location of the vegetation, severity and timing (e.g. summer or winter) of warming, its impacts on SWP and phenology of the vegetation. Each plant species has its own optimum temperature for g_s and the impact of warming on g_s will depend on which part of the temperature response function corresponds with the current ambient temperature. For example, in temperate, moist climates an increase in temperature is likely to result in an increase of the stomatal uptake of O_3, whereas in plants currently operating at their optimum temperature for g_s, warming is likely to result in a decrease of the stomatal uptake of O_3. The impacts of global warming on the canopy uptake of O_3 will also be affected by indirect effects, e.g. warming will result in an increase in VPD and decrease in SWP, which will generally result in a decrease in the stomatal flux of O_3 into leaves and warming will enhance plant development, which will reduce the stomatal flux of O_3 into leaves at a later stage of development. Little empirical data is available on the interactive impacts of O_3 and warming on vegetation, in particular at the field scale.

Many studies have shown that atmospheric CO_2-enrichment reduces g_s. Therefore, when plants were exposed to O_3 in the presence of elevated CO_2, the uptake of O_3 was often reduced. In general, elevated CO_2 ameliorates O_3-induced stress and the combined effects of O_3 and CO_2-enrichment on plant growth and physiology has often been near neutral as elevated CO_2 and O_3 affect vegetation in opposite ways. Effects of changes in precipitation patterns are likely to be mediated directly through (a) effects of VPD on g_s with increasing VPD causing a decrease in flux and (b) changes in SMD, with increasing SMD resulting in decreased stomatal flux and vice versa. The little data that is available on interactive impacts of elevated CO_2 and warming on vegetation indicate that the combined impacts of elevated CO_2 and temperature on vegetation might be complex.

10.3 Impacts of climate change on ozone detoxification within plants

In addition to the flux of O_3 into the stomata, subsequent detoxification of O_3 inside the leaves is a key determinant of the ultimate response of vegetation to O_3. Both the absolute level of antioxidants and the capacity to enhance antioxidant levels in response to O_3 might contribute to protect photosynthetic machinery and membrane functions from oxidative stress. Although CO_2-enrichment might primarily protect plants against O_3-induced damage by reducing the stomatal flux of O_3 (see above), additional detoxification of O_3 within the leaf cannot be excluded. However, a general ameliorating effect of CO_2-enrichment on O_3-induced oxidative stress via changes in the antioxidant status of leaves has not been proven as experimental data are inconclusive. Impacts of temperature on the antioxidant status of leaves have mainly been reported in relation to chilling or heat stress rather than the effects of a few degrees rise in temperature and thus cannot be used within a global warming context.

10.4 Ecosystem responses to combined ozone and climate change

Within ecosystems, vegetation responses to climate change are driven by complex interactions between abiotic and biotic factors such as atmospheric CO_2, temperature, nutrient and water availability, atmospheric pollutants, soil characteristics, land-use/management and species composition/diversity, and therefore are difficult to predict. Responses of plants to CO_2 enrichment, O_3 enrichment or warming are highly species-specific and the behaviour of species mixtures to these environmental changes can often not be predicted from responses by isolated plants. For example, in heterogeneous plant communities where the O_3 concentration decreases within the canopy with depth, species in the bottom layer of the canopy are exposed to lower O_3 concentrations and fluxes than species at the top of the canopy. Therefore, plant species identified as O_3-sensitive when grown as individual plants might not be so much affected by O_3 in a plant community if they grow in the bottom layer of the canopy. More knowledge is needed on the relationship between the responses of individual plant species and (semi-)natural plant communities to O_3 and climate change, because species competition is likely to influence the effects of O_3 and climate change on individual plant species. In addition, interactions between different species at various trophic levels are expected to change in a future climate. Because insect and plant species show individual responses to drivers of climate change, it is expected that climate change will affect the temporal and spatial association between species interacting at different trophic levels.

Due to high costs involved, data have emerged only in the last decade on the impacts of climate change on plant communities and ecosystems at the field scale under more natural conditions, i.e. without being confounded by artificial greenhouse-like conditions inside exposure chambers. As with exposure chambers, the majority of field studies have reported on the impacts of single drivers of climate change (such as elevated CO_2 or O_3 concentrations or warming) on plant communities and ecosystems, crops and trees in particular. Although Free Air Carbon dioxide/Concentration Enrichment (FACE) studies generally substantiate predictions based on chamber studies, some inconsistencies between the results of chamber and FACE studies have been reported. For example, grain crop yields increased far less than anticipated from prior enclosure studies and the responses of trees to elevated CO_2 might have been underestimated in chamber studies compared with FACE studies. Reported differences between the findings within FACE and prior chamber experiments show the need to aim for a wider use of FACE in both elevated CO_2 and elevated O_3 exposure studies and in particular with respect to (semi-)natural vegetation. However, large scale chamber studies are still required to further develop a mechanistic understanding of plant community responses to elevated O_3 and climate change, in particular for (semi-)natural vegetation.

In addition, vegetation responses to changes in single drivers of climate change cannot simply be scaled up to responses to changes in multiple drivers. There is a clear need for a combined approach of multifactorial experiments and modelling to improve predictions on the impacts of climate change on ecosystems in the long term. More field-scale experiments with (semi-)natural vegetation are needed to assess their long-term vulnerability to O_3 pollution and climate change. Elevated O_3 at relatively low concentrations can significantly reduce the growth enhancement by elevated CO_2 and therefore reduce C sequestration. This may mean that worldwide growth stimulations will not be as great as predicted from previous experimental and modelling studies with elevated CO_2. It is important to bring an understanding of O_3 as a moderator of climate change responses in global models of terrestrial net primary productivity and C sequestration.

10.5 Research recommendations

- The influence of climate change should be taken into account when predicting the future effects of O_3 on vegetation.

- In the first instance, the O_3-flux modelling procedures outlined in the Mapping Manual (LRTAP Convention, 2004) will need revising to include CO_2 concentration as a modifying factor.

- In the longer-term, it may be necessary to develop alternative modelling procedures since the current method is based on the stomatal responses to climatic and plant factors considered in isolation rather than in combination.
- There is a clear need for multi-factorial experiments to provide information for O_3-effect modelling. Because of the high cost involved with FACE systems, these may

only be possible using enclosed or semi-enclosed chamber systems especially where warming and CO_2 are considered as factors.

- As very little is known about the vulnerability of communities of (semi-)natural vegetation to O_3 and climate change, there is an urgent need for experiments that enhance our understanding of the impacts on vegetation responses and species competition. With little information available, much progress could be made in the short-term by conducting experiments with closed or semi-enclosed exposure systems. Despite the higher costs and increased complexity, the longer-term aim should be to conduct FACE experiments to assess the vulnerability of (semi-)natural vegetation to O_3 and climate change.

- There is a need for a wider and longer-term use of FACE for crops and forest trees to expand the range of species for which data exists and to determine the cumulative effects over several years.

- So far, FACE experiments have focussed on temperate ecosystems, while tropical, boreal and arctic systems have been largely ignored. FACE experiments with tropical forests, representing 50% of C in terrestrial biomass, are an obvious international need.

11. CONCLUSIONS AND FURTHER WORK

11.1 Conclusions and main findings

The Coordination Centre at CEH Bangor successfully managed the work programme of the ICP Vegetation with input from an increasing number of countries (currently 35) and participants. Numerous reports, technical documents and scientific papers were presented to Defra, the WGE of the LRTAP Convention and the scientific community, containing valuable information for the review of the LRTAP Convention Protocols.

The main conclusions/findings from work conducted under this contract are:

- For the first time stomatal flux-based critical levels of ozone were included for crops and forest trees in the Mapping Manual of the LRTAP Convention. At a recent ozone critical levels workshop it was decided that a simplified flux-modelling approach should be used for risk assessment in integrated assessment modelling for crops and forest trees within the EMEP domain.

- The concentration-based critical level of ozone for (semi-) natural vegetation and agricultural crops was exceeded at approximately 80% of the ICP Vegetation biomonitoring sites each year. Ozone-induced visible leaf injury on white clover was recorded widely across Europe, but at a higher intensity in 2003, a higher ozone year, than in 2004 and 2005. For white clover biomass reductions in the ozone-sensitive biotype relative to the resistant one were observed at sites where the three month AOT40 was in excess of c. 6 ppm.h.

- The ozone dose-response function for white clover biomass using modelled accumulated canopy flux as the dose parameter had a lower fit than that based on the AOT40. This may reflect uncertainty in estimating the development of leaf area index. For brown knapweed, two separate model parameterisations for Central Europe and Mediterranean Europe were needed to estimate the ozone flux to upper stem leaves.

- Although ozone new stomatal flux models were developed for grapevine, sunflower, tomato and maize, flux-response relationships could not be developed yet due to a lack of appropriate datasets describing the responses of these crop species to ozone.

- The ICP Vegetation database (OZOVEG) developed under this contract contains ozone dose-response functions for over 80 species of (semi-)natural vegetation. Available data indicate that species of the *Fabaceae* family and the therophyte life-form are most sensitive to ozone. Comparison of the relative sensitivity to ozone with Ellenberg Indicator values indicated that light-loving plants, plants from dry soils and plants which can tolerate moderately saline conditions are most sensitive to ozone. A model was developed that uses Ellenberg light and salinity Indicator values for a species to predict its sensitivity to ozone. There were no relationships between Ellenberg nitrogen, temperature or ‘reaction’ (pH) Indicator values and the relative ozone sensitivity of species. There was also no relationship between the ozone sensitivity of species and Grime’s plant functional types (CSR-strategy).

- Based on the responses of individual species, the following EUNIS (European Nature Information System) habitats were identified as potentially sensitive to ozone: Dry grasslands (E1), Mesic grasslands (E2), Seasonally-wet grasslands (E3), Alpine and sub-alpine grasslands (E4), Woodland fringes (E5) and Temperate shrub heathland (F4). The first pan-European map was produced showing the potential location of these communities using the harmonised SEI land-cover and European Environment Agency Corine land-cover datasets.

- Based on a literature review, nutrient-poor communities of (semi-)natural grassland communities were predicted to be at greatest risk from the combination of high ozone and high nitrogen deposition.

- Temporal trends (1977 – 2000) in the heavy metal concentration in mosses in five selected European countries were both metal- and country-specific. Lead was the only metal that had decreased significantly between 1990 and 2000 in all five countries, for the other metals no consistent significant changes with time were observed. The magnitude of decline in EMEP modelled lead and cadmium deposition (1990 – 2000) was generally in agreement with the decline in lead and cadmium concentration in mosses.

- A pilot study showed that mosses collected for the heavy metals survey can be used as biomonitors of atmospheric nitrogen deposition. For the first time, 18 countries participating in the European moss survey are determining the total nitrogen concentration in mosses.

- Long-term temporal trends (1829 – 2004) of the nitrogen concentration in mosses in the selected European countries showed no changes up to 1960. After 1960, there was a significant increase in the nitrogen concentration in mosses in Switzerland, but not in any of the other countries.

- Vegetation responses to changes in single drivers of climate change (including ground-level ozone concentrations) cannot simply be scaled up to responses to changes in multiple drivers. The influence of other climate change factors should be taken into account when predicting the future effects of ozone on vegetation. Results of a case study for winter wheat indicate that in a future climate the exceedance of the flux-based critical level of O_3 might be reduced across Europe, whilst exceedance of the AOT40-based critical level of O_3 will increase with the projected increase in tropospheric background O_3 concentration.

- An assessment of the economic impact of ozone on 23 arable crops in 47 countries in Europe has quantified ozone-induced yield losses at 6.7 (range: 4.4 – 9.3) billion Euros per year for year 2000 emissions. This represents losses equal to 2% of the arable agricultural production in Europe. These estimates do not account for damage via visible injury, changes in crop quality, interactions with pests or indirect effects on animal production.

11.2 Further work

The following have been identified as research aims for study in the next three years:

- An evidence-based assessment of the impacts of current ambient ozone on vegetation in Europe.

- To quantify the risk of ozone effects on (semi-)natural vegetation in Europe by (i) further developing the Ellenberg method and applying it across Europe, and (ii) including the modifying influence of nitrogen.

- To develop a flux-based assessment of the risk of ozone damage to (semi-) natural vegetation in Europe.

- To analyse the temporal trends of heavy metals in European mosses across Europe and compare the trends with deposition trends based on the EMEP model.

- To determine the nitrogen concentration in mosses across Europe, analyse spatial trends and compare these trends with deposition trends based on the EMEP model.

- To assess the evidence for impacts of nitrogen on vegetation in areas of Europe with high nitrogen deposition and identify areas where nitrogen critical loads are exceeded for specific EUNIS communities.

- To predict ozone effects on crops in a changing climate by modifying and applying the stomatal flux model (taken climate change factors such as atmospheric CO_2 enrichment into account) and predict crop yield losses in a changing climate.

- To develop methods to integrate our knowledge of the mechanisms of flux and flux-effect models (which exist only for a limited number of crops) to understand how best to modify concentration-effect models (which exists for a wide range of crops) and apply this to improve the economic impact assessment of ozone on crops in Europe.

12. REFERENCES

Achermann, B., Bobbink, R., 2003. Empirical Critical Loads for Nitrogen. Proceedings of Expert Workshop held in Berne, Switzerland 11 - 13 November 2002. Environmental Documentation No. 164. Air., Swiss Agency for the Environment, Forests and Landscape SAEFL, Berne.

