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Air Pollution and Vegetation

UNECE ICP Vegetation

Annual Report 2003/2004



Working Group on Effects of the UNECE Convention on Long-range Transboundary Air Pollution



Air Pollution and Vegetation

UNECE ICP Vegetation^{*} Annual Report 2003/2004

Harry Harmens, Gina Mills, Felicity Hayes, Philip Williams and the participants of the ICP Vegetation

ICP Vegetation Coordination Centre Centre for Ecology and Hydrology Bangor, Orton Building Deiniol Road, Bangor, Gwynedd, LL57 2UP, UK Tel: + 44 (0) 1248 370045, Fax: + 44 (0) 1248 355365, Email: <u>hh@ceh.ac.uk</u>

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Executive Summary

Background

The UNECE ICP Vegetation¹ has studied the impacts of air pollutants on crops and (semi-) natural vegetation in the ECE region for more than a decade. In recent 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. Two further pollution problems are recently being considered by the programme: plant responses to pollutant mixtures and the impacts of nitrogen pollutants on vegetation. The results of studies conducted by ICP Vegetation are reported to the Working Group on Effects (WGE) of the Convention on Long-Range Transboundary Air Pollution (LRTAP), 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 proposed revision 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. Thirty two parties to the LRTAP Convention participate in the programme. The 17th Task Force meeting of the Programme was held in Kalamata, Greece, February 2004, and was attended by 50 participants.

Biomonitoring of ozone impacts on vegetation

The year 2003 had generally a hot, dry summer across Europe and ozone concentrations at the ICP Vegetation biomonitoring sites were higher than in the previous, wetter year. The $AOT40^2$ for a three-month period ranged from 0.8 ppm h in Östad (Sweden) to 23.7 ppm h in Cadenazzo (Switzerland). The long-term critical level for (semi-) natural vegetation and agricultural crops for yield reduction, i.e. a three-month AOT40 of 3 ppm h, was exceeded at 80% of the sites and a three month AOT40 of 10 ppm h was exceeded at 60% of the sites.

Ozone-sensitive biotypes of white clover (*Trifolium repens* cv Regal) were used at 23 sites to monitor the frequency of occurrence of leaf injury caused by ozone episodes. At some sites visible injury due to ozone was observed almost every week during the main exposure period, whereas at other sites visible injury was observed intermittently throughout the growing season, reflecting the more episodic nature of ambient ozone at these sites. Visible injury was also recorded at sites where the critical level of ozone for yield reduction was not exceeded, including Östad (Sweden), Ascot and Bangor (UK) and Carlow (Ireland). Such data will be used to further validate the newly-defined short-term critical level for visible injury for crops.

In addition to leaf injury assessments, mainly 'high-ozone sites' determined the relationship between the biomass ratio of sensitive (NC-S) to resistant (NC-R) biotypes of white clover. The decrease in biomass ratio with increasing ozone exposure from the 2003 data fits the same trend as data from 1996 to 2002 and increased confidence in the dose-response relationship.

¹ The United Nations Economic Commission for Europe International Cooperative programme on Effects of Air Pollution on Natural Vegetation and Crops

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

An overview of the results from the clover biomonitoring experiments (1996 to 2003) has shown that the experiment has been successful in establishing that ambient ozone can cause both visible injury and biomass reductions in a sensitive species, and that these types of damage are both widespread across Europe and present in most years. Three modelling methods have been used to develop flux-effect relationships: multivariate statistics, artificial neural networks and multiplicative algorithms. Each has shown that many input factors are needed to describe ozone flux including climatic conditions, phenological factors and ozone. After conversion of ozone concentration to canopy height, the r^2 value for the three month dose-response relationship showed little difference between AOT40- and AF_{st}5³-based response functions ($r^2 = 0.58$ and 0.55 respectively). Critical levels for biomass reduction have been established using the response functions as an AOT40 of 2.2 ppm h or an AF_{st}5 of 1.7 mmol m⁻² PLA, both accumulated over three months. First efforts to apply the AOT40 response function to a risk assessment for Europe have indicated that biomass reductions potentially as high as 20% could be experienced in central and southern Europe. At most of the example sites investigated, AF_{st}5 appeared to be better correlated with biomass reduction than AOT40.

A biomonitoring programme with *Centaurea jacea* (brown knapweed) has been developed by the ICP Vegetation to evaluate the relationship between ozone exposure and effects on the performance and injury symptoms of native plant species. Across Europe ozone-specific leaf injury was observed on the *Centaurea jacea* plants at eight out of the 12 participating sites. At five of the sites, the modifying effects of nitrogen pollution on the impacts of ozone pollution on Centaurea jacea were also investigated. Unfortunately, not enough sites, particularly those with high ambient ozone conditions, participated in this pilot study to draw any definite conclusions. So far, it is unclear whether increasing nitrogen pollution decreased sensitivity of *Centaurea jacea* to ozone or had no effect. The protocol for exposure and assessment of *Centaurea jacea* to ozone in 2004 has been revised to take into account the experiences of the participants in 2003.

Revision of the critical levels for ozone

During the last year, the ICP Vegetation finalised the revision of Chapter 3 ("Mapping Critical Levels for Vegetation") of the UNECE Mapping Manual. The chapter provides an in depth description of the critical levels, their scientific bases and how to calculate exceedance. Two approaches for critical levels have been included: concentration-based critical levels and flux-based critical levels. Both approaches incorporate the concept that the effects of ozone are cumulative and that concentrations or fluxes should be accumulated over a period of time, that only concentrations or fluxes above a specified threshold are summed, and that ozone is measured or calculated for the top of the canopy. Concentration-based critical levels have been derived for effects on yield for agricultural and horticultural crops, and for biomass effects for (semi-) natural vegetation and forest trees. Flux-based critical levels have been derived for effects on the yield of wheat and potato, and provisionally for the biomass of sensitive tree species. A vapour pressure deficit-modified AOT30 has been defined for crops as a short-term critical level for the development of visible leaf injury. The flux-based critical levels take into account the varying influences of temperature, water vapour pressure deficit, light, soil water potential, ozone concentration and plant development on the stomatal flux of ozone and therefore provide a more realistic estimate of the critical amount of ozone entering through the stomata and reaching the sites of action inside the plant.

 $^{^{3}}$ Accumulated stomatal flux of ozone above a flux threshold of 5 nmol m⁻² PLA s⁻¹, where PLA is the projected leaf area

Heavy Metal Deposition to Vegetation

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. The results from the latest survey in 2000/01 were published in April, 2003. Since then, data have been received from Iceland and included in the ICP Vegetation database and the EMEP 50 x 50 km grid maps. A study on factors influencing the heavy metal concentration in mosses highlighted the strong correlation between the concentration of Cu and As, Cd and Pb, Cd and Zn, Cr and Ni and especially Fe and V. Both multivariate regression analysis and artificial neural networks indicated that there is a weak correlation between the heavy metal concentration in mosses and climatic parameters, analytical techniques and moss species. Preparations have started for the next European heavy metals in mosses survey, planned to start in 2005.

Future work

The ICP Vegetation will continue the ozone biomonitoring programme with white clover. The formulation and parameterisation of the stomatal conductance model for white clover will be further improved and data will be collected to help to scale up stomatal conductance models from single leaves to the whole canopy. The aim is to produce a canopy flux-effect model for clover. In addition, work will focus on the validation of flux-based critical levels for additional agricultural crop and tree species by reviewing the literature on effects of ozone on crops and tree species together with data on stomatal conductance-environmental parameter relationships for these species. Biomonitoring of the impact of ozone on (semi-) natural vegetation, using *Centaurea jacea* as a model species, will continue with the ultimate aim of completing a flux-effect model for this species for use in predicting impacts across Europe. The revised critical levels for ozone will be used to develop critical level exceedance maps, and further analysis of the uncertainties in concentration-based and flux-based methods will be performed. The economic impacts of exceedance on yield of potato and wheat in Europe will be established for concentration-based and flux-based critical level approaches for current and predicted future ozone scenarios.

For heavy metals, preparations for the European moss survey 2005 are already in progress. Analysis of the temporal trends in the concentration of heavy metals in mosses will be undertaken and these trends will be compared with trends in EMEP heavy metal deposition data. In addition, the concentration of heavy metals in mosses in the 2000/2001 survey will be compared with EMEP heavy metal deposition data for validation purposes. As in 2000 and 2002, coordinated experiments will be conducted again in 2004 to determine the atmospheric deposition of heavy metals to white clover, using an improved experimental protocol.

The ICP Vegetation will continue to investigate the modifying influence of nitrogen deposition on the impacts of ozone on vegetation. Spatial and temporal trends in the nitrogen concentration of mosses will be investigated as a first attempt to include nutrient nitrogen within the remit of the ICP Vegetation.

The further work described will thus allow the ICP Vegetation to continue to meet the requirements specified in the Medium-Term Workplan for the UNECE Working Group on Effects.

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1. Introduction

The ICP Vegetation

The UNECE ICP Vegetation¹ is an international programme that reports to the Working Group on Effects (WGE) of the Convention on Long-range Transboundary Air Pollution (LRTAP) on the effects of air pollutants on natural vegetation and crops. The WGE considers the effects of air pollutants on waters, materials, forests, vegetation, ecosystems, and health in Europe and North America. In recent years, the ICP Vegetation has focussed on two air pollution problems of particular importance in the ECE region: quantifying the risks to vegetation posed by ozone pollution and the atmospheric deposition of heavy metals to vegetation. Two further pollution problems are recently being considered by the programme: plant responses to pollutant mixtures and the impacts of nitrogen pollutants on vegetation. Thus, the work of the ICP Vegetation currently aims to provide information for future revision 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 150 scientists from 32 countries of Europe and North America contribute to the programme by conducting experiments, sampling vegetation or modelling pollutant deposition and effects. The ICP Vegetation is chaired by Dr Harry Harmens at the Coordination Centre at the Centre for Ecology and Hydrology, Bangor, UK, and the coordination is supported by the UK Department for Environment, Food and Rural Affairs (contract EPG 1/3/205).

The ICP Vegetation:

- Conducts coordinated experiments to determine the effects of ozone pollution on crops and (semi-) natural vegetation in Europe and North America.
- Develops computer models to quantify and interpret the influence of climatic conditions and environmental stresses on the responses of plants to ozone, and uses the models to establish critical levels for effects of pollutants.
- Develops maps showing where vegetation is at risk from ozone pollution within the ECE region, including areas where critical levels are exceeded.
- Assesses the economic losses caused by the effects of ozone on crops.
- Collates and reviews information on the effects of ozone on plant biodiversity.
- Collates and reviews monitoring data on the atmospheric deposition of heavy metals, and subsequent accumulation by mosses and higher plants.
- Considers the evidence for effects of nitrogen deposition on communities of (semi-) natural vegetation in Europe, including its modifying effect on the impacts of ozone.

¹ The United Nations Economic Commission for Europe International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops.

The specific objectives of the ICP Vegetation are presented in Annex I

Impacts of ozone on crops and (semi-) natural vegetation

As part of the work programme for the ICP Vegetation, information is collected on the effects of ambient ozone episodes on crops and example species of (semi-) natural vegetation in Europe and the USA by conducting biomonitoring experiments, and by assessing information in the scientific literature. Ozone episodes can cause short-term responses in plants such as the development of visible injury (small flecks on the upper surface of leaves) or reductions in photosynthesis. If episodes are frequent, longer-term responses such as reductions in growth and yield can occur.

Throughout the years, participants in the ICP Vegetation carried out systematic surveys of commercial crops for characteristic visible symptoms of ozone damage, on days following visible injury in the clover bioindicator system. Ozone injury was detected on the foliage of over 20 agricultural and horticultural crops including on crops such as lettuce, chicory, parsley and spinach for which such foliar damage results in loss in commercial value. Although ozone injury was mainly found in Mediterranean countries (Fumigalli *et al.*, 2001), leaf injury was also reported in Belgium, France and Switzerland. These surveys also indicated the importance of adequate soil moisture in determining the magnitude of ozone injury. For example, in Greece a chicory crop was severely injured by ozone when irrigated, but uninjured where irrigation was not in use. The documentation of the extent of visible injury due to ozone, both in field surveys and in the biomonitoring studies, provides important evidence for the significance of ozone as a phytotoxic pollutant across Europe.

The negotiations concerning ozone for the Gothenburg Protocol (1999) were based on exceedance of a then so-called level I long-term critical level of ozone for crops and (semi-) natural vegetation. This value, an $AOT40^2$ of 3 ppm h accumulated over three months was set at the Kuopio Workshop in 1996 (Kärenlampi and Skärby, 1996) and was considered to be the lowest AOT40 at which significant yield loss due to ozone could be detected, according to current knowledge (UNECE, 1996). However, several important limitations and uncertainties have been recognised for using the concentration-based approach. The real impacts of ozone depend on the amount of ozone reaching the sites of damage within the leaf, whereas AOTX-based critical levels only consider the ozone concentration at the top of the canopy. The Gerzensee Workshop in 1999 (Fuhrer and Achermann, 1999) recognised the importance of developing an alternative critical level approach based on the flux of ozone from the exterior of the leaf through the stomatal pores to the sites of damage (stomatal flux). This so-called level II approach required the development of mathematical models to estimate stomatal flux, primarily from knowledge of stomatal responses to environmental factors. In recent years, the terminology has changed such that 'level I' and 'level II' are no longer used and have been replaced by 'concentration-based' and 'flux-based', respectively (UNECE, 2004a).

In the first step towards an ozone-flux based critical level, Lisa Emberson and colleagues developed a multiplicative model of stomatal conductance of ozone (Emberson *et al.*, 2000a). This model includes functions for the effects of phenology, light, temperature, vapour pressure deficit (VPD) and soil water potential on the stomatal conductance of several species including wheat and potato, beech and birch. At the Gothenburg Workshop in 2002 (Karlsson

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

et al., 2003), it was concluded that for the time being it was only possible to derive flux-based ozone critical levels for the crops of wheat and potato. Since the Gothenburg Workshop, the parameterisation of the conductance model presented in Pleijel et al. (2002, 2003) and Danielsson et al. (2003) has been revised to achieve full compatibility with the EMEP model calibration (Emberson et al., 2000b). The new flux-based critical levels for crops are included in the revised Mapping Manual (UNECE, 2004a). Also included are provisional flux-based critical levels for the tree species, birch and beech. A vapour pressure deficit-modified concentration-based critical level was developed and included in the new Mapping Manual to define the short-term critical level for the development of visible injury on crops. New data collated since the Kuopio Workshop support the concentration-based critical level of 3 ppm h for agricultural crops. In addition, a separate concentration-based critical level was developed for horticultural crops, i.e. AOT40 of 6 ppm h, based on a growth period of 3.5 months. The critical level for horticultural crops has been derived from a dose-response function developed from a comprehensive data set of 14 tomato cultivars (UNECE, 2004a). Based on the analysis of the current experimental database, the concentration-based critical level of ozone for trees has been reduced from an AOT40 value of 10 ppm h (Kärenlampi and Skärby, 1996) to 5 ppm h. Further details on the new critical levels for ozone can be found in Chapter 2 of this report.

By conducting experiments in ambient air, the ICP Vegetation has established a unique database for developing the flux-based approach to critical levels. Since 1996, ozone-sensitive (NC-S) and ozone-resistant (NC-R) biotypes of white clover (*Trifolium repens* cv Regal) have been grown at each of the ICP Vegetation sites according to a standardised experimental protocol. Effects of ozone are recorded as a score for visible injury, and as the ratio of the weight of the dried clippings (biomass) of the NC-S to the NC-R biotype. The clover biotype system was chosen because the forage biomass for both biotypes was similar in conditions of low ozone, but lower for the NC-S biotype at high ozone concentrations (12h mean > 40-50 ppb, Heagle *et al.*, 1995). By exposing plants to ambient air, the reaction to ozone episodes could be considered without any confounding influence of a chamber on the flux of ozone to the plant. Trends in the impacts of ozone on clover between 1996 and 2003 are described in chapter 3 of this report.

In recent years, interest in the effects of ozone on (semi-) natural vegetation has increased considerably. Setting critical levels for this type of vegetation is far more complicated than for crops because of the diversity of species and ecosystems within the ECE region. In contrast to crops and trees, only limited experimental data are available for a small proportion of the vast range of species. Recently, ICP Vegetation has developed a new ozone biomonitoring system using *Centaurea jacea* (brown knapweed), which is currently being tested at several sites across Europe. Several attempts have been made to identify plant characteristics associated with ozone sensitivity in species of natural vegetation. Differences in ozone sensitivity between and within species could be related to differences in leaf conductance, with highest sensitivity found in species, ecotypes or clones with the highest leaf conductance (Nebel and Fuhrer, 1994; Reich, 1987; Becker *et al.*,1989). However, this distinction is by no means exhaustive and further work is needed to identify other plant characteristics associated with sensitivity to ozone, hence the need for more complex analysis and modelling. The ICP Vegetation has taken on the role of collating international databases in preparation for this type of modelling.

For (semi-) natural vegetation the provisional critical levels of AOT40s of 3 ppm h accumulated over three months and 7 ppm h accumulated over six months, set for annuals

and perennials respectively at the Gerzensee Workshop (Fuhrer and Achermann, 1999), were revised at the Gothenburg Workshop (Fuhrer *et al.*, 2003). New data, in particular from the European Framework Five BIOSTRESS programme (reviewed by Fuhrer *et al.*, 2003), meant that the time window of six months for perennial species was no longer supported. The time window for both annuals and perennials is now set to three months and the critical level of AOT40 of 3 ppm h is sufficient to protect the most sensitive annual and short-lived perennial species when grown in a competitive environment (UNECE, 2004a). Further study of factors influencing the stomatal uptake of ozone is required before a flux-based critical level for ozone can be established for (semi-) natural vegetation. Data from the ICP Vegetation database are used to identify species 'at risk' from ozone damage and the communities they represent and mapping procedures are being developed indicating where such species might be at risk from ozone.

Heavy metal deposition to vegetation

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 and 2002 ozone experiments have been analysed for lead, cadmium, arsenic and nickel content. Comparison of the heavy metal content 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. The experimental protocol for determining heavy metal deposition to white clover has been improved during 2003 and new data will be collated during 2004. 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 content at five-year intervals; it included almost 7,000 samples of mosses taken from 29 European countries in the 2000/2001 survey (Buse *et al.*, 2003b). ICP Vegetation is currently preparing for the next European heavy metals in mosses survey, to start in 2005.

Impacts of nitrogen deposition on (semi-) natural vegetation

The ICP Vegetation agreed at its 14th Task Force Meeting (January 2001) to include consideration of the impacts of atmospheric nitrogen deposition on (semi-) natural vegetation within its programme of work. This stemmed from concern over the impact of nitrogen on low nutrient ecosystems such as heathlands, moorlands, blanket bogs and (semi-) natural grassland. The empirical critical loads for nitrogen for different ecosystems were reviewed and revised at the UNECE Workshop on 'Empirical Critical Loads for Nitrogen Deposition on (Semi-) Natural Ecosystems', Bern, Switzerland, 11-13 November 2002. Impacts of nitrogen, and the interaction with ozone, now forms part of the work programme of the ICP Vegetation. At selected sites across Europe *Centaurea jacea*, the new ozone biomonitoring system, is being exposed to different levels of nitrogen pollution to determine the interactive effects of nitrogen and ozone pollution on (semi-) natural vegetation. In addition, moss

samples from the heavy metals in mosses survey have been collected from selected European countries for selected years for nitrogen analysis in order to determine trends in nitrogen deposition between 1977/1980 and 2000/2001. ICP Vegetation is currently investigating the feasibility of collecting moss samples from national herbaria for nitrogen analysis for establishing long-term trends in atmospheric nitrogen deposition in Europe.

Participation in the ICP Vegetation

With the inclusion of the heavy metals and mosses project within the work programme of the ICP Vegetation, the participation has increased to 32 Parties to the Convention (Table 1.1). The contact details of the participants are included in Annex 2. 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.

Austria	Lithuania
Belgium	The Netherlands
Bulgaria	Norway
The Czech Republic	Poland
Denmark	Portugal
Estonia	Romania
Faroe Islands	Russian Federation
Finland	Serbia and Montenegro
France	Slovakia
Germany	Slovenia
Greece	Spain
Hungary	Sweden
Iceland	Switzerland
Ireland	United Kingdom
Italy	Ukraine
Latvia	USA

Table 1.1 Countries participating in the ICP Vegetation

Web site

The ICP Vegetation web site can be found at icpvegetation.ceh.ac.uk

Aims of this report

It is the intention of this report to provide an overview of the main activities of the ICP Vegetation in 2003/2004 (chapter 2) and report in more detail about the analysis of clover biomonitoring data from 1996 to 2003 (chapter 3). Conclusions and future work are reported in chapter 4. Progress with contributions to the proposed deliverables to the WGE for the ICP Vegetation are also described in chapter 4.