Aerts, R., Bobbink, R., 1999. The impact of atmospheric nitrogen deposition on vegetation processes in terrestrial non-forest ecosystems. In: Langan, S.J. (Ed.), The impact of nitrogen deposition on natural and semi-natural ecosystems. Kluwer Academic Publishers, Dordrecht, pp. 85-122.

Aerts, R., Berendse, F., Decaluwe, H., Schmitz, M., 1990. Competition in heathland along an experimental gradient of nutrient availability. *Oikos,* 57(3), 310-318.

Aerts, R., 1989. Above-ground biomass and nutrient dynamics of Calluna vulgaris and Molinia caerulea in a dry heathland. *Oikos*, 56(1), 31-38.

Aerts, R., Berendse, F., 1988. The effect of increased nutrient availability on vegetation dynamics in wet heathlands. *Vegetatio*, 76 (1-2), 63-69.

Andersen, C.P., 2003. Source-sink balance and carbon allocation below ground in plants exposed to ozone. *New Phytologist*, 157(2), 213-228.

Andersen, C. P., Grulke, N. E., 2001. Complexities in understanding ecosystem response to ozone. *Human and Ecological Risk Assessment,* 7, 1169 – 1182.

Angadi, S.V., Entz, M.H. 2002. Water relations of standard height and dwarf sunflower cultivars. *Crop Science*, 42, 152-159.

Ashmore, M., Emberson, L.D., Terry, A.C., Buker, P., Cinderby, S., Toet, S., 2005. Developing and applying flux-based critical levels for semi-natural vegetation, Workshop report of the WGE CLRTRAP. Critical levels of ozone: further applying and developing the flux-based concept. 15-19 November, Obergurgl, Tyrol, Austria.

Ashmore, M., Bell, S., Fowler, D., Hill, M., Jordan, C., Nemitz, E., Parry, S., Pugh, B., Reynolds, B., Williams, J. 2002. Survey of UK metal content of mosses 2000. Part II of EPG 1/3/144 final contract report: development of a critical load methodology for toxic metals in soils and surface waters: stage II.

Ashmore, M., Coyle, M., Fowler, D., 2002. Implications of increasing tropospheric background ozone concentrations for vegetation in the UK. DEFRA report under contract EPG 1/3/173. http://www.atmosci.ceh.ac.uk/docs/o3trends_UKveg.htm.

Ashmore, M.R., Power, S.A., Cousins, D.A., Ainsworth, N., 1996. Effects of ozone on native grass and forb species: a comparison of responses of individual plants and artificial communities. In: Kärenlampi, L. and Skärby (Eds.), Critical levels for ozone in Europe: testing and finalising the concepts, UNECE Workshop report, Kuopio, Finland, pp. 104-108

Ashmore, M.R., Ainsworth, N., 1995. The effects of ozone and cutting on the species composition of artificial grassland communities. *Functional Ecology*, 9, 708-712.

Auvray, M., Bey, I., 2005. Long-range transport to Europe: Seasonal variations and implications for the European ozone budget. *Journal of Geophysical Research-Atmospheres*, 110 (D11).

Bakker, J.C. 1991. Leaf conductance of four glasshouse vegetable crops as affected by air humidity. *Agricultural and Forest Meteorology*, 55, 23-36.

Ball, G.R., Mills, G.E., Hayes, F., Ashmore, M., Emberson, L., Power, S., Bungener, P., Nussbaum, S., Fuhrer, J., 1999. Can artificial neural networks be used to identify ozone sensitivity in natural vegetation species. In: Fuhrer, J., Achermann, B. (Eds.). Critical levels for ozone - level II. Environmental Documentation No. 115. Swiss Agency for Environment, Forest and Landscape, Bern, Switzerland, pp. 261-264.

Bardgett, R.D., Lovell, R.D., Hobbs, P.J., Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biology & Biochemistry,* 31(7), 1021-1030.

Bardgett, R.D., Hobbs, P.J., Frostegård, Å., 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of upland grassland. *Biology and Fertility of Soils*, 22, 261-264.

Barnes, J.D., Bender, J., Lyons, T., Borland, A.M., 1999. Natural and man-made selection for air pollution resistance. *Journal of Experimental Botany*, 50(338), 1423-1435.

Bassin, S., Volk, M., Buchmann, N., Fuhrer, J., in press. The impact of combined ozone and nitrogen deposition on a species-rich alpine pasture:second year results. UNECE Workshop report “Critical levels of ozone: further applying and developing the flux-based concept”, Obergurgl, Austria, 15-19 November, 2005.

Bassin, S., Volk, M., Fuhrer, J., in press. Predicting the sensitivity to ozone of temperate European grasslands: an overview. *Environmental Pollution Special Issue*.

Bassin, S., Volk, M., Buchmann, N., Fuhrer, J., 2005. The influence of combined ozone and nitrogen deposition on a species-rich alpine pasture: second year results., Workshop report of the WGE CLRTRAP. Critical levels of ozone: further applying and developing the flux-based concept. 15-19 November, Obergurgl, Tyrol, Austria.

Batty, K., Ashmore, M.R., 2003. Lower threshold ozone exposures for effects on roots than shoots in wetland species, in: Karlsson *et al*. (Eds), Establishing ozone critical levels II, 2003 UNECE Workshop Report. IVL report B1523 IVL Swedish Environmental Research Institute, Gothenburg, Sweden. pp. 199–203.

Bender, J., Muntifering, R.B., Lin, J.C., Weigel, H.J., 2006. Growth and nutritive quality of *Poa pratensis* as influenced by ozone and competition. Environmental Pollution 142(1), 109-115.

Berendse, F., Schmitz, M., Devisser, W., 1994. Experimental manipulation of succession in heathland ecosystems. Oecologia 100 (1-2), 38-44.

Berg, T., Hjellbrekke, A., Rühling, Å., Steinnes, E., Kubin, E., Larsen, M.M., Piispanen, J. (2003). Absolute deposition maps of heavy metals for the Nordic countries based on the moss survey, TemaNord 2003:505, Nordic Council of Ministers, Copenhagen, Denmark.

Berg, T., Steinnes, E. (1997). Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: from relative to absolute values. Environmental Pollution 98, 61-71.

Berg, M.P., Kniese, J.P., Zoomer, R., Verhoef, H.A., 1998. Long-term decomposition of successive organic strata in a nitrogen saturated Scots pine forest soil. Forest Ecology and Management 107, 159-172.

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

Bergmann, E., Bender, J., Weigel, H.J., 1996. Ozone and natural vegetation: Native species sensitivity to different ozone exposure regimes. In: Kärenlampi, L. and Skärby (Eds.), Critical levels for ozone in Europe: testing and finalising the concepts, UNECE Workshop report, Kuopio, Finland, pp. 205–209.

Bergmann, E., Bender, J., Weigel, H.J., 1995. Growth responses and foliar sensitivities of native herbaceous species to ozone exposures. *Water Air and Soil Pollution*, 85, 1437-1442.

Bermejo, V., Sanz, J., Gimeno, B.S., 2005. The yield and reproductive response of the Mediterranean therophyte Trifolium striatum to ozone is modulated by soil nitrogen application, Workshop report of the WGE CLRTRAP. Critical levels of ozone: further applying and developing the flux-based concept. 15-19 November, Obergurgl, Tyrol, Austria

Bermejo, V., Gimeno, B.S., Grandos, I., Santamaria, J., Irigoyen, J.J., Bermejo, R., Porcuna, J.L., Mills, G., 2002. Investigating indices to explain the impacts of ozone on the biomass of white clover (*Trifolium repens* L. cv. Regal) at inland and coastal sites in Spain. *New Phytologist*, 156, 43-55.

Bermejo, V., Gimeno, B.S., Sanz, J., de la Torre, D., Gil, J.M., 2003. Assessment of the ozone sensitivity of 22 native plant species from Mediterranean annual pastures based on visible injury. *Atmospheric Environment*, 37, 4667-4677.

Bethenod, O., and Tardieu, F. 1996. Water use efficiency in field grown maize. Effect of soil structure. In Balltscheffsky, M. (ed.), Current research in Photosynthesis, vol. 4, Kluwer Academic Publishers, Dordrecht, pp. 737-740.

Bethenod, O. and Tardieu, F. 1990. Water use efficiency in field-grown maize. Effect of soil structure. In current Research in Photosynthesis. Vol. iv (ed. M. Baltscheffsky) pp. 737-740. Kluwer, Dordrecht.

Bobbink, R., 1998. Impacts of tropospheric ozone and airborne nitrogenous pollutants on natural and seminatural ecosystems: a commentary. *New Phytologist*, 139(1), 161-168.

Bobbink, R., Hornung, M., Roelofs, J.G.M., 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. A review. *Journal of Ecology*, 86(5), 717-738.

Bosac, C., Black, V.J., Roberts, J.A., Black, C.R., 1998. Impact of ozone on seed yield and quality and seedling vigour in oilseed rape (*Brassica napus* L.). *Journal of Plant Physiology*, 153, 127-134.

Boulard, T., Baille, A., Mermier, M. and Villette, F. 1991. Mesures et modelisation de la resistance stomatique foliare et de la transpiration d'un couvert de tomates de serre. *Agronomie*, 11, 259-274.

Brady, N.C., Weil, R.R., 1999. The nature and properties of soils. Twelfth edition. Prentice Hall, New Jersey.

Bull, K.R., 1991. The critical loads/levels approach to gaseous pollutant emission control. *Environmental Pollution*, 69,105-123.

Bungener, P., Balls, G.R., Nussbaum, S., Geissmann, M., Grub, A., and Fuhrer, J., 1999. Leaf injury characteristics of grassland species exposed to ozone in relation to soil moisture condition and vapour pressure deficit. *New Phytologist*, 142, 271-282.

Bungener, P., Nussbaum, S., Grub, A., Fuhrer, J., 1999b. Growth response of grassland species to ozone in relation to soil moisture condition and plant strategy. *New Phytologist*, 142, 283-293.

Buse, A., Norris, D., Harmens, H., Büker, P., Ashenden, T., Mills, G. 2003. Heavy metals in European mosses: 2000/2001 survey. UNECE ICP Vegetation. ISBN: 1 870393 70 8.

Caporn, S.J.M., Risager, M., Lee, J.A., 1994. Effect of nitrogen supply on frost hardiness in Calluna vulgaris. New Phytologist 128, 461-468.

Carroll, J.A., Caporn, S.J.M., Johnson, D., Morecroft, M.D., Lee, J.A., 2003. The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands, receiving long-term inputs of stimulated pollutant nitrogen deposition. *Environmental Pollution*, 121, 363-376.

Cinderby, S., Emberson, L. D., Ashmore, M. R., Bűker, P., Terry, A. C., in press. Harmonisation of land cover mapping. UNECE Workshop report “Critical levels of ozone: further applying and developing the flux-based concept”, Obergurgl, Austria, 15-19 November, 2005.

Cinderby, S., 2002. Description of 2002 revised SEI land-cover map. http://www.sei.se/atmospher/revised2002Landcover.pdf

Clapham, A.R., Tutin, T.G., Warburg, E.F., 1962. Flora of the British Isles. University Press, Cambridge, UK.

Connor, D.J. and Jones, T.R. 1985. Response of sunflower to strategies of irrigation II. Morphological and physiological responses to water stress. *Field Crops Research*, 12, 91-103.

Correia, M.J., Pereira, J.S., Chaves, M.M, Rodrigues M.L. and Pacheco, C.A. 1995. ABA xylem concnetrations determine maximum daily leaf conductance of field-grown *Vitis Vinifera* L. plants. *Plant, Cell and Environment*, 18, 511-521.

Danielsson, H., Gelang, J., Pleijel, H., 1999. Ozone sensitivity, growth and flower development in *Phleum* genotypes of different geographic origin in the Nordic countries. *Environmental and Experimental Botany*, 42, 41-49.

Davies, W.J., Tardieu, F. and Trejo, C.L. 1994. How do chemical signals work in plants that grow in drying soil? *Plant Physiology*, 104, 309-314.

Davison, A.W., Barnes, R.S.K., 1998. Effects of ozone on wild plants. *New Phytologist*, 139, 135-151.

de Smet, P.A.M, Hettelingh, J P., 2001. Intercomparison of current European Land Use/land cover databases. Coordination Centre for Effects Status Report 2001.

Duniway, J.M. 1971. Water relations of *Fusarium* wilt in tomato. *Physiological Plant Pathology*, 1, 537-546.

During, H. 1987. Stomatal responses to alterations of soil and air humidity in grapevines. *Vitis*, 26, 9-18

Eatherall, A., Sloan, W., Jenkins, A., Terry, A.C. 1992. The vegetation model. In “Modelling Climate Change Impacts on Biogeochemical and Ecological Systems: Core Model Project”, pp. 29-44. Institute of Hydrology progress report for the year to November 1992. Under contract to the Department of the Environment (PECD 7/12/69).

Eatough Jones, M., Paine, T.D., Fenn, M.E., Poth, M.A., 2004. Influence of ozone and nitrogen deposition on bark beetle activity under drought conditions. *Forest Ecology and Management,* 200(1-3), 67-76.

Efron, B., Gong, G., 1983. A leisurely look at the bootstrap, the jackknife, and cross-validation. *American Statistician*, 37, 36-48.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulissen, D., 1991. *Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica*, 18, 1-248.

Emberson, L.D. Simpson, D., Tuovinen, J.-P., Ashmore, M.R., and Cambridge, H.M. 2001. Modelling and Mapping ozone deposition in Europe. *Water, Air and Soil Pollution*, 130, 577-582.

EMEP 2005. Modelling of heavy metal airborne pollution in Europe: evaluation of the model performance. Technical report 8/2005, MSC – East (eds. Ilyin, I, Travnikov, O.). Available on http://www.msceast.org

EMEP 2004. EMEP assessment part 1 European perspective (eds. Lövblad, G., Tarrasón, L., Tørseth, K., Dutchak, S.). ISBN 82 7144 032 2. Available on http://www.msceast.org

Falkengren-Grerup, U., Schottelndreier, M., 2004. Vascular plants as indicators of nitrogen enrichment in soils. *Plant Ecology*, 172(1), 51-62.

FAO, AGL 2002. Crop Water Management Group.
http://www.fao.org/ag/agl/aglw/cropwater/maize.stm

Fambrini, M., Pugliesi, C., Vernieri, P., Pardossi, A. and Baroncelli, S. 1994. Characterization of a wilty sunflower (*Helianthus annuus* L.) mutant. I. Water relations, stomatal conductance, abscisic acid content in leaves and xylem sap of plants subjected to water deficiency. *Journal of Experimental Botany,* 45, 1809-1815.

Fay, P.A. and Knapp, A.K. 1996. Photosynthetic and stomatal responses to variable light in a cool-season and a warm-season prairie forb. *International Journal of Plant Science*, 157(3), 303-308.

Findlay, S., Jones, C.G., 1990. Exposure of cottonwood plants to ozone alters subsequent decomposition. *Oecologia*, 82, 248-250.

Flexas, J., Escalona, J.M. and Medrano, H. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. *Plant, Cell and Environment*, 22, 39 - 48.

Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. *Biological Reviews*, 63, 433-462.

Foot, J.P., Caporn, S.J.M., Lee, J.A., Ashenden, T.W., 1996. The effect of long-term ozone fumigation on the growth, physiology and frost sensitivity of *Calluna vulgaris*. *New Phytologist,* 133, 503-511.

Fowler, D., Leith, I.D., Binnie, J., Crossley, A., Inglis, D.W.F., Choularton, T.W., Gay, M., Longhurst, J.W.S., Conland, D.E., 1995. Orographic enhancement of wet deposition in the United Kingdom: Continuous monitoring. *Water Air and Soil Pollution*, 85(4), 2107-2112.

Franzaring, J., Tonneijck, A.E.G., Kooijman, A.W.N., Dueck, T.A., 2000. Growth responses to ozone in plant species from wetlands. *Environmental and Experimental Botany*, 44, 39-48.

Franzaring, J., Dueck, T.A., Tonneijck, A.E.G., 1999. Can plant traits be used to explain differences in ozone sensitivity between native European plant species? In: Fuhrer, J., Achermann, B. (Eds.), Critical Levels for Ozone - Level II. Proceedings of Expert Workshop held in Gerzensee, Switzerland 11 - 15 April 1999. Environmental Documentation No. 115. Air. Swiss Agency for the Environment, Forests and Landscape SAEFL, Berne, pp. 83-87.

Frissel, M.J. (Ed.), 1978. Cycling of mineral nutrients in agricultural ecosystems. Developments in agricultural and managed-forest ecology, 3. Elsevier, Amsterdam, 356 pp.

Fuhrer, J., Bassin, S., Volk, M., Mills, G., Jones, M.L.M., Hayes, F., Ashmore, M., 2005. Impacts of ozone on communities of (semi-)natural vegetation, Workshop report of the WGE CLRTRAP. Critical levels of ozone: further applying and developing the flux-based concept. 15-19 November 2005, Obergurgl, Tyrol, Austria.

Fuhrer, J., Ashmore, M.R., Mills, G., Hayes, F., Davison, A.W., 2003. Ozone critical levels for semi-natural vegetation. *In*: Establishing Ozone Critical Levels II (P.E. Karlsson, G.Selldén and H.Pleijel, Eds). UNECE Workshop Report, IVL Report B 1523, pp. 183-198. IVL Swedish Environmental Research Institute, Gothenburg, Sweden, 2003.

Gatz, D.F., 2003. Feasibility of forecasting surface ozone concentrations in the Chicago area. Report prepared for the Illinois Department of Natural Resources and Department of Commerce and Community Affairs, Illinois State Water Survey, Illinois.

Gavriilidou, M., Grünhage, L. and Jäger, H.-J. 2002. Derivation of critical absorbed doses for ozone using ozone sensitive clover clones. background document, UNECE Workshop “Establishing Ozone Critical Levels II”, Göteborg, Sweden, 19-22 Nov. 2002.

Gelang, J., Sellden, G., Younis, S., Pleijel, H., 2001. Effects of ozone on biomass, non-structural carbohydrates and nitrogen in spring wheat with artificially manipulated source/sink ratio. *Environmental and Experimental Botany*, 46 (2), 155-169.

Gimeno, B.S., Bermejo, V., Sanz, D., de la Torre, D., Elvira, S., 2004a. Growth response to ozone of annual species from Mediterranean pastures. *Environmental Pollution*, 132, 297-306.

Gimeno, B.S., Bermejo, V., Sanz, J., De la Torre, D., Gil, J.M., 2004b. Assessment of the effects of ozone exposure and plant competition on the reproductive ability of three therophytic clover species from Iberian pastures. *Atmospheric Environment*, 38, 2295-2305.

Gimeno, B.S., Porcuna, J.L., Jimenez, A., Bermejo, V., Jorda, C., 1999. Ozone exposure modifies the sensitivity of tomato plants to virus infestations. In: Fuhrer, J., Achermann, B. (Eds.), Critical Levels for Ozone - Level II. Proceedings of Expert Workshop held in Gerzensee, Switzerland 11 - 15 April 1999. Environmental Documentation No. 115. Air. Swiss Agency for the Environment, Forests and Landscape SAEFL, Berne, pp. 115-119.

Gollan, T., Passioura, J.B. and Munns, R. 1986. Soil water status effects the stomatal conductance of fully turgid wheat and sunflower leaves. *Australian Journal of Plant Physiology,* 13, 459-464.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., Bardgett, R.D., 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biology & Biochemistry*, 33(4-5), 533-551.

Grime, J.P., Hodgson, J.G., Hunt, R., 1988. Comparative Plant Ecology: A Functional Approach to Common British Species. Chapman and Hall, UK
Grime, J.P., 1973. Competitive exclusion in herbaceous vegetation. *Nature*, 242, 344-347.

Grulke, N.E., Dobrowolski, W., Mingus, P., Fenn, M.E., 2005. California black oak response to nitrogen amendment at a high O-3, nitrogen-saturated site. *Environmental Pollution*, 137 (3), 536-545.

Grulke, N.E., Balduman, L., 1999. Deciduous Conifers: High N Deposition and O3 Exposure Effects on Growth and Biomass Allocation in Ponderosa Pine. *Water, Air, & Soil Pollution,* 116(1 - 2), 235-248.

Guilioni, L., Cellier, P., Ruget, F., Nicoullaud, B. and Bonhomme, R. 2000. A model to estimate the temperature of a maize apex from meteorological data. *Agricultural and Forest Meteorology*, 100, 213-230

Hargreaves, P.R., Smith, J.U., Young, S., Goulding, K.W.T., 2005. Development of an empirical model to predict nitrogen dioxide concentrations from weather variables for sites across the UK. *Atmospheric Environment*, 39, 409-417.

Harmens, H. and the participants of the European moss survey 2005a. Heavy metals in European mosses: 2005/2006 survey. Monitoring manual. ICP Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK. Available on http://icpvegetation.ceh.ac.uk

Harmens, H., Mills, G., Hayes, F., Williams, P., De Temmerman, L. and the participants of ICP Vegetation 2005b. Air pollution and vegetation. ICP Vegetation Annual Report 2004/2005. ISBN: 1 870393 80 5.

Harmens, H., Buse, A., Büker, P., Norris, D., Mills, G., Williams, B., Reynolds, B., Ashenden,, T.W., Rühling, Å., Steinnes. E. 2004. Heavy metal concentration in European mosses: 2000/2001 survey. *Journal of Atmospheric Chemistry*, 49, 425-436.

Haupt-Herting, S. and Fock, H.P. 2000. Exchange of oxygen and its role in energy dissipation during drought stress in tomato plants. *Physiol. Plantarum*, 110, 489-495.

Hayes, F., Mills, G., Williams, P., Harmens, H., Büker, P. Impacts of summer ozone exposure on the growth and over-wintering of UK upland vegetation. Submitted (a) to *Atmospheric Environment*.

Hayes, F., Jones, M.L.M., Ashmore, M., Mills, G., in press 'Meta-Analysis of the relative sensitivity of semi-natural vegetation to ozone'. Submitted (b) to *Environmental Pollution*.

Heil, G.W., 1983. Change of Dutch heathland into grassland as a result of a heather beetle, Lochmaea suturalis (Thomson), infestation. *Acta Botanica Neerlandica*, 32, (3), 251-251.

Heil, G.W., Diemont, W.M., 1983. Raised nutrient levels change heathland into grassland. *Vegetatio*, 53, 113-120.

Herms, D.A., Mattson, W.J., 1991. The dilemma of plants: to grow or defend. *The Quarterly Review of Biology*, 67, 283-335.

Hicks, W.K., Leith, I.D., Woodin, S.J., Fowler, D., 2000. Can the foliar nitrogen concentration of upland vegetation be used for predicting atmospheric nitrogen deposition? Evidence from field surveys. *Environmental Pollution*, 107(3), 367-376.

Hill, M.O., Roy, D.B., Mountford, J.O., Bunce, R.G.H., 2000. Extending Ellenberg's indicator values to a new area: an algorithmic approach. *Journal of Applied Ecology*, 37 (1), 3-15.

Hill, M.O., Mountford, J.O., Roy, D.B., Bunce, R.G.H., 1999. Ellenberg's indicator values for British plants. ECOFACT: Vol. 2 Technical annex. Institute of Terrestrial Ecology, Huntingdon, UK.

Hirasawa, T., Wakabayashi, K., Touya, S. and Ishihara, K. 1995. Stomatal responses to water deficits and abscisic acid in leaves of sunflower plants (Helianthus annuus L.) grown under different conditions. *Plant Cell Physiology*, 36(6), 955-964.

Holland, M., Kinghorn, S., Emberson, L., Cinderby, S., Ashmore, M., Mills, G., Harmens, H., 2006.. Development of a framework for probabilistic assessment of the economic losses caused by ozone damage to crops in Europe. Defra Contract EPG 1/3/2005. Contract Report. January

Holland, M., Mills, G., Hayes, F., Buse, A., Emberson, L., Cambridge, H., Cinderby, S., Terry, A., and Ashmore, M., 2002. Economic Assessment of Crop Yield Losses from Ozone Exposure". pp. 1-76, 2002. Defra Air Quality Archive Reports.
http://www.airquality.co.uk/archive/reports/cat10/final_ozone_econ_report_ver2.pdf

Holton, M.K., Lindroth, R.L., Nordheim, E.V., 2003. Foliar quality influences tree-herbivore-parasitoid interactions: effects of elevated CO2, O3, and plant genotype. *Oecologia*, 137(2), 233-244.

Hurst, A.C., Grams, T.E.E., Ratajczak, R., 2004. Effects of salinity, high irradiance, ozone, and ethylene on mode of photosynthesis, oxidative stress and oxidative damage in the C-3/CAM intermediate plant *Mesembryanthemum crystallinum* L.. *Plant Cell and Environment,* 27, 187-197.

Jacobs, C.M.J., van den Hurk, B.J.J.M. and de Bruin, H.A.R. 1996. Stomatal behaviour and photosynthetic rate of unstressed grapevines in semi-arid conditions. *Agricultural and Forest Meteorology*, 80, 111-134.

Jondrup, P.M., Barnes, J.D., Port, G.R., 2002. The effect of ozone fumigation and different Brassica rapa lines on the feeding behaviour of Pieris brassicae larvae. *Entomologia Experimentalis et Applicata,* 104(1), 143-151.

Jones, G.V., Duchene, E., Tomasi, D., Yuste, J., Braslavska, O., Schultz, H., Martinez, C., Boso, S., Langellier, F., Perruchot, C. and Guimberteau, G. In press. Changes in European winegrape phenology and relationships with climate. GESCO.

Jones, M.L.M., Hayes, F., Mills, G., Sparks, T.H., Fuhrer, J., in press. Predicting community sensitivity to ozone using Ellenberg Indicator values. *Environmental Pollution*.

Jones, M.L.M., 2005. Nitrogen deposition in upland grasslands: Critical loads, management and recovery. PhD Thesis, University of Sheffield.

Jones, M.L.M., Wallace, H.L., Norris, D., Brittain, S.A., Haria, S., Jones, R.E., Rhind, P.M., Reynolds, B.R., Emmett, B.A., 2004. Changes in vegetation and soil characteristics in coastal sand dunes along a gradient of atmospheric nitrogen deposition. *Plant Biology*, 6(5), 598-605.

Jones, M.L.M., Oxley, E.R.B., Ashenden, T.W., 2002. The influence of nitrogen deposition, competition and desiccation on growth and regeneration of Racomitrium lanuginosum (Hedw.) Brid. *Environmental Pollution*, 120(2), 371-378.

Jones, M.L.M., Ashenden, T.W., 2001. Critical loads of nitrogen for acidic and calcareous grasslands in relation to management by grazing. Contract report to DEFRA., Centre for Ecology and Hydrology, Bangor.

Jones, H.G. 1992. Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology. 2nd edition. Cambridge University Press, Cambridge.