2. Overview of activities in 2003/4

Biomonitoring of ozone impacts on vegetation

The ICP Vegetation collates information on the effects of ambient ozone episodes on crops and (semi-) natural vegetation in Europe and the USA by conducting biomonitoring experiments, and by assessing information in the scientific literature. Since 1996, participants in the ICP Vegetation have detected effects of ambient ozone at sites across Europe and in the USA by growing ozone-sensitive (NC-S) and ozone-resistant (NC-R) biotypes of white clover (*Trifolium repens* cv Regal), and recording the occurrence of leaf injury and biomass differences between the two biotypes. Since 2002, the ozone biomonitoring programme has been extended to include pilot studies with brown knapweed (*Centaurea jacea*) as a representative of (semi-) natural vegetation. The response of the biomonitoring species at individual sites is compared with pollutant and climatic conditions during the experiment. The data from the 2003 experimental season of the ICP Vegetation has been added to the existing database. The ambient air experiments were the core activity for participants in 2003, and were conducted at 25 sites: 13 sites using white clover only, 2 sites using brown knapweed only and 10 sites using both species.

This section summarises the results from the 2003 biomonitoring experiments with clover and brown knapweed. A more detailed anlysis of the clover biomonitoring data from 1996 - 2003 is presented in chapter 3.

The clover biomonitoring experiment in 2003

Cuttings of ozone-sensitive (NC-S) and ozone-resistant (NC-R) biotypes of white clover (*Trifolium repens* cv Regal) were distributed by the Coordination Centre to participants of the programme. A standard protocol developed at the Coordination Centre was followed for establishment and subsequent exposure of the plants (UNECE, 2003). Individual plants were placed in individual 30 litre pots, which had an integral wick system for watering, and maintained at a field site away from local pollution sources and major roads. Plants were inspected at least weekly for ozone injury on leaves. At 28 day intervals the foliage was cut down to 7 cm above the soil surface, then dried and weighed to determine biomass. The plants were allowed to re-grow before a further harvest 28 days later. The period between the first and fourth harvest at each site equated to the three-month time period for calculation of AOT40 and other three-month based parameters. The ratio of the biomass of the NC-S biotype to that of the NC-R biotype indicated the extent of ozone damage at each site, with ratios of less than 1 showing that ozone was having a negative effect on the sensitive biotype.

At many of the sites in 2003, a second batch of NC-S clover was grown, using an identical protocol but 14 days later than the first batch. This ensured that there was always a full canopy of leaves on some clover plants at each site and allowed a more complete assessment of the development of visible injury at each site. Participants monitored the extent of visible leaf injury on the sensitive biotype of white clover frequently (generally on a weekly basis).

A wide range of climatic and pollution conditions are found over the network of biomonitoring sites in the ICP Vegetation. The range of sites in Europe extends from Sweden to Spain and covers both urban and rural locations. In the summer of 2003, participants conducted biomonitoring experiments at 25 sites. The data from each experimental site were

sent to the Coordination Centre for analysis. Data comprised measurements of biomass from four to five 28-day harvests, stomatal conductance measurements, assessments of plant health and weekly assessments of visible injury. Hourly means of climatic and pollution data including temperature, humidity, solar radiation, windspeed, ozone, NOx and SO_2 for a four to five-month period were also sent to the Coordination Centre for analysis.

Pollutant and climatic conditions in 2003

The year 2003 had generally a hot, dry summer across Europe and ozone concentrations were higher than in the previous year (Table 2.1, Figure 2.1), which was one of the wettest for many years in central and southern Europe (Buse *et al.*, 2003a). The mean daily maximum ozone in 2003 ranged from 38.1 ppb in UK-Ascot to 82.8 in Switzerland and the AOT40 for the three-month period ranged from 0.79 ppm h in Sweden-Östad to 23.72 ppm h in Switzerland-Cadenazzo. The long-term critical level for (semi-) natural vegetation and agricultural crops (a three month AOT40 of 3 ppm h) was exceeded at 80% of the sites where ozone was continuously monitored; data for selected sites are presented in Figure 2.2. An AOT40 of 10 ppm h was exceeded at 60% of the sites.

	Maaa	Ozone		Tempera	ature (°C)	Rai	nfall	VPD	(k Pa)
Site	Mean daily max (ppb)	24 hr mean (ppb)	AOT 40/ ppm h	Mean	Dayligh t mean	Total (mm)	No. of Days	24 hr mean	Dayligh t mean
Austria - Seibersdorf	(65.8)	(42.2)	(9.84)	21.3	24.0	-	-	1.31	1.84
Belgium - Tervuren	57.1	30.3	8.70	18.4	20.8	169	25	0.99	1.36
Germany - Trier Univ.	62.9	41.0	12.96	(20.8)	(23.8)	151	26	(1.27)	(1.77)
Greece - Kalamata	65.3	41.4	14.37	-	-	-	-	-	-
Ireland - Carlow	38.8	26.8	1.76	15.5	17.3	114	40	0.38	0.58
Italy - Naples	68.8	38.9	13.91	27.1	29.9	(12)	(3)	1.44	1.97
Italy - Rome	77.0	41.9	15.65	29.4	31.3	-	-	2.55	3.20
Spain - Ebro Delta	48.5	30.2	3.04	25.9	27.4	-	-	0.46	0.69
Sweden - Östad	38.2	22.5	0.79	15.8	18.4	-	-	0.40	0.62
Switzerland - Cadenazzo	82.8	46.4	23.72	21.9	24.4	261	30	1.07	1.51
UK - Ascot	38.1	18.8	1.63	15.8	18.6	83	22	-	-
UK - Bangor	40.9	29.1	2.90	(16.5)	(18.4)	(281)	(28)	-	-

Table 2.1Climatic and pollution conditions over the three-month experimental period at
selected ICP Vegetation biomonitoring sites in 2003. Within brackets = data
available for two months only, - = data unavailable



Figure 2.1 A comparison of the mean daily maximum ozone concentration at ICP Vegetation biomonitoring sites in the 2002 and 2003 experimental seasons. The data is presented as a histogram of the percentage of 28 days harvest intervals (HI) that fall within each 10 ppb ozone concentration interval



Figure 2.2 Three month AOT40 (ppm h) at selected ICP Vegetation sites in 2003

Effects of ambient ozone on white clover

A major component of the biomonitoring work with Trifolium repens in 2003 was to monitor the incidences of visible leaf injury (fine bronze or pale yellow specks on leaf surfaces) for the ozone-sensitive biotype 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. The range of injury scores and those recorded at each harvest for selected sites is presented in Figure 2.3. Visible injury due to ozone was observed almost every week during the main exposure period at sites including Rome (Italy) (Figure 2.4a), Ljubljana (Slovenia) and Östad (Sweden). At other sites, e.g Tervuren (Belgium) (Figure 2.4b), visible injury was observed intermittently throughout the growing season, reflecting the more episodic nature of ambient ozone at that site. Visible injury was also recorded at sites where the critical level of ozone for yield reduction was not exceeded, including Östad (Sweden), Ascot and Bangor (UK) and Carlow (Ireland). Other sites showed less injury than expected; e.g. Ljubljana (Slovenia) had a 3-month AOT40 exceeding 17 ppm h, but had less leaf injury than Ebro Delta (Spain) with a 3-month AOT40 of 3 ppm h (Figures 2.2 and 2.3). The visible injury data will be used in conjunction with the associated climate and pollution data and developed stomatal conductance models to validate the newly accepted VPDmodified AOT40 short-term critical level for visible injury development (UNECE, 2004a) and with a view to developing a flux-based short-term critical level.



Figure 2.3 The extent of visible injury due to ozone on the sensitive biotype of *Trifolium* repens at harvests 1 to 4 at a range of sites across Europe. Leaf injury scores: 1 = <1%, 2 = 1%-5%, 3 = 5%-25%, 4 = 25%-50%, 5 = 50%-90%, 6 = 90%-100% of leaves affected. * = data unavailable for harvest 4; otherwise a score of 0 indicates no leaf injury.

In addition to leaf injury assessments, mainly 'high-ozone sites' determined the relationship between the biomass ratio of sensitive (NC-S) to resistant (NC-R) biotypes of white clover. The decrease in biomass ratio with increasing ozone exposure from the 2003 data fits the same trend as data from 1996 to 2002 (Figure 2.5) and increased confidence in the

relationship as the new points are from the higher AOT40 region of the curve. The r^2 of the trendline increased from 0.43 to 0.54; there was a 5% reduction in biomass for each 2 ppm h increase in the three month AOT40.



Figure 2.4 Weekly assessments of visible injury on *Trifolium repens* at (a) Italy-Rome and (b) Belgium-Tervuren. The injury score is as described in Figure 2.3



Figure 2.5 Response of the NC-S/NC-R biomass ratio of white clover to AOT40 over three months

Effects of ambient ozone on (semi-) natural vegetation

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 (Buse *et al.*, 2003a). Last summer, *Centaurea jacea* was 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 the 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 (UNECE, 2003) provided by the participants from Switzerland. The plants were transferred to the field site 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.

Twelve sites participated in the *Centaurea* experiment in 2003, representing all regions of Europe. The ozone exposure to the *Centaurea jacea* plants at these participating sites is shown in Table 2.2 and ranged from an AOT40 of 0.3 ppm h (Finland-Jokioinen) to 33.9 ppm h (Switzerland-Cadenazzo). 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 pilot study were exposed to ambient air for the same length of time and this consequently affects the AOT40.

Ozone-specific injury was observed on the *Centaurea jacea* plants at seven participating sites. These were Austria-Seibersdorf, Germany-Hohenheim, Greece-Kalamata, Spain-Ebro Delta, Sweden-Östad, Switzerland-Cadenazzo and UK-Ascot. In Finland-Jokioinen visible injury to the plants was observed, but this was thought to be non-specific injury. No injury was recorded on the plants in Germany-Trier, Ireland-Carlow, or UK-Bangor. The protocol for exposure and assessment of *Centaurea jacea* to ozone in 2004 has been revised to take into account the experiences of the participants in 2003 (UNECE, 2004b).

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 - Ebro Delta	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

Table 2.2Ozone exposure of *Centaurea jacea* at sites across Europe

Interaction between ozone and nitrogen pollution on (semi-) natural vegetation

In a pilot study, five of the European sites participating in the ozone biomonitoring study with *Centaurea jacea* exposed the plants to three levels of nitrogen: control (no added N), moderate (30 kg ha⁻¹ yr⁻¹) and high (80 kg ha⁻¹ yr⁻¹) at fortnightly intervals during the course of an eight week exposure to ambient air. The nitrogen was added in the form of NH_4NO_3 and all plants also received non-N nutrients in the form of Hoagland solution to make sure that no other nutrient was limiting plant growth. Visible injury was recorded over the period of exposure to ambient air at some of the participating sites. At the end of the exposure, the plants were harvested and the above-ground biomass was recorded.

It is possible that the extent of ozone-specific visible injury on *Centaurea jacea* plants was decreased with increased nitrogen fertilisation, however, not enough sites, particularly those with high ambient ozone conditions, participated in this aspect of the study to draw definite conclusions. All five sites recorded biomass at the end of the exposure and none of the sites observed an increased sensitivity to ozone with increasing nitrogen fertilisation. However, it is unclear whether increasing nitrogen fertilisation decreased sensitivity of *Centaurea jacea* to ozone or had no effect (Table 2.3). The study will be repeated in 2004 with more sites participating, including sites with high ambient ozone conditions and using an improved protocol (UNECE, 2004b).