Katerji, N., van Hoorn, J.W., Hamdy, A. and Mastrorilli, M. 1998. Response of tomatoes, a crop of indeterminate growth, to soil salinity. *Agricultural Water Management*, 38, 59-68.

Karlsson *et al.*, 2003. Proceedings Hindas workshop.

Karnosky, D.F., Gagnon, Z.E., Dickson, R.E., Coleman, M.D., Lee, E.H., Isebrands, J.G., 1996. Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of Populus tremuloides clones and seedlings. *Canadian Journal of Forest Research*, 26, 23-37.

Kim, J.S., Chappelka, A.H., Miller-Goodman, M.S., 1998. Decomposition of blackberry and broomsedge bluestem as influenced by ozone. *Journal of Environmental Quality*, 27, 953-960.

Kohut, R.J., Laurence, J.A., Amundson, R.G., 1988. Effects of ozone and sulfur-dioxide on yield of red-clover and timothy. *Journal of Environmental Quality*, 17, 580-585.

Kopper, B.J., Lindroth, R.L., 2003a. Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. *Oecologia*, 134(1), 95-103.

Kopper, B.J., Lindroth, R.L., 2003b. Responses of trembling aspen (Populus tremuloides) phytochemistry and aspen blotch leafminer (Phyllonorycter tremuloidiella) performance to elevated levels of atmospheric CO2 and O-3. *Agricultural and Forest Entomology*, 5(1), 17-26.

Korner, C., Arnone, J.A.I., 1992. Responses to elevated carbon dioxide in artificial tropical ecosystems. *Science*, 257, 1672-1675.

Korner, C.H., Scheel, A. and Bauer, H. 1979. Maximum leaf diffusive conductance in vascular plants. *Photosynthetica*, 13(1), 45-82.

LRTAP Convention, 2004. Revised manual on methodologies and criteria for mapping critical levels/loads and geographical areas where they are exceeded, Chapter 3: Mapping Critical Levels for Vegetation. pps. 53, 2004. Editor: G Mills. Umweltbundesamt, Berlin, Germany. http://www. icpmapping.org

LRTAP Convention, 1996. Manual on methodologies and criteria for mapping critical levels/loads and geographical areas where they are exceeded. Texte 71/96, Umweltbundesamt, Berlin, Germany.

Lu, P., Yunusa, I.A.M., Walker, R.R., Mueller, W.J. 2003. Regulation of canopy conductance and transpiration and their modelling in irrigated grapevines. *Functional Plant Biology*, 30, 689-698.

Lyons, T.M., Barnes, J.D., 1998. Influence of plant age on ozone resistance in Plantago major L. *New Phytologist*, 138, 83-89.

Lyons, T.M., Barnes, J.D., Davison, A.W., 1997. Relationship between ozone resistance and climate in European populations of *Plantago major*. *New Phytologist*, 136, 503-510.

Machado, E.C., Lagoa, A.M.M.A. 1994. Trocas gasosas e condutancia estomatica em tres especies de gramineas.Bragantia, *Campinas*, 53(2), 141-149.

Makela, P. Munns, R., Colmer, T.D., Condon, A.G., and Peltonen-Sainio, P. 1998. Effect of foliar applications of glycinebetaine on stomatal conductance, abscisic acid and solute concentrations in leaves of salt- or drought-stressed tomato. *Australian Journal of Plant Physiology*, 25, 655-663.

Mascart, P., Taconet, O., Pinty, J-P. and Mehrez, M.B. 1991. Canopy resistance formulation and its effect in mesocale models: a HAPEX perspective. *Agricutural and Forest Meteorology*, 54(2-4), 319-351.

Massman, W.J., Pederson, J., Delany, A., Grantz, D., den Hartog, G., Oncley, S.P., Pearson, Jr. R, Shaw, R. .1994. An evaluation of the RADM surface module for ozone uptake at three sites in the San Joaquin Valley of California. *Journal of Geophysical Research*, 99, 8281-8294.

Matsumoto, K., Ohta, T., Tanaka, T., 2005. Dependence of stomatal conductance on leaf chlorophyll concentration and meteorological variables. *Agricultural and Forest Meteorology*, 132 (1-2), 44-57.

McDonald, E.P., Kruger, E.L., Riemenschneider, D.E., Isebrands, J.G., 2002. Competitive status influences tree-growth responses to elevated CO2 and O-3 in aggrading aspen stands. *Functional Ecology,* 16(6), 792-801.

McKee, I.F., Long, S.P., 2001. Plant growth regulators control ozone damage to wheat yield. *New Phytologist*, 152(1), 41-51.

Medrano, H., Escalona J.M., Cifre, J., Bota, J. and Flexas, J. 2003. A ten-year study of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Functional Plant Biology*, 30, 607-619.

Mills, G., Harmens, H., Hayes, F., Williams, P., Emberson, L., Cinderby, S., Terry, A., Ashmore, M., Holland, M., Green, E., Power, S. 2005. The UNECE International Cooperative Programme on Vegetation. Defra contract EPG 1/3/205. Progress Report (April, 2004 – March, 2005).

Mills, G., Harmens, H., Hayes, F., Williams, P., Emberson, L., Cambridge, H., Cinderby, S., Terry, A., Ashmore, M., Holland, M., Green, E., Power, S. 2004. The UNECE International Cooperative Programme on Vegetation. Defra contract EPG 1/3/205. Progress report (April, 2003 – March, 2004).

Mills, G., Holland, M., Buse, A., Cinderby, S., Hayes, F., Cambridge, F., Ashmore, M., Terry, A., 2003. Introducing response modifying factors into a risk assessment for ozone effects on crops in Europe. In: Karlsson, P.E., Sellden, G., Pleijel, H. (Eds.), Establishing ozone critical levels II. UN-ECE Workshop Report. IVL Report B 1523. IVL Swedish Environmental Research Institute, Gothenburg, pp. 74-88.

Mills, G., Ball, G., Hayes, F., Fuhrer, J., Skarby, L., Gimeno, B., De Temmerman, L., Heagle, A., 2000. Development of a multi-factor model for predicting the effects of ambient ozone on the biomass of white clover. *Environmental Pollution*, 109(3), 533-542.

Mills, G., Hayes, F., Buse, A., and Reynolds, B. 2000. Air Pollution and Vegetation. UNECE ICP Vegetation Annual Report 1999/2000, NERC (ISBN 1-870393-55-4)

Mills, G., Hayes, F., Jones, M.L.M., Cinderby, S., in press. Identifying ozone-sensitive communities of (semi-)natural vegetation suitable for mapping exceedance of critical levels. *Environmental Pollution Special Issue*

Miller, J.D., Arteca, R.N., Pell, E.J., 1999. Senescence-associated gene expression during ozone-induced leaf senescence in Arabidopsis. *Plant Physiology*, 120, 1015-1024.

Moorsheet, S. and Yocum, C.S. 1972. A condensation type porometer for field use. *Plant Physiology*, 49, 944-949.

Morecroft, M.D., Sellers, E.K., Lee, J.A., 1994. An experimental investigation into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. *Journal of Ecology,* 82, 475-483.

Mortensen, L., Nilsen, J., 1992. Effects of ozone and temperature on growth of several subalpine plant species. *Journal of Agricultural Science*, 6, 195-204.

Mulholland, B.J., Fussell, M., Edmondson, R.N., Burns, I.G., McKee, J.M.T. and Basham, J. 2000. Effect of humidity and nutrient feed K/Ca ratio on physiological responses and the accumulation of dry matter, Ca and K in tomato. *Journal of Horticultural Science and Biotechnology*, 75(6), 173-722.

Naor, A., Wample, R.L. 1995. A rapid field method for measuring net assimilation rate-stoamtal conductance relationship: a feasibility test using grapevine leaves. *Scientia Horticulturae*, 60, 287-292.

Nebel, B., Fuhrer J., 1994. Inter- and Intraspecific differences in ozone sensitivity in semi-natural plant communities. *Angewandte Botanik*, 68, 116-121.

Nordin, A., Näsholm, T., Ericson, L., 1998. Effects of simulated N deposition on understorey vegetation of a boreal coniferous forest. *Functional Ecology*, 12, 691-699.

Novak, K., Skelly, J.M., Schaub, M., Krauchi, N., Hug, C., Landolt, W., Bleuler, P., 2003. Ozone air pollution and foliar injury development on native plants of Switzerland. *Environmental Pollution*, 125, 41-52.

Olff, H., Berendse, F., Devisser, W., 1994. Changes in nitrogen mineralization, tissue nutrient concentrations and biomass compartmentation after cessation of fertilizer application to mown grassland. *Journal of Ecology*, 82(3), 611-620.

Olioso, A., Bethenod, O., Rambal, S. and Thamitchian, M. 1995. Comparison of empirical leaf photosynthesis and stomatal conductance models. In 10th International Photosynthesis Congress, Montpellier (FRA), 20-25 August 1995, 4pp.

O'Neill, E.G., Norby, R.J., 1996. Litter quality and decomposition rates of foliar litter produced under CO2 enrichment. In: Koch, G.W., Mooney, H.A. (Eds.), Carbon dioxide and terrestrial ecosystems. Academic Press, London.

Ozier-Lafontaine, H., Lafolie, F., Bruckler, L., Tournebize, R., and Mollier, A. 1998. Modelling competition for water in intercrops: theory and comparison with field experiments. *Plant and Soil*, 204, 183-201.

Padro, J., Massman, W.J., den Hartog, G. and Neumann, H.H. 1994. Dry deposition velocity of O_3 over a vineyard obtained from models and observations: The 1991 California Ozone Deposition Experiment. *Water Air & Soil Pollution*, 75, 307-323.

Patakas, A., Kofidis, G., Bosabaldis A.M. 2003. The relationship between CO_2 transfer mesophyll resistance and photosynthetic efficiency in grapevine cultivars. *Scientiia Horticultura*, 97, 255-263.

Pate, J.S., 1983. Patterns of nitrogen metabolism in higher plants and their ecological significance. In: Lee, J.A., McNeill, S., Rorison, I.H. (Eds.), Nitrogen as an ecological factor. 22nd symposium of the British Ecological Society. Blackwell Scientific, Oxford, pp. 225-256.

Pearce, I.S.K., van der Wal, R., 2002. Effects of nitrogen deposition on growth and survival of montane Racomitrium lanuginosum heath. *Biological Conservation*, 104(1), 83-89.

Perkins, D.F., 1978. The distribution and transfer of energy and nutrients in the Agrostis-Festuca grassland ecosystem. In: Heal, O.W., Perkins, D.F. (Eds.), Production ecology of British moors and montane grasslands. Ecological Studies, 27. Springer-Verlag, Berlin, pp. 374-395.

Pirker, K.F., Reichenauer, T.G., Pascual, E.C., Kiefer, S., Soja, G. and Goodman, B.A. 2003. Steady state levels of free radicals in tomato fruit exposed to drought and ozone stress in a field experiment. *Plant, Physiology and Biochemistry,* 41(10), 921-927.

Pitcairn, C.E.R., Skiba, U.M., Sutton, M.A., Fowler, D., Munro, R., Kennedy, V., 2002. Defining the spatial impacts of poultry farm ammonia emissions on species composition of adjacent woodland groundflora using Ellenberg Nitrogen Index, nitrous oxide and nitric oxide emissions and foliar nitrogen as marker variables. *Environmental Pollution,* 119, 9-21.

Pitcairn, C.E.R., Fowler, D., Grace, J., 1995. Deposition of fixed atmospheric nitrogen and foliar nitrogen content of bryophytes and Calluna vulgaris (L.) Hull. *Environmental Pollution*, 88, 193-205.

Pleijel, H, Ojanpera, K., Mortensen, L., (1997) Effects of tropospheric ozone on the yield and grain protein content of spring wheat (*Triticum aestivum* L) in the Nordic countries. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science*. 47(1): 20-25

Pleijel, H., Danielsson, H., 1997. Growth of 27 herbs and grasses in relation to ozone exposure and plant strategy. *New Phytologist*, 135, 361-367.

Power, S.A., Ashmore, M.R., 2002. Responses of fen and fen-meadow communities to ozone. *New Phytologist*, 156, 399-408.

Power, S.A., Ashmore, M.R., Cousins, D.A., Sheppard, L.J., 1998. Effects of nitrogen addition on the stress sensitivity of Calluna vulgaris. *New Phytologist*, 138, 663-673.

Putnam, D.H., Oplinger, E.S., Hicks, D.R., Durgan, B.R., Noetzel, D.M., Meronuck, R.A., Doll, J.D. and Schulte, E.E. 2000. Alternative Field Crops Manual.
http://www.hort.purdue.edu/newcrop/afcm/sunflower.html

Quick, W.P., Chaves, M.M., Wendler, R., David. M., Rodrigues, M.L., Passahariho, J.A., Pereria, J.S., Adcock, M.D., Leegood, R.C. and Stitt, M. 1992. The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. *Plant, Cell and Environment*, 15, 25-35.

Reiling, K., Davison, A.W., 1995. Effects of ozone on stomatal conductance and photosynthesis in populations of *Plantago major* L. *New Phytologist,* 129, 587-594.

Reiling, K., Davison, A.W., 1992. The response of native herbaceous species to ozone – growth and fluorescence screening. *New Phytologist*, 120, 29-37.

Rennenberg, H., Herschbach, C., Polle, A., 1996. Consequences of air pollution on shoot-root interactions. *Journal of Plant Physiology*, 148, 296-301.

RGAR, 1997. Acid deposition in the United Kingdom 1992-1994. Fourth report of the UK Review Group on Acid Rain., Department of the Environment Transport and Regions, London.

Rivelli, A. R., Lovelli, S. and Perniola, M. 2002. Effects of salinity on gas exchange, water relations and growth of sunflower (*Helianthus annuus*). *Functional Plant Biology*, 29(12), 1405-1415.

Rochette, P., Pattey, E., Desjardins, R.L., Dwyer, L.M., Stewart, D.W. and Dubé, P.A. 1991. Estimation of maize (*Zea mays* L.) canopy conductance by scaling up leaf stomatal conductance. *Agricultural and Forest Meteorology*, 54(2-4), 241-261.

Rodriguez, J.L. and Davies, W.J. 1982. The effects of temperature and ABA on stomatal of *Zea Mays*. L. *Journal of Experimental Botany*, 33(136), 977-987.

Rodwell, J. S., 1992. British Plant Communities. Volume 3. Grasslands and montane communities. Cambridge, Cambridge University Press, UK.

Romero-Aranda, R., Soria, T. and Cuartero, J. 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Science*, 160, 265-272.

Rorison, I.H., Peterkin, J.H., Clarkson, D.T., 1983. Nitrogen source, temperature and the growth of herbaceous plants. In: Lee, J.A., McNeill, S., Rorison, I.H. (Eds.), Nitrogen as an ecological factor. 22nd symposium of the British Ecological Society. Blackwell Scientific, Oxford, pp. 189-210.

Rühling, Å. 1994. Atmospheric heavy metal deposition in Europe – estimation based on moss analysis. NORD 1994:9. Nordic Council of Ministers, Copenhagen.

Rühling, Å., Steinnes, E. 1998. Atmospheric heavy metal deposition in Europe 1995-1996. NORD 1998:15. Nordic Council of Ministers, Copenhagen.

Sadras, V.O., Villalobos, F.J., Fereres, E. and Wolfe, D.W. 1993. Leaf responses to soil water deficits: Comparative sensitivity of leaf expansion rate in field-grown sunflower (*Helainthus annuus* L.). *Plant and Soil*, 153, 189-194.

Saleem, A., Loponen, J., Pihlaja, K., Oksanen, E., 2001. Effects of long-term open-field ozone exposure on leaf phenolics of European silver birch (Betula pendula Roth). *Journal of Chemical Ecology*, 27, 1049-1062.

Sanguineti, M.C., Tuberosa, R., Landi, P., Salvi, S., Maccaferri, M., Casarini, E. and Conti, S. 1999. QTL analysis of drought-related traits and grain yield in relation to genetic variation for leaf abscisic acid concentration ion field grown maize. *Journal of Experimental Botany*, 50(337), 1289-1297.

Sanders, G.E., Clark, A.G., & Colls, J.J. 1991. The influence of open-top chambers on the growth and development of *Vicia faba*. *New Phytologist*, 117, 439-447.

Sanz, J., Muntifering, R.B., Bermejo, V., Gimeno, B.S., Elvira, S., 2005. Ozone and increased nitrogen supply effects on the yield and nutritive quality of *Trifolium subterraneum*. *Atmospheric Environment*, 39, 5899-5907.

Schultz, H.R. 2003. Extension of a Farquar model for limitations of leaf photosynthesis induced by light environment, phenology and leaf age in grapevines (*Vitis vinifera* L.cvv. White Riesling and Zinfandel). *Functional Plant Biology*, 30, 673 - 687.

Schultz, H.R. 2003a. Differences in hydraulic architecture caccount for near-isohydric and anisohydric behaviour of two field-grown *Vitis Vinifera* L. cultivars during draught. *Plant, Cell and Environment*, 26, 1393-1405.

Schurr, U., Gollan, T. and Schulze E.-D. 1992, Stomatal response to drying soil in relation to changes in the xylem sap composition of Helianthus annuus. II. Stomatal sensitivity to abscisic acid imported from the xylem sap. *Plant, Cell and Environment*, 15, 561-567.

Sild, E., Pleijel, H., Sellden, G., 2002. Elevated ozone (O-3) alters carbohydrate metabolism during grain filling in wheat (Triticum aestivum L.). *Agriculture Ecosystems & Environment*, 92 (1), 71-81.

Simpson, D., Ashmore, M.R., Emberson, L., Tuovinen, J.-P., in press. A comparison of two different approaches for mapping potential ozone damage to vegetation. A model study. *Environmental Pollution Special Issue*.

Simpson, D., Fagerli H., Jonson J.E., Tsyro S., Wind, P., 2003. Transboundary Acidification, Eutrophication and Ground Level Ozone in Europe. Part I - Unified EMEP Model Description. Norwegian Meteorological Institute, Oslo, EMEP MSC-W Note 1/2003. http://www.emep.int/publ/common_publications.html, 104p.

Sims, D.A., Cheng, W., Luo, Y., Seemann, J.R. 1999. Photosynthetic acclimation to elevated CO2 in a sunflower canopy. *Journal of Experimental Botany*, 50(334), 645-653.

Sinclair, T.R., Bingham, G.E., Lemon, G.E. and Allen, L.H. Jr. 1975. Water use efficiency of field-grown maize during moisture stress. *Plant Physiological*, 56, 245-249.

Soja, G., Reichenauer, T.g., Eid, M., Soja, A.-M., Schaber, R., Gangl., H., 2004. Long-term ozone exposure and ozone uptake of grapevines in open-top chambers. *Atmospheric Environment*, 38, 2313 – 2321.

Soja, G., Barnes, J., Vandermeiren, K., Pleijel, H., Mills, G., 1999. Phenological weighting of ozone exposures in the calculation of critical levels for non-woody species. In: Fuhrer, J., Achermann, B. (Eds.), Critical Levels for Ozone - Level II. Proceedings of Expert Workshop held in Gerzensee, Switzerland 11 - 15 April 1999. Environmental Documentation No. 115. Air. Swiss Agency for the Environment, Forests and Landscape SAEFL, Berne, pp. 89-94.

Sparks, T.H., Greatorex-Davies, J.N., Mountford, J.O., Hall, M.L., Marrs, R.H., 1996. The effects of shade on the plant communities of rides in plantation woodland and implications for butterfly conservation. *Forest Ecology and Management*, 80, 197-207.

Spence, R.D., Rykiel, E.J., Sharpe Jr, P.J.H., 1990. Ozone alters carbon allocation in loblolly pine: Assessment with carbon-11 labeling. *Environmental Pollution*, 64, 93-106.

Starck, Z., Niemyska, J., Bogdan, J. and Akour Tawalbeh, R.N. 2000. Response of tomato plants to chilling stress in association with nutrient or phosphorous starvation. *Plant and Soil*, 226, 99-106.

Steduto, P., Albrizio, R., Giorio, P. and Sorrentino, G. 2000. Gas-exchange response and stomatal and non-stomatal limitations to carbon assimilation of sunflower under salinity. *Environmental and Experimental Botany*, 44, 243-255.

Steinnes, E., Rühling, Å., Lippo, H., Mäkinen, A. 1997. Reference material for large-scale metal deposition surveys. *Accreditation and Quality Assurance*, 2, 243-249.

Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J., 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303(5665), 1876-1879.

Stigter, C.J. and Lammers, B. 1974. Leaf diffusion resistance to water vapour and its direct measurement . III. Results regarding the improved diffusion porometer in growth rooms and fields of Indian corn (*Zea mays*). Med. Landbouwhogesch. Wageningen 74-21, 1-76.

Strengbom, J., Walheim, M., Näsholm, T., Ericson, L., 2003. Regional differences in the occurrence of understorey species reflect nitrogen deposition in Swedish forests. *Ambio*, 32, 91-97.

Strengbom, J., Nordin, A., Nasholm, T., Ericson, L., 2002. Parasitic fungus mediates change in nitrogen-exposed boreal forest vegetation. *Journal of Ecology*, 90, 61-67.

Takemoto, B.K., Bytnerowicz, A., Fenn, M.E., 2001. Current and future effects of ozone and atmospheric nitrogen deposition on California's mixed conifer forests. *Forest Ecology and Management*, 144(1-3), 159-173.

Tardieu, F., Zhang, J. and Davies, W.J. 1992a. What information is conveyed by an ABA signal from maize roots in drying field soil. *Plant, Cell and Environment*, 15, 185-191.

Tardieu, F., Katerji, H., Bethenod, O., Zhang, J. and Davies, W.J. 1991. Maize stomatal conductance in the field: it's relationship with soil and plant water potentials, mechanical constraints and ABA concentration in the xylem sap. *Plant, Cell and Environment*, 14, 121-126.

Terry, A.C., Woodward, F.I. 1994. Predicting grassland responses to climate and CO_2. In "Demonstrating Climate Change Impacts in the UK: the Department of the Environment Core Model Programme 1990-1994" (Parr, T. and Eatherall, A., eds) ISBN 1 870393 23 6.

The British Tomato Growers Association http://www.britishtomatoes.co.uk.

Thomas, V.F.D., Braun, S., Fluckiger, W., 2005. Effects of simultaneous ozone exposure and nitrogen loads on carbohydrate concentrations, biomass, and growth of young spruce trees (Picea abies). *Environmental Pollution*, 137(3), 507-516.

Thwaites, R., 1997. The effects of tropospheric ozone on calcareous grassland communities, University of London.

Tingey, D.T., Rodecap, K.D., Lee, E.H., Hogsett, W.E., Gregg, J.W., 2002. Pod development increases the ozone sensitivity of Phaseolus vulgaris. *Water Air and Soil Pollution*, 139, 325-341.

Tingey, D.T., Anderson, C.P., 1991. The physiological basis of differential plant sensitivity to changes in atmospheric quality. In: Jr, T.G.E., Pitekla, L.F., Clegg, M.T. (Eds.), Ecological genetics and air pollution. Springer-Verlag, New York, pp. 209-235.

Tisdale, S.L., Nelson, W.L., Beaton, J.D., Havlin, J.L., 1993. Soil fertility and fertilisers. 5th edition. Macmillan Publishing Company, New York.

Tonneijck, A.E.G., Franzaring, J., Brouwer, G., Metselaar, K., Dueck, T.A., 2004. Does interspecific competition alter effects of early season ozone exposure on plants from wet grasslands? Results of a three-year experiment in open-top chambers. *Environmental Pollution*, 131, 205-213.

Tuebner, F. 1985. Messung der photosynthese und transpiration an weizen, kartoffel und sonnenblume. Diplomarbeit University Bayreuth, West Germany.

Tukey, J.W., 1958. Bias and confidence in not quite large samples. *Annals of Mathematical Statistics*, 29, 614.

Turner, N.C., Schulze, E.-D. and Gollan, T. 1985. The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content II. In the mesophytic herbaceous species *Helianthus annuus*. *Oecologia*, 65, 348-355.

Turner, N.C., Schulze, E.-D. and Gollan, T. 1984. The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content I. Species comparison at high soil water contents. *Oecologia*, 63, 338-342.

Turner, N.C., and Begg, J.E. 1973. Stomatal behaviour and water status of maize, sorghum, and tobacco under field conditions. *Plant Physiology*, 51, 31-36.

Turner, N.C. 1970. Response of adaxial and abaxial stomatal to light. *New Phytologist*, 69, 647-653.

Uddling, J., Gunthardt-Goerg, M.S., Matyssek, R., Oksanen, E., Pleijel, H., Sellden, G., Karlsson, P.E., 2004. Biomass reduction of juvenile birch is more strongly related to stomatal uptake of ozone than to indices based on external exposure. *Atmospheric Environment*, 38, 4709-4719.

van der Maarel, E., Boot, R., van Dorp, D., Rijntjes, J., 1985. Vegetation succession on the dunes near Oostvoorne, The Netherlands; a comparison of the vegetation in 1959 and 1980. *Vegetatio*, 58, 137-187.

Vandermeiren, K., de Temmerman, D., Hookham, N., 1995. Ozone sensitivity of Phaseolus vulgaris in relation to cultivar differences, growth stage and growing conditions. *Water Air and Soil Pollution*, 85, 1455-1460.

Volk, M., Bungener, P., Contat, F., Montani, M., Fuhrer, J., 2006. Grassland yield declined by a quarter in five years of free-air ozone fumigation. *Global Change Biology*, 12, 74-83.

Volk, M., Geissmann, M., Blatter, A., Contat, F., Fuhrer, J., 2003. Design and performance of a free-air exposure system to study long-term effects of ozone on grasslands. *Atmospheric Environment*, 37, 1341-1350.

Wample, R.L. and Thornton, R.K. 1984. Differences in the response of sunflower (Helianthus annuus) subjected to flooding and drought stress. *Physiologia Plantarum*, 61, 611-616.

Ward, D.A. and Bunce, J.A. 1986. Responses of net photosynthesis and conductance to independent changes in the humidity environments of the upper and lower surfaces of leaves of sunflower and soybean. *Journal of Experimental Botany*, 37, 1842-1853.

Wang, Y.H., Shim, C.S., Blake, N., Blake, D., Choi, Y., Ridley, B., Dibb, J., Wimmers, A., Moody, J., Flocke, F., Weinheimer, A., Talbot, R., Atlas, E., 2003. Intercontinental transport of pollution manifested in the variability and seasonal trend of springtime O-3 at northern middle and high latitudes. *Journal of Geophysical Research-Atmospheres*, 108 (D21).

Warwick, K. R., Taylor, G., 1995. Contrasting effects of tropospheric ozone on five native herbs which coexist in calcareous grassland. *Global Change Biology*, 1, 143-151.

Weiss, A. and Norman, J.M. 1985. Partitioning solar radiation into direct and diffuse, visible and near-infra-red components. *Agricultural and Forest Meteorology*, 34: 205-213.