Table 2.3Impacts of nitrogen pollution on the sensitivity to ozone of the biomass
production of *Centaurea jacea* for ozone sensitive and resistant plants. The
values in the columns refer to the number of biomonitoring sites showing the
impact of nitrogen

	Ozone sensitive	Ozone resistant
N increases sensitivity to ozone	0	0
N decreases sensitivity to ozone	2	1
N has no effect on sensitivity to ozone	3	3

Revision of the critical levels for ozone

The critical level values for ozone have been set, reviewed and revised at a series of UNECE workshops: Bad Harzburg (1988); Bad Harzburg (1989); Egham (1992; Ashmore and Wilson, 1993); Bern (1993; Fuhrer and Achermann, 1994); Kuopio (1996; Kärenlampi and Skärby, 1996), Gerzensee (1999; Fuhrer and Achermann, 1999) and Gothenburg (2002; Karlsson *et al.*, 2003). During this 15 year period, the critical levels for ozone have evolved from daily and seasonal means, to accumulated concentrations above a threshold of X ppb (AOTX), and at the most recent Workshop in Gothenburg, to accumulated stomatal fluxes above a threshold of Y nmol m⁻² s⁻¹(AF_{st}Y). During the last two years, the text related to ozone in the previous version of the Mapping Manual (UNECE, 1996) has been substantially revised to reflect the decisions made at the Gothenburg 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 have been included in the Mapping Manual (UNECE, 2004a) 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 new mapping manual can be downloaded from the following web site: www.icpmapping.org.

The critical levels and methods described for ozone in chapter 3 "Mapping critical levels for vegetation" of the revised Mapping Manual (UNECE, 2004a) 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. As described in the chapter, the level I and level II terminology is no longer considered appropriate to describe the critical levels for ozone and these terms are not included. Instead, the critical levels are described as either concentration-based using AOTX as the ozone parameter or stomatal flux-based using AF_{st}Y as the ozone parameter. 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.

The terminology for the revised critical levels is reproduced in this report in Table 2.4 and the concentration-based and stomatal flux-based critical levels are reproduced in Table 2.5 Since the 22nd Session of the Working Group on Effects the content of chapter 3 of the Mapping

Manual has undergone major editorial changes under the leadership of Gina Mills, but without many changes in the substance. The terminology has been changed slightly since the previous annual report of ICP Vegetation (Buse *et al.*, 2003a). In addition, the concentration-based critical level of ozone for yield reduction in horticultural crops was changed from an AOT40 of 5 ppm h over a time period of four months to an AOT40 of 6 ppm h over a time period of 3.5 months.

In agreement with earlier versions of the Mapping Manual, the revised version indicates that AOTX-based critical levels are well suited to assessing the risk of damage to vegetation over large geographical regions. However, several important limitations and uncertainties are recognised for this approach since AOTX-based critical levels only consider the ozone concentration at the top of the canopy, but the real impacts of ozone depend on the amount of ozone reaching the sites of damage within the leaf. The Gerzensee Workshop in 1999, recognised the importance of developing an alternative critical level approach based on the flux of ozone from the exterior of the leaf through the stomatal pores to the sites of damage (stomatal flux). This approach required the development of mathematical models to estimate stomatal flux, primarily from knowledge of stomatal responses to environmental factors. Thus, stomatal flux-based critical levels (Cle_f) for ozone take into account the varying influences of temperature, water vapour pressure deficit (VPD), light (irradiance), soil water potential (SWP), ozone concentration and plant development (phenology) on the stomatal flux of ozone. They therefore provide an estimate of the critical amount of ozone entering through the stomata and reaching the sites of action inside the plant. This is an important new development in the derivation of critical levels because, for example, for a given ozone concentration, the stomatal flux in warm, humid conditions with moist soil can be much greater than that in hot, dry conditions with dry soil because the stomatal pores will be more widely open. Concentration-based critical levels do not differentiate between such climatic conditions and would not indicate the increased risk of damage in warm, humid conditions.

For further details about how the new concentration-based and flux-based critical levels were derived, we refer to the Mapping Manual (UNECE, 2004a) and last years' ICP Vegetation annual report (Buse *et al.*, 2003a). A technical report on the scientific basis of the new flux-based critical levels of ozone was prepared for 23rd session of the WGE (EB.AIR/WG.1/2004/8).

Assessment of economic impacts of ozone on crops in Europe

In a previous study, an economic assessment of the impacts of ozone on crop yield in Europe has been carried out in the UK under the direction of the ICP Vegetation Coordination Centre (Buse *et al.*, 2002; Holland *et al.*, 2002). The study was based on the responses of crops to ozone using the AOT40 approach. UK Defra is currently funding a new study being jointly conducted by L Emberson and colleagues at SEI-York and M Holland (EMRC, Reading). The study aims to compare economic assessments made using concentration-based methodologies with those made using the stomatal-flux based methodologies. Sensitivity analyses will be performed on both methodologies where appropriate. Secondly, the study aims to further develop the economic analysis methods employed in such assessments by refining the modelling system and performing scenario-based modelling and uncertainty analysis. The economic impact of exceedance of the revised ozone critical levels for crops across Europe for various scenarios will be considered. The new study will be completed during 2005 and will be included in this report next year.

Term	Abbreviation	Units	Explanation
Terms for concent	ration-based crit	ical levels	<u>^</u>
Concentration	AOTX	ppm h	The sum of the differences between the hourly
accumulated over		PP	mean ozone concentration (in ppb) and X ppb
a threshold ozone			when the concentration exceeds X ppb during
concentration of			daylight hours, accumulated over a stated time
X ppb			period. Units of ppb and ppm are parts per billion $(mmel mel^{-1})$ and parts per million $(mmel mel^{-1})$
			(nmol mol ⁻¹) and parts per million (μ mol mol ⁻¹)
			respectively, calculated on a volume/volume
-			basis.
Concentration-	CLe _c	ppm h	AOTX over a stated time period, above which
based critical			direct adverse effects on sensitive vegetation may
level of ozone			occur according to present knowledge.
Concentration	AOTX _{VPD}	ppm h	The sum of the differences between the hourly
accumulated over			mean ozone concentration (in ppb) modified by a
a threshold ozone			vapour pressure deficit factor ($[O_3]_{VPD}$), and X
concentration of			ppb when the concentration exceeds X ppb
X ppb modified			during daylight hours, accumulated over a stated
by vapour			time period.
pressure deficit			Porto a.
(VPD)			
(VID)	<u>.</u>		
Terms for flux-base	ed critical levels		
Projected leaf	PLA	m ²	The projected leaf area is the total area of the
area		111	sides of the leaves that are projected towards the
arca			sun. PLA is in contrast to the total leaf area,
			which considers both sides of the leaves. For flat
Ctown to 1 Classic C	Г	12	leaves the total leaf area is simply 2*PLA.
Stomatal flux of	F _{st}	nmol m ⁻²	Instantaneous flux of ozone through the stomatal
ozone		PLA s ⁻¹	pores per unit projected leaf area (PLA). F_{st} can
			be defined for any part of the plant, or the whole
			leaf area of the plant, but for this manual, F_{st}
			refers specifically to the sunlit leaves at the top of
			the conony E is normally calculated from hourly
1			the canopy. F_{st} is normally calculated from hourly
			mean values and is regarded here as the hourly
Stomatal flux of	F _{st} Y	nmol m ⁻²	mean values and is regarded here as the hourly
Stomatal flux of ozone above a	F _{st} Y	nmol m ⁻² PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux
ozone above a	F _{st} Y	nmol m ⁻² PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol $m^{-2} s^{-1}$, through the stomatal
ozone above a flux threshold of	F _{st} Y	nmol m ⁻² PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol $m^{-2} s^{-1}$, through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be
ozone above a flux threshold of Y nmol m ⁻² PLA	F _{st} Y	nmol m ⁻² PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol $m^{-2} s^{-1}$, through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf
ozone above a flux threshold of	F _{st} Y	nmol m ⁻² PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. F_{st} Y can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual F_{st} Y refers
ozone above a flux threshold of Y nmol m ⁻² PLA	F _{st} Y	nmol m ⁻² PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. F_{st} Y can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual F_{st} Y refers specifically to the sunlit leaves at the top of the
ozone above a flux threshold of Y nmol m ⁻² PLA	F _{st} Y	nmol m ⁻² PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual $F_{st}Y$ refers specifically to the sunlit leaves at the top of the canopy. $F_{st}Y$ is normally calculated from hourly
ozone above a flux threshold of Y nmol m ⁻² PLA	F _{st} Y	nmol m ⁻² PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol $m^{-2} s^{-1}$, through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual $F_{st}Y$ refers specifically to the sunlit leaves at the top of the canopy. $F_{st}Y$ is normally calculated from hourly mean values and is regarded here as the hourly
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹		PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual $F_{st}Y$ refers specifically to the sunlit leaves at the top of the canopy. $F_{st}Y$ is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata.
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Accumulated	F _{st} Y AF _{st} Y	PLA s ⁻¹ mmol m ⁻²	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual $F_{st}Y$ refers specifically to the sunlit leaves at the top of the canopy. $F_{st}Y$ is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Accumulated stomatal flux of		PLA s ⁻¹	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. F_{st} Y can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual F_{st} Y refers specifically to the sunlit leaves at the top of the canopy. F_{st} Y is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Accumulated stomatal flux of ozone above a		PLA s ⁻¹ mmol m ⁻²	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. F_{st} Y can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual F_{st} Y refers specifically to the sunlit leaves at the top of the canopy. F_{st} Y is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time period during daylight hours. Similar in concept
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Accumulated stomatal flux of ozone above a flux threshold of		PLA s ⁻¹ mmol m ⁻²	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual $F_{st}Y$ refers specifically to the sunlit leaves at the top of the canopy. $F_{st}Y$ is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time
Accumulated stomatal flux of ozone above a flux threshold of y nmol m ⁻² PLA		PLA s ⁻¹ mmol m ⁻²	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. F_{st} Y can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual F_{st} Y refers specifically to the sunlit leaves at the top of the canopy. F_{st} Y is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time period during daylight hours. Similar in concept
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Accumulated stomatal flux of ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹	AF _{st} Y	PLA s ⁻¹ mmol m ⁻² PLA	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. F_{st} Y can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual F_{st} Y refers specifically to the sunlit leaves at the top of the canopy. F_{st} Y is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time period during daylight hours. Similar in concept to AOTX.
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Accumulated stomatal flux of ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Flux-based		PLA s ⁻¹ mmol m ⁻² PLA mmol m ⁻²	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual $F_{st}Y$ refers specifically to the sunlit leaves at the top of the canopy. $F_{st}Y$ is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time period during daylight hours. Similar in concept to AOTX.
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Accumulated stomatal flux of ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹	AF _{st} Y	PLA s ⁻¹ mmol m ⁻² PLA	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. F_{st} Y can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual F_{st} Y refers specifically to the sunlit leaves at the top of the canopy. F_{st} Y is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time period during daylight hours. Similar in concept to AOTX.
ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Accumulated stomatal flux of ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Flux-based	AF _{st} Y	PLA s ⁻¹ mmol m ⁻² PLA mmol m ⁻²	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual $F_{st}Y$ refers specifically to the sunlit leaves at the top of the canopy. $F_{st}Y$ is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time period during daylight hours. Similar in concept to AOTX.
Accumulated stomatal flux of ozone above a flux threshold of stomatal flux of ozone above a flux threshold of Y nmol m ⁻² PLA s ⁻¹ Flux-based critical level of	AF _{st} Y	PLA s ⁻¹ mmol m ⁻² PLA mmol m ⁻²	mean values and is regarded here as the hourly mean flux of ozone through the stomata. Instantaneous flux of ozone above a flux threshold of Y nmol m ⁻² s ⁻¹ , through the stomatal pores per unit projected leaf area. $F_{st}Y$ can be defined for any part of the plant, or the whole leaf area of the plant, but for this manual $F_{st}Y$ refers specifically to the sunlit leaves at the top of the canopy. $F_{st}Y$ is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata. Accumulated flux above a flux threshold of Y nmol m ⁻² s ⁻¹ , accumulated over a stated time period during daylight hours. Similar in concept to AOTX.