Werner, W. Büker, P. and Schulze, E. 1998. Stomatal response of the two clover clones . Poster presented at the ICP-Crops Task Force meeting, Jan. 14-16, Wagenningen, The Netherlands.

Whitfield, C.P., Davison, A.W., Ashenden, T.W., 1998. The effects of nutrient limitation on the response of Plantago major to ozone. *New Phytologist*, 140(2), 219-230.

Willis, A.J., Yemm, E.W., 1961. Braunton Burrows: Mineral nutrient status of the dune soils. *Journal of Ecology*, 49, 377-390.

Wilson, E.J., Wells, T.C.E., Sparks, T.H., 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition? - an experimental determination of critical load. *Journal of Ecology*, 83, 823-832.

Wingler, A., Einig, W., Schaeffer, C., Wallenda, T., Hampp, R., Wallander, H., Hylund, J.-E., 1994. Influence of different nutrient regimes on the regulation of carbon metabolism in Norway spruce (Picea abies [L.] Karst.) seedlings. *New Phytologist*, 128, 323-330.

Winkel, T. and Rambal, S. 1993. Influence of water stress on grapevines growing in the field: from leaf to whole-plant response. *Australian Journal of Plant Physiology*, 20, 143-157.

Winkle, T. and Rambal, S. 1990. Stomatal conductance of some grapevines growing in the field under a Mediterranean environment. *Agric. For. Meteorol.* 51, 107-121

Wookey, P.A., Atkinson, C.J., Mansfield, T.A. and Wilkinson, J.R. 1991. Control of plant water deficits using the "Snow and Tingey system" and their influence on the water relations and growth of sunflower. *Journal of Experimental Botany*, 42, 589-595.

Zhang, L., Moran, M.D. and Brook, J.R. 2001. A comparison of models to estimate in-canopy photosynthetically active radiation and their influence on canopy stomatal resistance. *Atmospheric Environment*, 35, 4463-4470.

Zhang, J., and Davies, W.J. 1989. Sequential response of whole plant water relations to prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behaviour of sunflower plants. *New Phytologist*, 113, 167-174.

Zheng, Y., Shimizu, H., Barnes, J.D., 2002. Limitations to CO_2 assimilation in ozone-exposed leaves of *Plantago major*. *New Phytologist*, 155, 67-78.

ANNEX 1

WORKSHOP ON CRITICAL LEVELS OF OZONE: FURTHER APPLYING AND DEVELOPING THE FLUX-BASED CONCEPT

Report prepared by the organizers with the assistance of the secretariat

INTRODUCTION

1. The workshop on the critical levels of ozone and the further application and development of the flux-based concept took place on 15–19 November 2005 in Obergurgl, Austria. It was organized by the Federal research and training centre for forests, natural hazards and landscape (BFW).

2. The workshop was attended by 97 experts from the following Parties to the Convention: Austria, Belgium, Canada, Denmark, Finland, France, Germany, Greece, Italy, the Netherlands, Poland, Slovenia, Spain, Sweden, Switzerland, the United Kingdom and the United States of America. An expert from Australia also attended. The International Cooperative Programme (ICP) on Forests, ICP Vegetation, the EMEP Meteorological Synthesizing Centre - West (MSC-W) and the UNECE secretariat were represented.

I. AIMS OF THE WORKSHOP

3. The overall purpose of the workshop was to confirm the flux-based approach, and the primary objectives were to:

   a) Further develop methods for applying flux-effect relationships for impact assessments at different geographical levels, including consideration of uncertainties;
   b) Review the provisional flux-based critical level for forest trees, and to consider progress in establishing flux-based critical levels for crops not currently included in the mapping manual;
   c) Assess progress in the development of canopy and stand level ozone flux-effect models and methods for crops and forest trees;
   d) Assess progress in the development of flux-effect models for (semi-)natural vegetation; and
   e) Identify areas of further work for crops, (semi-)natural vegetation and forest trees.

4. The workshop was opened by Mr. Gerhard Wieser (Austria). He briefly presented the background and main aims of the workshop.

II. CONCLUSIONS AND RECOMMENDATIONS

A. General

5. The workshop noted the new evidence for confirmation of the flux-based approach, which had not been available at the previous workshop in 2002. The confirming data

comprised mainly processes at the leaf level, such as stomatal conductance. Progress in the development of flux methodology was presented. It was not yet possible to confirm the flux-effect relationships developed from ozone-exposure experiments with field data, although harmful effects of ozone, such as ozone injury to leaves, have been detected in the field in ambient ozone concentrations.

6. The workshop agreed that its recommendations would mainly be applicable to the EMEP modelling domain, which covers most of Europe. However, close cooperation should be maintained with ozone researchers in North America.

7. The workshop proposed the flux-based approach as a common method to assess the risk of effects of ozone on ecosystems in integrated assessment modelling. The quantitative indicator for flux is $AF_{st}Y$, the accumulated stomatal flux of ozone above a flux threshold of Y nmol m^{-2} s^{-1} per unit projected leaf area. However, the flux-based approach could not currently be quantified for effects on semi-natural vegetation; critical levels remained based on the concentration-based approach (AOTX, accumulated ozone concentration over the threshold of X parts per billion (ppb) over a stated time period) for this receptor.

8. There was no new information on crops and forest trees that might necessitate revision of the critical levels currently found in the *Manual on methodologies and criteria for modelling and mapping critical loads and levels and air pollution effects, risks and trends*. New experimental evidence of effects was presented for (semi-)natural vegetation, and the workshop agreed on a new critical level for communities dominated by perennial species.

9. The workshop noted the need of the research community to identify main areas of uncertainties in the ozone impact pathway. The defined uncertainties and possible biases should be quantified and clearly communicated to policy-makers. The confirmation activities on measuring effects could be classified according to three main levels: canopy (or ecosystem), leaf and cellular responses. The workshop recommended encouraged that the ICPs discuss and list relevant impact endpoints that would be meaningful for policy-making. It noted the need to engage national contact points to encourage assessment of ozone risks using the suggested methods, in order to gain experience in local applications.

B. Crops

10. One "model" crop species was suggested for applying the flux-based approach in integrated assessment modelling. It should have a simplified phenology, which was considered a major difficulty in continent-wide mapping, and was assumed to be well-irrigated. Practical ways were identified to define the growing seasons or periods across Europe, using means such as climatological maps, temperature sums and latitude functions. The model species could indicate the relative distribution of ozone damage risk across Europe in support of policy-making, using integrated assessment modelling. It should not be used to estimate yield losses.

11. Detailed local-scale applications for specific species and locations were recommended for parallel studies of Europe-wide flux-based approach. The workshop agreed that concentration-based approach, possibly modified by factors important for the exposure dose, could still be useful on a local scale, but few new data were available. Local

climatic data could be used for cultivars within specific climatic regions. The workshop recognized the problem that ozone concentrations were still not used at canopy height.

12. The need to distinguish random uncertainties from systematic ones was noted. In the concentration-based approach, the latter included the lack of environmental limitations. Systematic uncertainties in the flux approach have yet to be sufficiently well identified.

13. The workshop agreed that the limitations and uncertainties of existing flux- and concentration-based response relationships for crops should be clarified in the *Mapping manual.* In local applications, existing locally derived relationships should be used; otherwise the limitations of applying the relationships of the *Mapping manual* should be clearly explained. New data that might be included in future response functions existed, for example, for maize, sugar beets, grapevines, tomatoes and alfalfa.

14. The main limitation for assessing uncertainty was the lack of observations. The potential future use of molecular markers in the validation process was noted.

C. Forest trees

15. The workshop noted that formulation and parameterization for forest trees had been improved in the EMEP stomatal ozone flux model, partly due to new data sets being available. Models that have been developed and validated for other applications, such as forest growth, climate change and the water cycle, could provide information to improve the EMEP flux model further. A full validation and sensitivity analysis should be conducted within the EMEP flux model development and implementation. The workshop concluded that the use of the stomatal uptake model was reasonable for risk assessment within the EMEP domain. Other options were required for local assessments.

16. The workshop considered impact endpoint and the quantitative indicator of negative impacts as important issues. In particular, the appropriateness of the use of growth as a surrogate to represent impacts on natural woodland ecosystems was questioned. Ozone impacts on mature forest trees over a wide geographical area should be verified to support the use of critical levels.

17. The workshop agreed a quadrant framework, which comprised risk assessment and impacts evaluation on the local and EMEP domain scales. Different approaches were suggested for the four combinations of available methods and geographical scales.

18. The workshop noted that guidance for assessments at the local scale, defined as national or sub-national, was advisory only. Detailed local-scale risk assessment applications were recommended using the concentration-based (AOTX) approach, the maximum permissible ozone concentration (MPOC) approach or the flux-based approach ($AF_{st}Y$). The application of these approaches should be optimized to local conditions and species. The workshop made no specific recommendations or advice on local-scale impact evaluation.

19. The workshop noted the procedure for optimizing emission reductions to protect forests in Europe was to be based only on $AF_{st}1.6$ in integrated assessment models. Maps should be produced on the basis of both $AF_{st}1.6$ and AOT40, but maps based on the latter should

be used for selected key scenarios only. The workshop agreed that the threshold value Y = 1.6 nmol m^{-2} s^{-1} per unit projected leaf area, above which the stomatal flux should be accumulated, was to be retained as no scientific evidence was presented to justify a change.

20. The workshop recommended estimates of $AF_{st}1.6$ should be made for a model tree species representing all forests in the EMEP domain for use in integrated assessment modelling. The parameterization of the model tree stomatal uptake module should be reviewed by appropriate experts, with a focus on additional input data to reflect regional variation.

21. The workshop agreed the critical level based on $AF_{st}1.6$ should not be used in integrated assessment modelling. The workshop agreed that ozone uptake to leaves and needles was potentially harmful but that, at present, the uncertainties associated with quantifying the impacts of ozone on forests on the European scale were high.

22. The workshop agreed the flux-based approach in its present form should only be used for relative risk assessments in support of policy-making. It should not be used to derive quantitative estimates of the negative effects of ozone on forests on this scale; therefore, no specific recommendations or advice can be made on impact evaluation.

23. The workshop agreed the implementation of the flux-based approach for Europe-wide integrated assessment modelling was to provide a biologically meaningful approach which could enhance the geographical representativity of ozone risks for forests and underlying damage mechanisms. However, high uncertainties were still associated with the formulation, parameterization and validation of the model and the link between stomatal flux and responses.

24. The workshop adviced that additional text was required in the *Mapping manual* to reflect changes in guidance for integrated assessment modelling and concerns over the use of the flux-based approach. Additional information was required on the estimation of the ozone flux accumulation period in different parts of Europe.

25. The workshop noted the need appropriate experts on forest trees to prepare concrete proposals on practical modelling details and proposals for changes in the *Mapping manual*. Mr. Per-Erik Karlsson (Sweden) offered to organize experts for this work. The workshop requested him to report to all appropriate ICP Task Force meetings in spring 2006.

D. (Semi-)natural vegetation

26. The workshop decided that the concentration-based (AOT40) critical level of 3 ppm.h (parts per million x hours) over three months for communities dominated by annual species was still valid and recommended its continued use.

27. Results from recent studies suggested a new critical level for communities dominated by perennial species. Because of the longer growth period of these communities, the AOT40 should be calculated over a six-month growth period. The workshop recommended new critical level of an AOT40 of 5 ppm.h over six months to prevent adverse effects.

Assessment of the exceedance of this critical level should be based on mean values of AOT40 for five consecutive years.

28. The workshop agreed AOT40 values for three months (for annuals-dominated communities) and six months (for perennials-dominated communities) should be calculated over the period of active growth, which depends on climatic zones. Revised start and end dates for these periods were suggested to replace the current values in the *Mapping manual*. If a single map was necessary for integrated assessment modelling, the six-month value of 5 ppm.h should be used.

29. The workshop agreed that receptors were grouped according to the European nature information system (EUNIS) classification of ecosystems. Species-level data suggested the highest risk of adverse effects for dry grassland (E1), mesic grassland (E2), wet grassland (E3), alpine grassland (E4), woodland fringes (E5), dehesa (E7.3) and heathland (F4). For E1 and E2 this classification of sensitivity was supported by experimental evidence of changes in plant community studies.

30. Experimental data were not available to support the development of critical levels based on the flux-based approach for semi-natural vegetation. The workshop agreed models available to map ozone flux to semi-natural communities were not sufficiently developed and well parameterized for risk assessment at this stage. However, progress had been made in developing a flux model parameterized for productive grasslands dominated by perennial ryegrass (*Lolium perenne*), under different management and nutrient regimes.

III. MAIN FUTURE RESEARCH PRIORITIES

31. The workshop identified the following priorities for future research:

a) Establishment of a Europe-wide research network with field sites on plant exposure and ozone deposition studies (which would significantly help flux model validation and development and further research);
b) Development of epidemiological methods based on crop statistics to study ozone effects;
c) Detailed specification of the “model” crops for integrated assessment modelling;
d) Field experiments on trees, including open-release systems, which cover the range of climatic conditions represented across Europe;
e) Identification of ozone impacts on forest trees using existing databases, including assessment of dose-response indicators other than tree growth;
f) Gather new data to validate the stomatal flux model for forests of all ages and types in all geographical regions;
g) Field-release experiments in a range of intact semi-natural vegetation communities to confirm that critical levelsare appropriate for field application, including the derivation of dose-response relationships;
h) New experimental studies to assess interactions between nitrogen deposition and ozone, especially in nutrient-limited communities, and also considering below-ground effects andimpacts by nutrient status;
i) Experimental confirmation of adverse effects in regions where critical levels are exceeded, including bio-indicators, historical impact analysis and evolution of ozone-tolerant genotypes.

ANNEX 2

CONVENTION ON LONG-RANGE TRANSBOUNDARY AIR POLLUTION

WORKING GROUP ON EFFECTS

INTERNATIONAL COOPERATIVE PROGRAMME ON EFFECTS OF AIR POLLUTION ON NATURAL VEGETATION AND CROPS (ICP VEGETATION)

Minutes of the 19th Task Force Meeting

The nineteenth meeting of the Programme Task Force was held from 30th January to 2nd February, 2006, Caernarfon, Wales, United Kingdom.

1. The meeting was attended by 52 experts from the following Parties to the Convention: Austria, Belgium, Finland, France, Germany, Italy, Latvia, Lithuania, Norway, Russian Federation, Slovenia, Spain, Sweden, Switzerland, United Kingdom. In addition, the chairman and the secretary of the Working Group on Effects (WGE), two representatives from the ICP Forests, one representative from EMEP/MSC-West, one guest from India and one guest from South-Africa attended the meeting.

2. Mr H Harmens (chairman of the ICP Vegetation, UK) welcomed all participants on behalf of the ICP Vegetation Coordination Centre and thanked the UK Department for Environment, Food and Rural Affairs (Defra) and the Centre for Ecology and Hydrology (CEH) for supporting the meeting. Ms B Emmett (Head of site, CEH Bangor) welcomed the participants to North Wales on behalf of CEH and gave an overview of the five scientific programmes in CEH (biogeochemistry, water, biodiversity, climate change and sustainable economies). Ongoing research at CEH Bangor includes catchment science, biogeochemistry of carbon and nitrogen, sustainable use of resources, impacts of ozone on vegetation and biomonitoring of ozone and heavy metal pollution.

3. The Programme Task Force adopted the agenda of the meeting.

4. Mr H Gregor (chairman of the WGE) gave an overview of the status of work on the effects of major air pollutants on human health and the environment as discussed at the 24th session of the Working Group on Effects (31 August – 2 September 2005, Geneva) and other relevant developments. He highlighted major achievements of the ICPs, Task Force on Health and Joint Expert Group on Dynamic Modelling as presented at the 24th session of the WGE and thanked the ICP Vegetation for its valuable input to the work under the LRTAP Convention. The Convention intends to produce a new draft on data rules for its work, to be submitted to the Executive Body of the Convention in December 2006 for approval. Mr H Gregor concluded with summary statements related to work conducted for the Multi-pollutant multi-effect Protocol, Heavy Metals Protocol, POPs Protocol and work regarding particulate matter (PM) and stressed the need for stable funding to ensure the success of the new long-term strategy of the WGE (2005-2015).

5. Mr M Johansson (secretary of the WGE) reported that Albania has joined the LRTAP Convention, which now has 50 Parties. He gave an overview of the organisation of the Convention and the eight Protocols, all in force and three under review. He stressed the importance of support to the participation of countries in the EECCA region (Eastern Europe, the Caucasus and Central Asia). He reported on the main draft conclusions from the Convention workshops on ozone and nitrogen in late 2005, including the workshop in Obergurgl, Austria (15-19 November) on 'Critical levels of ozone: further applying and developing the flux-based concept', in particular to consolidate its results in programmes' Task Force meetings. He gave an overview of the main outcome of the 23rd session of the Executive Body and informed the meeting that Mr M Williams (UK) is its new chair. Mr M Johansson concluded with a summary of the data requirements and priorities as set out in the new long-term strategy of the WGE.

6. Mr H Harmens (ICP Vegetation Coordination Centre, UK), Chairman of the ICP Vegetation, gave an overview of ICP Vegetation activities and achievements in 2005. He described the biomonitoring programmes for ozone damage on white clover and *Centaurea jacea* (brown knapweed) and showed that the ozone levels across much of Europe were lower in 2005 than 2004. Nevertheless, the concentration-based critical level of ozone for crops and (semi-)natural vegetation was still exceeded at ca. 80% of the biomonitoring sites, resulting in frequent occurrences of leaf injury on the biomonitors in 2005. He continued with a summary of the outcome of the discussions in the three working groups (forest trees, semi-natural vegetation and crops/application of flux-based models) at the ozone critical levels workshop in Obergurgl. Mr H Harmens reported on the current status of the European heavy metals in mosses survey 2005/6 and informed the meeting that 32 Parties to the Convention participate, collecting mosses from ca. 7000 sites. In addition, 17 Parties to the Convention will determine the nitrogen concentration in the mosses at ca. 3200 sites. For lead he showed that its concentration in mosses had decreased in general between 1990 and 2001. The ICP Vegetation moss data base was used for the first time by EMEP/MSC-East to verify the performance of the EMEP deposition model. For lead significant positive correlations were observed between the modelled total deposition and the concentration in mosses, in particular for areas in Scandinavia that are not influenced by local emission sources (see EMEP/MSC-East Technical Report 8/2005, available from http://www.msceast.org). Mr H Harmens showed encouraging examples of the application of mosses as biomonitors of atmospheric nitrogen deposition for Scandinavian countries, reported on the trends of the nitrogen concentration in herbarium moss samples for selected European countries (1829-2000) and discussed potential confounding factors in the use of mosses as biomonitors of atmospheric nitrogen pollution. Finally, he listed the reports and publications produced by the ICP Vegetation Coordination Centre during 2005, informed the meeting that all deliverables to the WGE in 2005 were achieved, described the ICP Vegetation work plan for 2006 and reported on developing areas. He thanked the UK Department for Environment, Food and Rural Affairs for their continuing financial support to the ICP Vegetation Coordination Centre and the participants for all their contributions to the programme.

7. Ms M Sanz (Spain) and Mr M Schaub (Switzerland) represented the ICP Forests Working Group on Air Quality and gave an overview on the work conducted during the three-year test phase (2001-2003) on the relationship between passive monitoring of the atmospheric ozone concentration and visible leaf injury in trees and shrubs. Based on the surveys on ozone injury and concentrations carried out in the Level II plots of the ICP Forest Pan

European network, the ozone visible injury and high levels of ozone are widespread. However, despite the frequent occurrence of visible leaf injury and the observed relatively high concentrations of ozone, it is still difficult to establish a relationship between both. A significant role of ozone as predictor of defoliation was identified and the potential of using ICP Forests Level II data for flux modelling was verified and offered to ICP Vegetation in case it is interested in collaborating in this field. The 20th ICP Forests Task Force Meeting (Rome, 2005) adopted the continuation of ozone concentration measurements at ICP Forests Level II plots for another three years (2006-2008) using passive sampling with a reference exposure period of 14 days. The Working Group on Air Quality offered the collaboration with the ICP Vegetation and the ICP Modelling and Mapping regarding ozone exposures and ozone risk assessment, including symptom assessment and ozone flux modelling.

8. Ms G Mills (ICP Vegetation Coordination Centre, UK), Head of the Programme Centre, gave an overview of ozone research conducted at CEH. Ozone research at CEH Edinburgh includes measurements of ozone fluxes to vegetation (both stomatal and non-stomatal) applying the Eddy Covariance technique and mapping of ozone concentrations and fluxes in the UK. Ozone research at CEH Bangor includes quantifying the effects of the changing ozone profile (increasing background and reduced peak concentrations) on upland vegetation using solardomes, population-specific responses of *Anthoxanthum odoratum* to ozone (growth, plant physiology and molecular genetics), impacts of ozone on wetlands (plant physiology, carbon cycling, microbial processes) and predicting ozone-sensitive communities at the European scale using the ICP Vegetation database (OZOVEG).

9. The following presentations reported on areas of research that the ICP Vegetation would like to develop further in the future: reporting to the Convention on impacts of nitrogen on vegetation and links with air pollution networks outside the ECE region. Ms B Emmett (UK) gave an overview of the impacts of nitrogen deposition on vegetation. In many areas in the UK the current nitrogen deposition rate is 20 kg ha^{-1} y^{-1} or more. The critical load approach indicates that currently 55% of the UK habitat is at risk from nitrogen pollution. Surveys and experimental work indicate that a major shift in species composition has already occurred, i.e. towards species with a higher fertility score. The further development of dynamic models will be key to forecasting impacts of clean air policies. Ms L Emberson (UK) gave an update on the RAPIDC (Regional Air Pollution In Developing Countries) project and discussed in more detail APCEN (Air Pollution Crops Effect Network). She presented plans for a provisional risk assessment and the use of white clover as biomonitor of ozone pollution in southern Africa and South-Asia (biomonitoring work has already started in South-Africa). Ms M Agrawal (India) gave a presentation on ‘Air pollution and agriculture: an Indian scenario’. After information on the major pollution sources and trends in air pollutant concentrations in India, she described the major approaches to air pollution research with crops (surveys, long-term field studies and experimental exposure studies) and presented some results in more detail. Ozone poses the greatest threat to agriculture in India and she emphasized the need for yield response relationships (applicable in various environmental conditions) and the need to develop bioindicator protocols for impact evaluation. Mr G Krüger (South Africa) followed with a presentation on ‘Air pollution in South Africa with reference to quantification of the impact of C2-hydrocarbons’. He reported on the main pollution sources in southern Africa, impacts on vegetation (e.g. trichloroacetic acid concentrations in leaves) and quantification of those impacts. There is very little conclusive evidence of

air pollution damage in sourthern Africa. A ozone biomonitoring study with white clover has started in South-Africa.

10. Sixteen posters were presented at the meeting. These showed the results of a variety of ozone themes, e.g. results of ozone biomonitoring studies with white clover, the use of Ellenberg numbers in predicting ozone sensitivity of plant species, impacts of ozone (and interactions with nitrogen) on Mediterranean crop yield, impacts of ozone on hedgerow communities, experimental approaches to detect early ozone injury, short-term critical levels of ozone. Regarding heavy metals/nitrogen, results were presented on the temporal trends (1990-2001) of heavy metals in mosses across Europe, copper accumulation and defence mechanisms in a lichens growing on copper-rich rock and mosses as bioindicators of nitrogen deposition.

11. The meeting split into parallel sessions considering the ozone and heavy metals/nitrogen sub-programmes.

12. Ms G Mills (UK) opened the ozone session with an introduction to the aims of the 'post-Obergurgl' session in which developments since the Obergurgl Workshop were discussed and a procedure for updating the LRTAP Convention Mapping Manual was agreed. The chairs/rapporteurs of the three working groups in Obergurgl summarised the outcome of the ozone critical levels workshop: Mr PE Karlsson (Sweden) reported on forests, Mr M Ashmore (UK) on semi-natural vegetation and Mr H Pleijel (Sweden) on crops/application of flux-based models. Three presentations followed to streamline and aid further discussion on ozone critical levels. Mr D Simpson (EMEP/MSC-West) gave a presentation on 'European-scale modelling: implications for flux approaches' in which he clarified the needs of the various users of flux-based methods, which were also distributed on paper titled 'Suggestions for large-scale modeling and IAM requirements concerning AOT and/or fluxes'. It was followed by Ms L Emberson (UK) with a presentation on 'Evaluation of the DO_3SE model for crops and application at the European scale' and Mr H Pleijel (Sweden) reported on 'A simplified flux-based method for Integrated Assessment Modelling'.

13. The ozone session continued with discussions on possible changes to the ozone critical levels for vegetation as described in chapter 3 of the LRTAP Convention Mapping Manual and the timescale for implementation of these changes. A full description of the decisions made is provided in Annex I. In summary, it was agreed that for integrated assessment modelling, generic flux models would be developed for crops (one model applied to all of Europe) and trees (one model for Mediterranean Europe, one for other areas) and that changes would be made by drafting teams (see Annex I) and presented for incorporation in the Mapping Manual as an annex in time for the ICP Modelling and Mapping Task Force Meeting in April, 2006.

14. Mr I Gonzalez-Fernandez (Spain) described how genetic variation in stomatal conductance introduces uncertainty in Pan-European ozone risk assessment. Ms V Picchi reported on photosynthetic responses of two wheat varieties exposed to chronic ozone fumigation. Mr PE Karlsson (Sweden) presented results of an economic assessment of ozone impacts on vegetation in Sweden, followed by Mr M Holland (UK) who presented results of an economic assessment of crop losses from ozone exposure in Europe which included an analysis of uncertainty in the use of AOT40-based dose-response functions.

15. The ozone session continued with presentations and discussions on ozone biomonitoring experiments. Ms F Hayes (ICP Vegetation Coordination Centre, UK) gave an overview of the results of the ICP Vegetation biomonitoring experiments with white clover and brown knapweed, followed by a presentation from Mr J Fuhrer (Switzerland) on progress with the micropropagation technique of brown knapweed and plans for a pilot study in 2006. The session included a discussion on the requirements and plans for ozone biomonitoring experiments in 2006 and beyond. It was agreed that during the next two years, effort would be placed on collating evidence for effects of ozone on crops and semi-natural vegetation in the ECE region with the aim of producing a glossy report in 2008. This would involve a review of the literature, an in depth analysis of the results ICP Vegetation ambient air experiments and an assessment of evidence existing within national surveys and assessments that are not widely available. Participants from each of the five climatic regions agreed to assist in the collation of such data. These were: Mr B Gimeno or a colleague (West Mediterranean), Mr F Batic (East Mediterranean), Mr J Fuhrer (Continental Central Europe), Ms F Hayes (Atlantic Central Europe) and Mr H Pleijel and Mr P Karlsson (Northern Europe). An offer of assistance from the ICP Forests representative, Ms M Sanz, was gratefully received.