Table 2.4Terminology for critical levels of ozone (UNECE, 2004a)

Approach		Crops	(Semi-) natural vegetation	Forest trees
Stomatal flux- based critical level	CLe _f	Wheat: An AF _{st} 6 of 1 mmol m ⁻² PLA Potato: An AF _{st} 6 of 5 mmol m ⁻² PLA	Not available	<i>Birch and beech:</i> Provisionally AF _{st} 1.6 of 4 mmol m ⁻² PLA
	Time period	Wheat: Either 970°C days, starting 270°C days before mid- anthesis (flowering) or 55 days starting 15 days before mid-anthesis <i>Potato</i> : Either 1130°C days starting at plant emergence or 70 days starting at plant emergence		One growing season
	Effect	Yield reduction		Growth reduction
Concentration- based critical level	CLe _c	Agricultural crops: An AOT40 of 3 ppm h Horticultural crops: An AOT40 of 6 ppm h	An AOT40 of 3 ppm h	An AOT40 of 5 ppm h
	Time period	Agricultural crops: 3 months Horticultural crops: 3.5 months	3 months (or growing season, if shorter)	Growing season
	Effect	Yield reduction for both agricultural and horticultural crops	Growth reduction in perennial species and growth reduction and/or seed production in annual species	Growth reduction
VPD-modified	CLe _c	An AOT30 _{VPD} of 0.16	Not available	Not available
concentration-		ppm h		
based critical level	Time period	Preceding 8 days		
	Effect	Visible injury to leaves		

Table 2.5Critical levels for ozone. The methods for calculating each critical level are
described in the Mapping Manual (UNECE, 2004a)

Note: The recommendations of the Gothenburg Workshop (2002), the 16th Task Force Meeting of the ICP Vegetation and the 17th Task Force Meeting of the ICP Modelling and Mapping were:

<u>For agricultural crops</u>: use stomatal flux-based critical levels based on $AF_{st}6$ if the necessary inputs are available for a quantitative assessment of impacts on wheat and potato, and the AOT40-based critical level to assess the risk of yield reduction if only ozone concentration is available and a risk assessment for all crops is needed. The AOT30_{VPD} critical level should be used to assess the risk of visible ozone injury and cannot be used to indicate the risk of yield reduction.

<u>For horticultural crops:</u> use the AOT40-based critical level to assess the risk of effects on yield. The AOT 30_{VPD} critical level should be used to assess the risk of visible ozone injury and cannot be used to indicate the risk of yield reduction.

For (semi-) natural vegetation: use the AOT40-based critical level.

<u>For forest trees</u>: use the AOT40-based critical level to assess the risk of growth reduction. The provisional stomatal flux-based critical level is provided for guidance only.

The concentration of heavy metals in mosses in Europe

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.*, 2003b). 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., 2003b). However, since then data has been received from Iceland and included in the ICP Vegetation database and the EMEP 50 x 50 km grid maps. ICP Vegetation was invited to give a key note lecture on the European moss survey at the 3rd International Workshop on Biomonitoring of Atmospheric Pollution, Bled, Slovenia, 21-25 September 2003. The lecture was given as an introduction to the session 'Case studies using different biomonitors'. A full paper of the presentation was published in the proceedings of the workshop on CD and accepted for publication (Harmens et al., in press). The paper shows the EMEP 50 x 50 km grid maps for cadmium, lead and mercury, including the data received from Iceland. A report has been submitted to the Secretariat of the WGE regarding factors influencing the heavy metal concentration in mosses (Harmens et al., 2004). This study showed that the variation in the European heavy metal in mosses dataset of 2000/2001 was high. Multivariate regression analysis highlighted the strong correlation between the concentration of Cu and As, Cd and Pb, Cd and Zn, Cr and Ni and especially Fe and V. Both multivariate regression analysis and artificial neural networks indicated that there are weak correlations between the heavy metal concentation in mosses and climatic parameters, analytical techniques and moss species.

Preparations were started for the next European heavy metals in mosses survey, planned to start in 2005. The experimental protocol for the moss survey (UNECE, 2001) was discussed at the 17th ICP Vegetation Task Force Meeting in Kalamata, Greece, and a first draft of the revised protocol is being written and will be circulated to all participants in the 2005 survey for comments. The final version of the experimental protocol will be distributed among participants by the end of 2004. At the 17th ICP Vegetation Task Force Meeting in Kalamata, Greece, Eero Kubin (Finnish Forest Research Institute, Muhos Research Station, Finland) kindly agreed to distribute standards used in the 1995 survey (Steinnes *et al.*, 1997) amongst all participants at a reduced cost. This will improve the quality assurance of the data from the 2005 moss survey compared with the 2000/2001 moss survey.

Task Force Meeting

Each year, the ICP Vegetation holds a Task Force Meeting in one of the participating countries to consider recent results and to plan the future work programme. The most recent 17th Task Force Meeting of the ICP Vegetation was held in Kalamata, Greece, from the 10 - 13 February 2004, and was hosted by Professor Dimitris Velissariou (Technological Educational Institute, Kalamata) and Dr Pavlina Drogoudi (Pomology Institute, National Agricultural Research Foundation, Naoussa). Fifty participants attended the meeting,

representing 19 parties to the Convention, together with the Secretary of the Working Group on Effects (WGE) and a representative of the ICP on Modelling and Mapping. Presentations, poster sessions and working group discussions addressed the following topics:

- biomonitoring of ozone pollution using crops and (semi-) natural vegetation;
- recent developments in modelling ozone fluxes;
- finalising chapter 3 of the UNECE Mapping Manual ('Mapping critical levels for vegetation'), including flux-based critical levels for ozone;
- the use of flux-based and concentration-based ozone dose-response relationships for crops for cost-benefit analysis (in collaboration with the European Union Clean Air for Europe (CAFE) programme);
- interactions between the impacts of ozone and nitrogen on (semi-) natural vegetation;
- biomonitoring of heavy metal pollution using mosses and crops;
- preparations for the next European heavy metal in mosses survey, planned for 2005.

Near the end of the meeting, seven small discussion groups considered ongoing and developing areas of interest for the ICP Vegetation. The subjects covered were ozone flux modelling, ozone injury assessment methods, ozone and nitrogen interactions, new biomonitoring systems, ICP Vegetation contributions to health impact assessments for heavy metals, climate change as a modifier of pollutant impacts and developing links with Asia. Finally, the short and medium-term objectives of the ICP Vegetation were revised (see Annex I) and after ten years of chairing the ICP Vegetation, Gina Mills handed over the chair of ICP Vegetation to her colleague Harry Harmens. On behalf of the ICP Vegetation community, Harry Harmens thanked Gina Mills for her invaluable contributions and commitment to the work of ICP Vegetation over the years and emphasized the growth of ICP Vegetation in recent years under her leadership.

Publicity

Reports

- Harmens, H., Mills, G., Hayes, F., Williams, P. and the participants of the ICP Vegetation (2004). Air Pollution and Vegetation: UNECE ICP Vegetation Annual Report 2003/4. UNECE ICP Vegetation
- 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.
- 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. Progress report. Defra contract EPG1/3/205.
- UNECE (2004). The scientific basis of the new flux-based critical levels of ozone. Technical Report (EB.AIR/WG.1/2004/8) by ICP Vegetation.

Contributions were made to the following reports:

UNECE (2004) Review and assessment of present air pollution effects and their recorded trends.

UNECE (2004) Joint Report of the International Cooperative Programmes and the Task Force on Health Apects of Air Pollution (EB.AIR/WG.1/2004/3 and add. 1).

Papers and conference proceedings

- 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. *In press*.
- 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.

A Special Issue of Atmospheric Environment was published in 2004 on "New Methods of risk assessment for ozone impacts on vegetation", Volume 38 (15), pages 2211 – 2438.

Web site

The web site has been updated during the year and can be found at: <u>icpvegetation.ceh.ac.uk</u>

3. The clover biomonitoring experiment (1996 – 2003)

Background

Ever since the ICP Vegetation was first established in 1988 (formerly named ICP Crops), a main objective has been to quantify the effects of ambient ozone in the ECE region using an inexpensive biomonitoring system. Early investigations, using first radish (*Raphanus sativus*) and then subterranean clover (*Trifolium subterraneum*) showed that this was possible using ethylene diurea (EDU) as a protectant against ozone injury (e.g. Ball et al, 1998). In the meantime, a biomonitoring system that did not involve the use of chemical treatments was sought. Following a pilot study in 1995, a programme of experiments has been conducted each year since 1996 using ozone-sensitive (NC-S) and ozone-resistant (NC-R) biotypes of white clover (*Trifolium repens* cv Regal) selected by Heagle *et al* (1995). This biomonitoring system was chosen because the forage biomass for both biotypes was similar at low ozone concentrations, but lower for the NC-S biotype at high ozone concentrations (12h mean > 40-50 ppb, Heagle *et al*, 1995). By exposing plants to ambient air, the reaction to ozone episodes could be considered without any confounding influence of an exposure chamber on the flux of ozone to the plant. This chapter reviews the results from the biomonitoring experiments conducted with NC-S and NC-R clover during the period 1996 to 2003.