16. Ms G Mills (UK) informed the ozone group and subsequently the Task Force in plenary that regretfully, because of the extra time involved in preparing the evidence report, the ICP Vegetation Coordination Centre could no longer send plant material and equipment to participants that had a poor record in returning data. In future, pollutant and climate data could only be processed if received as a complete season-long file in the Excel format requested by the Coordination Centre, with filled gaps clearly identified within the file.

17. Mr E Steinnes (Norway) opened the heavy metals/nitrogen session with a presentation on ‘Long-range atmospheric transport of trace metals to Norway as evident from moss analysis and other studies’. Mr E Kubin (Finland) followed with a presentation on ‘The development of specimen banking and database for heavy metal surveys in Finland’ and Mr R Pesch (Germany) reported on ‘Classification and regression trees relating metal accumulation in mosses with site specific and regional land characteristics’.

18. Mr H Harmens (UK) gave an introduction to the data rules that currently apply to work conducted under the LRTAP Convention (see ECE/EB.AIR/42, annex V) and which are relevant to the ‘heavy metals in European mosses database’. Currently the data provided to the ICP Vegetation Coordination Centre by the participants are not made available for use outside the Convention without the approval of the relevant participant. However, as the objective of the effect-oriented activities is to supply the best available data to Parties for the negotiations of Protocols in a transparent way, the Working Group on Effects (WGE) is currently reviewing its data rules for discussion at the Extended Bureau of the WGE (1 - 3 March 2006, Geneva, Switzerland) and the 25th session of the WGE (30 August – 1 September 2006, Geneva, Switzerland). Mr H Harmens will seek further advice from the participants of the moss survey in preparation for the discussion on data rules at the 25th session of the WGE. During the discussion in Caernarfon mixed opinions were aired on enhancing the public availability of the moss data.

19. Ms M Frontasyeva (Russian Federation) informed the heavy metal session about the outcome of air pollution studies in Macedonia using the moss biomonitoring technique, NAA, AAS and GIS technology, followed by a presentation from Ms A-M Rusu (UK) on

'Investigating biogeochemical signatures in lichens and other environmental samples immediately following closure of a major polluting source in Romania'.

20. Mr H Harmens (UK) gave an update of the status of European moss survey 2005/2006, followed by a general discussion on data requirements and further processing. Currently 32 countries are participating in the heavy metals in mosses survey and more than half of the participating countries (17) have indicated that they will determine the nitrogen concentration in mosses as well. Mr E Steinnes (Norway) suggested to include antimony in the moss survey as a good indicator of heavy metal pollution from anthropogenic sources, which was accepted by the other participants. Data for the moss reference material (including nitrogen) will be further processed by Mr E Kubin (Finland) and Mr E Steinnes (Norway) for quality assurance purposes and countries were encouraged to participate in cross-border calibration exercises with neighbouring countries. Based on results of the interlaboratory calibration exercise with certified moss reference material, it was agreed that obvious outliers will be excluded from further data processing. The heavy metal sub-group suggested to include the 90th percentile of median values for each metal in the report of the 2005/6 moss survey and decided that only EMEP maps (50 km x 50 km grids) will be presented in the report and no 'dot maps'. Mr H Harmens will remind all participants via e-mail about the data requirements for the European moss survey 2005/6, as described in the moss monitoring manual (see http://icpvegetation.ceh.ac.uk/publications.htm).

21. Ms Z Jeran reported on 'Nitrogen concentrations and δ^{15}N in Hypnum cupressiforme collected in 2001 survey in Slovenia', followed by a detailed presentation from Mr H Harmens (UK) on the use of mosses as biomonitors of nitrogen pollution in Europe (see paragraph 6 for further details). Data presented as a poster by Mr J Santamaria (UK) indicated that short- and long-term temporal trends in the nitrogen concentration in mosses might be confounded by high spatial variability of the nitrogen concentration in mosses. The sub-group agreed on further developing the work on mosses as biomonitors of atmospheric nitrogen deposition.

22. Mr L de Temmerman (Belgium) reported on heavy metal deposition (arsenic, cadmium, lead and mercury) and the potential contamination of food crops and Mr H Harmens (UK) finished with a presentation on the temporal trends of heavy metals in mosses (1990 – 2001) in comparison with temporal trends in anthropogenic emissions and measured wet depositions for the metals cadmium, lead and mercury. In general, the lead concentration in mosses across Europe had decreased more with time than the cadmium concentration in mosses, in agreement with the emission and wet deposition trends reported by EMEP/MSC-East (see EMEP/MSC-East Technical Report 8/2005, available from http://www.msceast.org). For mercury not enough data were available for mosses to identify clear temporal trends. The group stressed and acknowledged the importance of the close collaboration with EMEP/MSC-East.

23. In the final plenary session Mr H Harmens (UK) gave a brief overview of the presentations and the outcome of discussions in the heavy metal/nitrogen sub-group (as described above), followed by brief summary from Ms G Mills (UK) on the conclusions and recommendations from the discussion in the ozone sub-group (as decribed above and in Annex I). The meeting took note of the conclusions and recommendations of the heavy metal/nitrogen and ozone sub-groups. The Task Force took note of the ICP Vegetation work plan and the agreed deliverables to the WGE for 2006 as described in document

EB.AIR/WG.1/2005/4/Rev. 1 (see Annex II). The meeting revised the objectives for the programme as indicated in Annex III. Mr M Johansson, secretary of the WGE, emphasized the need to streamline work plans and deliverables within the WGE and the LRTAP Convention. Therefore, the Task Force will consider in the future how it can translate its objectives into a medium-term work plan (3 years) with concrete deliverables to the Convention. It decided to present its current and following year's work plan in the format requested by the Convention.

24. Ms M Frontasyeva (Russian Federation) kindly offered to host the 20th ICP Vegetation Task Force Meeting in Dubna (Moscow Region), which was gratefully accepted by the Task Force. The meeting was provisionally scheduled for the middle of March 2007. Mr H Harmens (UK) closed the meeting by thanking his colleagues of the ICP Vegetation Coordination Centre for organising the meeting and their contributions to the programme, the UK Department for Environment, Food and Rural Affairs (Defra) and the Centre for Ecology and Hydrology (CEH) for financially supporting the meeting and the ICP Vegetation and last but not least the participants for their continuing support of the programme.

ANNEX I (of minutes)

19th Task Force Meeting of the ICP Vegetation
Ozone Flux Modelling Discussion (31 January, 2006)
Summary of Key Decisions

Needs of users – Large scale modelling using flux models for generic species

In response to the points raised by CIAM & EMEP, the group decided that for use by Integrated Assessment Modelling within the RAINS model of IIASA:

- Risk based on generic $AF_{st}Y$ models should be used for trees and crops (comparable to application of SOMO35 approach for health effects);
- AOTX-based critical levels should be used for semi-natural vegetation;
- A generic $AF_{st}3$ crop flux model will be developed using simplified wheat parameterisation, but having a longer accumulation period of three months;
- For trees, two $AF_{st}1.6$ generic models would be developed, one for deciduous species which would use the beech parameterisation as a starting point and one for evergreen species to be parameterised for application to the Mediterranean area. The details of the parameterisation for these models would be decided within the Forests sub-group;
- All three generic models would use latitude or temperature models for growing seasons and a long accumulation period;
- The text related to generic flux models and new semi-natural vegetation will be provided in an Annex to Chapter 3 of the Mapping Manual;
- Decisions on parameterisation of the generic flux models will be included in the Annex.

Needs of users – Local, national and regional scale

Default parameterisations of the full flux model for selected crop and forest species are currently included in the Mapping Manual within the critical levels text. It was decided that a note would be added to the Mapping Manual that local/national/regional parameterisations may be used for application at these geographical scales, but that the details of such parameterisations would not be included in the Mapping Manual in the proposed revision.

Guidance on appropriate EUNIS categories to use for the species/communities identified in the critical levels text will be added to the Mapping Manual.

Include parameterisation for individual species in ICP Vegetation Annual Report.

Procedure for making the annex to the Mapping Manual

It was agreed that the following groups would be responsible for the revision process:

Editorial group

- ☐ Liaise with ICP M&M, ICP Forests, EMEP, CIAM & WGE Secretariat
- ☐ Insert text changes
- ☐ Finalise text

Receptor-specific Advisory Groups

- ☐ Parameterisations for the generic species
- ☐ EUNIS land-use categories that can be used for each representative and generic species
- ☐ Suggest text changes for Mapping Manual

	Lead person	**Members**
Editorial Group	Gina Mills	Per Erik Karlsson, Hakan Pleijel, Mike Ashmore.
Forest tree advisory group	Per Erik Karlsson	Ben Gimeno; Maria Sanz; Gaby Deckmyn; Lisa Emberson; Marcus Schaub; Sabine Braun; Gerhard Wieser; Marco Ferretti; Håkan Pleijel; Mark Broadmeadow.
Crop advisory group	Håkan Pleijel	David Simpson, Lisa Emberson, Ben Gimeno or colleague.
Semi-nat. veg. advisory group	Mike Ashmore	Jürg Fuhrer, Gina Mills.

Changes in Mapping Manual to be circulated to all participants for comments.

Timescale for implementing changes to Chapter 3

2006	Deliverables
Discussed at ICP Vegetation TFM	Workshop report, summarising key decisions.
Confirmation of ICP Modelling and Mapping TFM (5 -7 April)	New annex for Mapping Manual chapter 3. Sub-groups to agree changes by end of Feb, and circulate for comment within relevant ICPs. Circulation of draft Mapping Manual annex in mid-March for ICP M&M delegates.
Presented at ICP Forests TFM (22-24 May)	Meeting acknowledged unfortunate timing and agreed that forest sub-group decisions would be circulated to ICP Forests members for comment at all stages in the revision process.
Noted by Working Group on Effects and recommended for use (30 Aug – 1 Sep)	Report.
Noted by Executive Body (11-15 Dec)	

ANNEX II of minutes: Work plan of the ICP Vegetation for 2006

(EB.AIR/WG.1/2005/4/Rev. 1)

Items common to all ICPs	Ozone	Heavy Metals	Nutrient N
• Report on support of effects-based approaches for the review and possible revision of the Convention protocols (to be defined by the Working Group on Strategies and Review); • Summary report of current information on dose-response functions and stock at risk; • Review report of links between field observations and critical loads.	• Report on the extent of ozone damage to ozone-sensitive species of crops and (semi-) natural vegetation in 2005 using standardized experiments; • Refined maps of exceedances of critical ozone levels, based on the new critical levels of ozone (with EMEP/MSC-West); • Report on the impacts of ozone on vegetation in a changing climate; • Interim report on risk assessment and mapping procedures for communities of (semi-) natural vegetation at risk from ozone; • Proceedings of the workshop: "Critical levels of ozone: further applying and developing the flux-based concept".	• Report on the temporal trends in the European heavy metals in mosses database.	• Report on the long-term (about 100 years) temporal trends in the nitrogen concentrations in mosses using herbarium material; • Report on the interactive impacts of ozone and nitrogen on crops and (semi-) natural vegetation;

In addition:

- Nineteenth meeting of the Programme Task Force to be held in Caernarfon, United Kingdom, from 30 January to 2 February 2006.

ANNEX III of minutes: Objectives of the ICP Vegetation

(Updated 1-2-06)

Long-term objectives

1. To meet the requirements of the UNECE Convention on Long-range Transboundary Air Pollution for information on the responses of (semi-) natural vegetation and crops to atmospheric pollutants.

2. To evaluate data on the responses of (semi-)natural vegetation and crops to air pollutants to validate the critical levels and methods defined in the mapping manual and to show the effects of exceedance.

3. To provide information for the further development of effects-driven protocols with respect to (semi-)natural vegetation and crops.

Short- and medium-term objectives

1. To validate maps of exceedance and risk by monitoring the impacts of ambient ozone on various crops and (semi-)natural vegetation.

2. To produce a state of knowledge report on evidence of impacts of ambient ozone in the ECE region.

3. To further develop and apply the concept of concentration-based and flux-based methods and critical levels of ozone for crops, (semi-)natural vegetation and trees.

4. To produce maps of exceedance of the revised ozone critical levels and risk (in collaboration with ICP Forests, EMEP/MSC-West and the ICP on Modelling and Mapping).

5. To provide further information on response functions and land cover for use in an economic assessment of crop losses due to ozone.

6. To conduct literature reviews and specific experiments to provide further information on the critical levels for, and risk of damage by, air pollutants for selected plants, plant communities and biodiversity.

7. To conduct literature reviews and experiments on the accumulation of atmospheric deposition of heavy metals by vegetation and the transfer of heavy metals into the human food chain (in collaboration with TF Health).

8. To conduct the 2005/6 survey of heavy metal and nitrogen concentrations in mosses in Europe.

9. To investigate methods for estimating and mapping heavy metal deposition from the heavy metal concentration in mosses data (in collaboration with EMEP/MSC-East).

10. To study the spatial and temporal trends in the atmospheric deposition of nitrogen by determining the nitrogen concentration in mosses.

11. To review the literature on, and conduct studies of, the interactions between ozone and nitrogen.

12. To consider the possibility of including within the programme experimental and modelling work on the effects of ozone on vegetation in a changing climate.

13. To consider the feasibility of including nutrient nitrogen effects on (semi-) natural vegetation within the programme of work.

14. To collaborate on air pollution effects research outside the UNECE region (e.g. Asia and southern Africa).