The experiment was conducted at 20 - 35 sites across Europe and the USA each year since 1996. Impacts of ozone have been monitored by weekly assessments of the health of the leaves, including the presence of ozone injury (small pale yellow spots on the leaf) and measurements of the biomass of the foliage and stems at the end of each 28 day exposure period. The results from these experiments have drawn attention to the widespread occurrence of ozone injury-causing episodes across Europe, and have been used to establish a dose-response relationship between AOT40 and biomass reduction in the sensitive biotype. From 1998-2001, participants from nine sites collected stomatal conductance measurements according to a standard protocol. Together with the biomass-effect data, these have been used to develop a flux-effect relationship for clover that can be used in mapping the effects of ozone across Europe.

The biomonitoring system and data quality assurance

The experiment was performed each year as described in chapter 2, with participants growing NC-S and NC-R biotypes of white clover from cuttings distributed by the Coordination Centre, and exposing the plants to ambient ozone according to a standard protocol (e.g. UNECE, 2003). Plants were assessed at least once per week for ozone injury using a standard key based on the proportion of injured leaves per plant. At 28 day intervals, the extent of ozone injury was assessed and the foliage was cut down to 7 cm above the soil surface, then dried and weighed to determine biomass. The plants were allowed to re-grow before a further harvest 28 days later; four to six such harvests were performed at each site each year. Harvest intervals 1-2, 2-3 and 3-4 were used in the data analysis presented here.

At nine sites, stomatal conductance measurements were made on fully developed leaves positioned in full sun; the fourth leaf from the tip of a stolon was selected for consistency. At each site, measurements were made on several days during the season, allowing a range of climatic conditions to be represented in the dataset, and normally between 10 am and 4 pm (several complete days of data are also included in the dataset). The conductance values were standardised to account for the different ways in which different instruments measure stomatal conductance. A relationship between the stomatal conductance of the upper and

lower leaf surfaces was determined (data not presented), allowing stomatal conductance to be calculated for the missing surface for those sites where measurements where only recorded for one of the leaf surfaces. All conductance measurements from all sites were then expressed as total conductance of the upper and lower surface of the leaf, based on the projected leaf area. The resulting database was subjected to a rigorous quality check. Datasets were excluded from the database if one or more of the climatic or ozone parameters were missing, and where values were 'erratic' suggesting instrumentation problems.

Each year, the data from each experimental site were sent to the ICP Vegetation Coordination Centre for analysis. Data comprised measurements of hourly means of climatic and pollution data, including temperature, humidity, solar radiation, wind speed, ozone, NO_x and SO_2 , for the four to six month period covering the experiments. For each site, data were standardised into appropriate units where necessary, and hourly VPD was calculated from temperature and humidity data for those sites where the VPD information was not already available.

All plant, climatic and pollutant data received by the Coordination Centre at CEH Bangor were subjected to a rigorous quality assurance procedure (see Mills *et al*, 2000, for details), ensuring that only data from healthy plants and well-maintained and calibrated pollutant and climatic monitors were included in the dataset.

Pollution and physical climate at ICP Vegetation sites

Measurements made at the ICP Vegetation sites indicated that ozone was a widespread pollutant across Europe. Table 3.1 illustrates the three month AOT40s for selected sites over the period 1997 to 2003 and indicates a geographical trend with the highest AOT40s occurring in southern Europe, medium AOT40s in central Europe and the lowest AOT40s occurring in northern Europe. No specific trends in time were observed over the seven years with considerable variation from year to year; for example, Italy – Naples had a range in AOT40 in the selected three-month period from 9.4 ppm.h in 2002 to 32.4 ppm.h in 1998. The three month AOT40 exceeded the critical level for crops of an AOT40 of 3 ppm h every year at six of the nine sites shown in Table 3.1. AOT40s were more than five times the critical level at Italy-Isola Serafini (1998, 1999 and 2000), Italy-Naples (1998 and 2000) and Switzerland-Cadenazzo (1998, 1999 and 2003).

Following a rigorous quality check, data from the 28d intervals at each site were used in the development of dose-response functions as described later. A subset of the data for the sites in Table 3.1 is included in Table 3.2 to provide examples of the range of 28d values for daylight mean temperature, total rainfall, and AOT40 at ICP Vegetation sites. Mean daylight temperature over 28d for these sites ranged from 13 °C at UK-Bangor to 28.1 °C at Italy-Naples with the highest rainfall totals of 238.5 and 191 mm being recorded at Austria-Seibersdorf and Belgium-Tervuren respectively. With the exception of the Italian sites and Austria, the 28d AOT40 was very low (≤ 0.6 ppm h) at least once at each of the other sites illustrated in Table 3.2. In contrast, 28 day AOT40s in excess of 5 ppm h were recorded at least once at five of the sites.

Frequency of occurrence of ozone injury at ICP Vegetation sites

This overview of data from 1996 to 2003 has allowed the frequency of incidences of ozone injury to be investigated. Long-term trends are difficult to determine due to both the year to year variation in ozone concentrations at individual sites (see above) and also due to practical reasons that include moving of sites or gaps in funding for participants. Table 3.3 provides data for those sites with a relatively long record of participation in this activity. With the

exception of Belgium-Tervuren and UK-Bangor, ozone injury was detected on at least one 28d harvest at all sites in all years during the period 1996 to 2003. Injury was detected on at least 75% of harvests in all years for which data is available at Germany-Trier, Italy-Isola Serafini and Slovenia-Ljubljana, and additionally on 60% or more of the harvests every year at Switzerland-Cadenazzo. In northern Europe, ozone injury was frequently detected at Finland-Jokioinen and Sweden-Ostad, but infrequently detected at UK-Bangor.

Site	1997	1998	1999	2000	2001	2002	2003
Austria - Seibersdorf	9.2	7.1	9.4	13.1	10.3	8.1	9.8
Belgium - Tervuren	4.2	1.3	4.6	1.0	n.a	1.7	8.7
Germany -							
Deuselbach	9.0	7.9	10.0	4.8	8.4	n.a.	n.a.
Italy - Isola Serafini	n.a.	32.8	20.4	17.3	n.a.	n.a.	n.a.
Italy - Naples	n.a.	32.4	12.5	19.2	12.2	9.4	13.9
Sweden - Östad	2.1	0.5	1.9	0.3	0.8	0.3	0.8
Switzerland -							
Cadenazzo	14.0	22.5	18.0	n.a.	12.9	n.a.	23.7
UK - Bangor	n.a.	0.8	1.2	0.1	2.0	0.4	2.9

Table 3.1AOT40 (ppm.h) over 3 months at selected rural ICP Vegetation sites during
the period 1996 – 2002

Table 3.2Examples of the ranges of temperature, rainfall, AOT40 and NC-S/NC-R
biomass ratio for the 28 day periods included in the analysis of data from 1996
to 2002

Country	Daylight T (°C)	Total rainfall (mm)	AOT40 (ppb.h)	NC-S/NC-R biomass ratio
Austria - Seibersdorf	13.8 - 25.7	17.7 – 238.5	1.65 - 5.64	0.71 – 1.10
Belgium - Tervuren	16.0 - 22.5	30.6 - 191.0	0.01 – 2.29	0.83 - 1.23
Germany- Deuselbach	15.2 - 21.8	19.2 – 141.7	0.57 – 5.71	0.73 – 1.12
Italy-Isola Serafini	23.8 - 27.1	1.85 - 62.0	2.98 - 10.31	0.23 - 0.86
Italy -Naples	25.1 - 28.1	0 - 148.0	2.24 - 6.93	0.57 - 0.95
Sweden - Ostad	15.9 - 21.8	23.0 - 120.0	0.05 - 1.48	0.92 - 1.25
UK - Bangor	13.0 - 14.4	32.7 - 77.6	0.04 - 0.23	0.95 - 1.18

AOT40-based dose-response functions for effects on biomass

Quality checks of the ozone data has revealed that ozone is measured at different heights at the different sites, ranging from <1 m to > 5 m above ground level (Figure 3.1). This variation has arisen because some participants cannot provide data for the recommended height of 3m (see experimental protocol) because of using a national ozone monitoring site where the measurement height is fixed, or are measuring ozone at a different height for another experiment being conducted alongside the ICP Vegetation ones.

Table 3.3Frequency of occurrence of ozone injury on white clover (*Trifolium repens*) at
selected ICP Vegetation biomonitoring sites (1996 – 2003). Data is presented
as percentage of 28-day harvests per site per year when injury was detected

Site	1996	1997	1998	1999	2000	2001	2002	2003
Austria-Seibersdorf	100	33	33	50	50	60	80	n.a.
Belgium-Terveuren	100	n.a	20	80	33	17	0	80
Finland-Jokioinen	0	25	n.a.	100	66	33	n.a.	66
Germany-Trier	n.a.	n.a.	n.a.	100	75	80	100	100
Italy - Isola Serafini	n.a [.]	75	100	100	100	100	75	n.a.
Slovenia-Ljubljana	100	n.a.	100	100	100	75	n.a.	100
Sweden-Östad	100	100	80	75	33	75	100	100
Switzerland-Cadenazzo	75	75	100	83	n.a.	60	100	n.a.
UK-Bangor	n.a.	n.a.	25	25	0	20	0	50

Since an ozone gradient exists over crops and grasslands meaning that concentrations can be 10% lower at 1m than at 5m (UNECE, 2004a), this variation in measurement height could introduce error into AOT40-based dose response functions especially at sites where the ozone concentration is frequently close to 40 ppb. The first phase in the development of dose-response functions was conducted in collaboration with W. Werner (University of Trier, Germany) and involved investigating ways of standardising the ozone data for a canopy height of 1m.



Figure 3.1 Histogram of ozone measurement heights used at ICP Vegetation sites (1996 – 2002).

Two methods are described in the Mapping Manual (UNECE, 2004a) for converting ozone concentration to canopy height, depending on availability of meteorological data:

- a) If no meteorological data is available, a simple gradient can be used that has been estimated from the ozone deposition module (Emberson et al, 2000a) for an artificial 1m crop.
- b) If wind speed is available, neutral stability profiles can be used to estimate the ozone concentration using a constant flux assumption and aerodynamic resistance.

Although both methods involve several assumptions, method (b) is the preferred method and was the first choice for standardisation of ozone data in the ICP Vegetation dataset. However, hourly mean wind speed data was only available for 9 of the 20 sites included in the analysis and was not available for the highest ozone sites with 28d AOT40 values above 6.1 ppm h. Figure 3.2a illustrates the measured AOT40 against the wind-speed corrected AOT40 for those sites for which this was possible. The importance of the conversion of the data is reflected in the scatter within this figure ($r^2 = 0.74$). As wind speed data was not available for all sites within the data set, it was necessary to use the concentration gradient method (method (a)) which provided a much closer relationship with the measured AOT40 as indicated in Figure 3.2b ($r^2 = 0.98$). The gradient-corrected canopy height AOT40s were on average 26% lower than the measurement height ozone concentration.



Figure 3.2 AOT40 measured against AOT40 corrected for ozone measurement height using (a) neutral stability profiles (UNECE 2004a) for those sites where hourly mean wind speed is available, and (b) Tabulated values for ozone gradient (UNECE 2004a) for all sites.

Using the gradient corrected values for ozone had little effect on the fit of the data in the dose-response relationship, with linear regression providing an r^2 of 0.58 for the three month gradient corrected AOT40 and 0.56 for the uncorrected AOT40 (Figure 3.3). However, the gradient correction reduced the AOT40 values as shown in Figure 3.2, and thus the slope of the regression is steeper for Figure 3.3b than for Figure 3.3a. The importance of this difference becomes apparent if the regression is used to calculate the critical level for a 5% reduction in the biomass of the NC-S biotype relative to that of the NC-R biotype. The critical level without correction is 2.8 ppm h whilst that with correction is lower at 2.2 ppm h. Unfortunately, there was insufficient data from the high ozone sites to enable a response function to be derived for wind-speed corrected AOT40 (data not presented).



Figure 3.3 Dose-response functions for effects on biomass using the three month (a) uncorrected AOT40 and (b) ozone gradient corrected AOT40 (data from 1996 – 2002).

Flux-based dose-response functions for effects on biomass

For four experimental seasons, participants at nine sites collected stomatal conductance (g_s) measurements for climatic conditions ranging from the cool/low VPD conditions typically found at UK-Bangor and Sweden-Östad to the hot/high VPD climates typical of Italy-Milan and Italy-Rome. Three methods have been used to develop stomatal conductance models using this data: multivariate statistical analysis (G Mills, CEH Bangor, UK), artificial neural networks (P Bűker and W Werner, University of Trier, Germany) and multiplicative algorithm (L Emberson, SEI-York, UK). A brief review of results is presented here.

The stomatal conductance dataset comprised of over 5000 quality assured measurements for stomatal conductance which are accompanied by measurements of photosynthetically active radiation (PAR), air temperature (T_{air}), vapour pressure deficit (VPD) and ozone concentration together with the AOT40 since previous harvest, days since previous harvest (DSLH), time (hour), date and harvest interval during which measurements were made. Mean g_s values were in the range 178 (Austria-Seibersdorf) to 585 mmol H₂O m⁻² s⁻¹ (Italy-Milan). Initial analysis of the dataset revealed, not surprisingly, that VPD and temperature were linearly correlated ($r^2 = 0.85$) and that the hourly mean ozone concentration was correlated with temperature ($r^2 = 0.51$) and AOT40 since previous harvest ($r^2 = 0.42$). There was a significant influence of site on g_s (p<0.001), but as there was also a significant influence of site on VPD (p<0.001), one of the main drivers of g_s , it was considered reasonable to combine the data from the nine sites when needed for subsequent analysis.

Multiple linear regression

Analysis of variance (ANOVA) of the whole dataset revealed that g_s was significantly lower for the NC-R biotype (n = 2418, mean = 339 ± 199 mmol H₂O m⁻² s⁻¹) than for the NC-S biotype (n=2639, mean = 393 ± 209 mmol H₂O m⁻² s⁻¹, and p <0.001 for the difference between the means). After splitting the data by biotype, best subsets multiple linear regression provided relationships between five input factors and g_s that accounted for 22.4% and 25.7% of the variation for NC-R and NC-S respectively (data not presented). The dataset was processed further by calculation of hourly means for each parameter, and exclusion of data outside the period used in the biomass effects measurements. Furthermore, g_s data from Austria-Seibersdorf and Germany-Essen were omitted because they were approximately half of those from other sites and were found to be from a different population (ANOVA, p<0.001) even though T_{air} and VPD at these sites were not statistically significant from the rest of the sites (p = 0.62 and 0.94 respectively).

For the remaining 286 sets of data, ANOVA again showed that there was a significant difference (p<0.001) between g_s for the NC-R biotype (n=133, mean=400 ± 138 mmol H₂O m⁻² s⁻¹) and the NC-S biotype (n=153, mean=500 ± 155 mmol H₂O m⁻² s⁻¹). Thus all subsequent analysis was conducted separately for the two biotypes. Best subsets analysis showed that air temperature and vapour pressure deficit were the most important influencing factors, that phenology was important (indicated by days since last harvest), and that ozone concentration and AOT40 since last harvest had a somewhat lower influence (data not presented). The best fit using multiple linear regression was established using the following equations:

NC-R ($R^2 = 46.8\%$, p <0.001) $g_s = 27.8 - (1.84*DSFH) + (23.8*Tair) - (111*VPD) + (0.0318*AOT40)$ [1] NC-S ($R^2 = 44.4\%$, p<0.001) $g_s = -136 - (1.04*DSFH) + (37.4*Tair) - (158*VPD) + (0.0087*AOT40)$ [2]

However, the plots of measured versus calculated g_s from these equations were skewed towards the measured values (data not presented). Thus, the regression equations were underestimating g_s for conditions which lead to high g_s values. This statistical approach seems suitable for development of parsimonious models of g_s , with reasonable fit to the data, but is limited by the non-linear nature of the effects of the parameters on g_s . A major benefit of this approach has been the identification of those factors that are the prime drivers of g_s in each biotype of clover. This information was used to guide the multiplicative flux modelling described later.

Artificial neural network modelling

The modelling described in this section was conducted by Patrick Büker and Willy Werner (University of Trier, Germany) as a "contribution in kind" to the ICP Vegetation.

The software package, Neuroshell 2.0 (Ward System Group) was used to develop back propagation neural networks using multi-layer perceptrons. Seventy percent of the g_s data was used to train the model and 20% was used to test the accuracy of the model by comparing the predicted g_s with the actual g_s values. The remaining 10% of the data were used to cross-validate the results of each network with data the network had never "seen" before, and networks were trained until the error for the test data could not be reduced further. Each possible influencing factor was used as an input variable, while the stomatal conductance in mmol $H_2O \text{ m}^{-2} \text{ s}^{-1}$ was the output. In order to optimise the effectiveness of the network, the number and combination of inputs and the network parameters (hidden neurons, learning rate, momentum and initial weight) were varied until the optimum performance was achieved. The resulting ANN-equation, a complex linear equation with as many weighting factors as connections between neurons, was expressed as a C-program that was executed as a subprogram in MS Excel.

Following comprehensive training and optimisation procedures, the ANN model with the best performance for each biotype included all eight input parameters and nine hidden neurons.

The models had r² values for the test data of 0.78 for the NC-S biotype (named ANN_{NC-S}) and 0.75 for the NC-R clover biotype (named ANN_{NC-R}). Neuroshell-2 provided information on the percentage contribution of each of the inputs into the models. DSLH, VPD and Tair had the highest percentage contribution for both biotype ANNs (13.4-15.9%); time of day, PAR and ozone concentration had the lowest contribution for the ANN_{NC-S} (10.1 – 11.6%) whilst time, DSFH and AOT40 were lowest for ANN_{NC-R} (9.1 -10.9). However, it is notable that no one factor dominated, and that the percentage contribution per input factor only varied from 10.1 to 14.7% for ANN_{NC-S} and 9.11 – 15.9% for ANN_{NC-R}. This confirmed the message from multivariate statistical analysis that stomatal conductance in clover is influenced by several factors in a complexity of interactions.

Multiplicative stomatal conductance modelling

The multiplicative algorithm (MA) that has been used to model stomatal flux has been described previously in Emberson *et al.* (2000a,b) and is presented here as equation [3]:

$$g_s = g_{max} * g_{pot} * g_{light} * max\{g_{min}, (g_{temp} * g_{VPD})\}$$
[3]

where g_s is the actual stomatal conductance in mmol H₂O m⁻² s⁻¹; g_{max} is defined as the average maximum stomatal conductance expressed in mmol H₂O m⁻² s⁻¹ on a total leaf area basis. The parameters g_{pot} , g_{light} , g_{temp} and g_{VPD} are all expressed in relative terms as a proportion of g_{max} where:

 g_{pot} represents the modification to g_{max} due to phenological changes. g_{light} represents the modification of g_{max} by irradiance described by PAR (µmol m⁻² s⁻¹) g_{temp} represents the modification of g_{max} by temperature (°C) g_{VPD} represents the modification of g_{max} by vapour pressure deficit (VPD) (kPa) g_{min} represents the minimum g_s that occurs during the daylight period.

The original stomatal flux model also included a soil water parameter. Since the protocol used to collect clover g_s data stipulates that plants be grown under well-watered conditions, it was not considered necessary to include this parameter in the flux modelling. Parameterisation was achieved using a boundary line analysis technique whereby all g_s data points are plotted against each model variable (e.g. irradiance, temperature and VPD) individually. A boundary line was then fitted according to generic functions that have been predefined for each model variable (described in Emberson et al. (2000a,b)). In previous studies, the boundary line was fitted by eye; in this study we attempted a less subjective fitting procedure based on statistical principles. First, the gs data were divided into classes with the following step-wise increases for each variable: 50 μ mol m⁻² s⁻¹ for irradiance, 1 °C for temperature and 0.1 kPa for VPD. For each class with greater than 10 points, a normal distribution was assumed and the mean, maximum and minimum were used to determine the 90th percentile using the "range" rule whereby the range is approximately 4 s.d. and z is 1.64 as follows: 90% ile = mean +(1.64*(0.5*(max-mean))). The boundary line was fitted around the 90% ile data (excluding any obvious outliers) using the generic functional shapes described in Emberson et al. (2000a,b).

The initial aim of this work was to use the clover g_s dataset to produce one model of g_s per biotype that was applicable to all of Europe. However, it soon became apparent that this approach might not be appropriate since boundary line analysis would be dominated by data from southern Europe (Figure 3.4). For this reason, the data were separated into a North

dataset (Austria, Belgium, Germany-Essen, Germany-Trier, UK, Sweden) and a South dataset (Italy Milan (only these data were used for g_{max} and g_{min} derivation), Italy Rome, Spain) for all subsequent model development. Separate parameterisations were established for each biotype for each region, and the models were named MA_{NC-S South} etc. (Table 3.4).



- **Figure 3.4** The ICP Vegetation stomatal conductance data, illustrated as VPD versus stomatal conductance (nmol m⁻² s⁻¹), and separated into data from northern sites (Austria, Belgium, Germany-Essen, Germany-Trier, UK, Sweden) and southern sites (Italy Milan, Italy Rome, Spain).
- **Table 3.4**Parameterisations for the multiplicative algorithms used to model gs for white
clover

No	rth	South		
NC-R	NC-S	NC-R	NC-S	
753	780	816	826	
0.1	0.12	0.11	0.14	
-0.007	-0.013	-0.013	-0.015	
10	5	$n.a.^{1}$	$n.a^1$.	
28	23	$n.a.^1$	$n.a.^1$	
1.8	2.6	3.7	2.6	
4	4.8	6.1	7.2	
	NC-R 753 0.1 -0.007 10 28	$\begin{array}{cccc} 753 & 780 \\ 0.1 & 0.12 \\ -0.007 & -0.013 \\ 10 & 5 \\ 28 & 23 \\ 1.8 & 2.6 \end{array}$	NC-RNC-SNC-R7537808160.10.120.11-0.007-0.013-0.013105n.a.12823n.a.11.82.63.7	

¹Polynomial functions used, see equations [4] and [5]

The figures describing g_{temp} show that for the biotypes of the southern dataset the generic g_{temp} relationship described by Emberson *et al*, 2000a, b will significantly overestimate g_s as temperature increases (data not presented). Thus, a polynomial function was fitted though the boundary data points and was used in place of the generic function as follows:

NC-R:
$$g_{temp} = max (g_{min}, -0.000161*T^{o}C^{3} + 0.0079*T^{o}C^{2} - 0.0463*T^{o}C - 0.37)$$
 [4]
NC-S: $g_{temp} = max (g_{min}, -0.00002*T^{o}C^{3} - 0.0009*T^{o}C^{2} + 0.1145*T^{o}C - 1.08)$ [5]
Flux-effect modelling

The NC-S and NC-R flux algorithms described in the previous section were used to develop flux-effect relationships for the 28d and three month data for the nine sites contributing stomatal conductance data. The stomatal ozone flux, F_{st} (mol O₃ m⁻² s⁻¹) was first calculated as g_s (mol H₂O m⁻² s⁻¹) * O₃ concentration (mol mol⁻¹)* 0.613, where 0.613 is the ratio of diffusivities for water vapour and ozone, and is used to convert g_s from mol H₂O m⁻² s⁻¹ to mol O₃ m⁻² s⁻¹.

The NC-S/NC-R biomass ratio was poorly correlated with the ratio of the fluxes for the two biotypes, regardless of threshold used (Table 3.5). Use of 40 ppb or 5 nmol m⁻² s⁻¹ as a threshold improved the linear regression using the accumulated fluxes for either biotype. The highest correlations with NC-S/NC-R biomass ratio were found for AF_{st}Y calculated using the MA_{NC-R South} model with either 40 ppb ($r^2 = 0.459$) or 5 nmol m⁻² s⁻¹ ($r^2 = 0.499$) as a threshold.

All of the preceding analysis used ozone concentrations that were uncorrected for measurement height. To provide a flux-response relationship for the whole dataset, ozone concentration was first corrected for the concentration gradient as described above, and the AF_{st}5 was calculated for three months for each site using the MA_{NC-R South} model. The r^2 for the resulting linear regression was 0.55 (Figure 3.5). The critical level for a 5% reduction in the biomass ratio, calculated using this function was an AF_{st}5 of 1.7 mmol m⁻² PLA.

Flux or AOT Time period for accumulation						
Causal parameter	threshold	28	28 d		onths	
		MA north	MA_{south}	MA north	MA_{south}	
NC-S/NC-R FO ₃ ratio	0	0.096	0.068	0.160	0.118	
NC-S/NC-R FO ₃ ratio	40 ppb	0.052	0.023	0.099	0.041	
NC-S/NC-R FO ₃ ratio	5 nmol m^{-2}	0.058	0.040	0.073	0.070	
NC-S FO ₃	0	0.180	0.238	0.248	0.400	
NC-S FO ₃	40ppb	0.221	0.252	0.338	0.421	
NC-S FO ₃	5 nmol m^{-2}	0.200	0.258	0.275	0.439	
NC-R FO ₃	0	0.199	0.252	0.299	0.449	
NC-R FO ₃	40 ppb	0.222	0.265	0.342	0.459	
NC-R FO ₃	5 nmol m $^{-2}$	0.219	0.277	0.328	0.499	

Table 3.5Regression coefficients (r²) for ozone fluxes calculated using Multiplicative
Algorithms (MA) against NC-S/NC-R biomass ratio accumulated over 28
days or 3 months, using various thresholds

Developing a risk assessment for clover in Europe

The biomonitoring experiments have indicated the extent of damage at sites across Europe and the resulting response functions could be used to establisd critical levels for clover. Methods for applying the response functions to mapping ozone impacts across Europe have been investigated by H Cambridge and S Cinderby at SEI-York, UK. Their progress in devoping such a risk assessment is described here.



Figure 3.5 Flux-effect relationship developed using the $MA_{NC-R \text{ south}}$ model to accumulate $AF_{sl}5$ over three months. Data are for all sites in the network (1996 to 2002) and hourly mean concentrations of ozone have been corrected to canopy height using the concentration gradient method.

The first stage involved mapping the potential location of white clover (*Trifolium repens*) in Europe. This species grows best in humid areas of temperate zones during cool, moist seasons with optimum growth occurring on fertile soils which have adequate soil moisture availability and where competition from other plants is minimized by grazing or clipping. As the white clover used in the experimental studies is a commercial variety and typically sown on managed grasslands, the location of areas of potential growth could be delineated through re-classifying the improved pasture classes (and irrigated meadow as found in Russia) into a single class of potential clover growth. The layer was then aggregated to an EMEP 50km x 50km grid to enable combination with ozone deposition data. However, it is recognised that there are still many uncertainties associated with this potential clover map and efforts to further develop methods for mapping clover are continuing.

To map the potential impacts on clover using the AOT40 dose-response function, AOT40 data for the year 1990 for four three-month periods of the year were obtained from EMEP model output via IIASA. For this exercise, the AOT40 values for the period July, August and September were used in association with the AOT40-response relationship for NCS/NCR biomass ratio. This time period was chosen since it was considered most appropriate to represent the experimental period over which the response relationships were derived.

Figure 3.6 illustrates the spatial distribution of the biomass ratios estimated on the 150x150 km EMEP grid using the AOT40 response relationship described earlier for areas of potential white clover distribution. It shows that the greatest risk for clover biomass reduction due to ozone exposure occurs across France and parts of central Europe with additional "risk areas" located in the Mediterranean region of northern and southern Italy.

The application of the clover flux model on a European scale requires that the clover multiplicative model is ultimately embedded in the EMEP ozone deposition model. So far, it has been possible to model potential stomatal ozone flux at the ICP Vegetation sites contributing stomatal conductance data for those years for which the necessary continuous meteorological data were available, and to relate this to NC-S/NC-R biomass ratios. This work was conducted before the ozone concentration gradient corrections were made, and used a flux-effect model based on AF_{st}5 for MA_{NC-R south} with $r^2 = 0.499$ (Table 3.5). The ability of AF_{st}5 to predict biomass reduction has been compared with that of AOT40 at selected locations (Figure 3.7).



Figure 3.6 NCS/NCR biomass ratios predicted using 1990 AOT 40 for July, August and September for areas of potential clover growth.



Figure 3.7 Accumulated fluxes >5 nmol $m^{-2} s^{-1}$ (AF_{st}5 in mmol m^{-2} PLA) and AOT40 (ppb.h) based ozone indices in relation to NCS/NCR biomass ratios for selected ICP vegetation sites located across Europe.

For interpretation of Figure 3.7 it is worth considering the ideal situation. If both the AOT40 and $AF_{st}5$ indices are "doing well" at describing when damage is likely to occur (i.e. the biomass ratio is reduced) all variables shown on the graphs would adhere to the following trend - lower biomass ratios would be associated with higher AOT40 and $AF_{st}5$. It is useful to look at one of the sites where biomass reduction occurs in Figure 3.7 in detail to explain further how the information provided in this figure can be interpreted. The graphic for Trier city shows biomass reduction to be greatest in 1999, followed by 1997 and 2000 in turn. The magnitude of $AF_{st}5$ inversely mimics this trend, as would be expected if flux is a good descriptor of ozone damage. In contrast, the year with most biomass reduction has a lower AOT40 in comparison to the year with intermediate biomass reduction. This particular example would suggest that the flux index is a better descriptor of biomass reduction than AOT40. However, across the different sites and years there is considerable variability in the relative merits of both indices. On balance, it would appear that $AF_{st}5$ is slightly better at predicting damage compared to AOT40.

Conclusion

The results presented in this chapter provide an overview of the analysis performed so far on the data from the clover biomonitoring experiments (1996 to 2003). The experiment has clearly been successful in establishing that ambient ozone can cause both visible injury and biomass reductions in a sensitive species, and that these types of damage are both widespread across Europe and present in most years. Three modelling methods have been used to develop flux-effect relationships: multivariate statistics, artificial neural networks and multiplicative algorithms. Each has shown that many input factors are needed to describe ozone flux including climatic conditions, phenological factors and ozone. After conversion of ozone concentration to canopy height, the r² value for the three month dose-response relationship showed little difference between AOT40- and AF_{st}5-based response functions (r^2 = 0.58 and 0.55 respectively). However, the two functions are not strictly comparable as the AOT40 function includes high ozone site data from 2003 which is not currently included in the AFst5 function. At this stage in the ongoing analysis, critical levels for biomass reduction could be established as an AOT40 of 2.2 ppm h or an $AF_{st}5$ of 1.7 mmol m⁻² PLA, both accumulated over three months. First efforts to apply the AOT40 response function to a risk assessment for Europe have indicated that biomass reductions potentially as high as 20% could be experienced in central and southern Europe. At most of the example sites investigated, AF_{st}Y appeared to be better correlated with biomass reduction than AOT40. Planned further analysis of the biomonitoring data and development of mapping procedures are described in the next chapter.

4. Conclusions and Further Work

In recent years, the ICP Vegetation has focussed on two air pollution problems of particular importance in the ECE region: quantifying the risks to vegetation posed by ozone pollution and the atmospheric deposition of heavy metals to vegetation. Two further pollution problems are now being considered: plant responses to pollutant mixtures, in particular ozone and nitrogen pollution, and the impacts of nitrogen pollutants on vegetation. Over 150 scientists from 32 countries of Europe and North America contribute to the programme by conducting experiments, sampling vegetation or modelling pollutant deposition and effects. The most recent 17th Task Force Meeting of the ICP Vegetation (Kalamata, Greece, February 2004) attracted 50 participants representing 19 parties to the Convention. During 2003/2004, the ICP Vegetation continued the coordination of the revision of the 'Mapping Critical Levels for Vegetation' chapter of the UNECE Mapping Manual (UNECE, 2004a), which is now available on the web (www.icpmapping.org).

Biomonitoring of ozone impacts on vegetation

Monitoring of the impacts of ambient ozone on vegetation in Europe continued during 2003 using the NC-S (ozone-sensitive) and NC-R (ozone-resistant) biotypes of white clover. A major component of the biomonitoring work was to monitor the incidences of visible leaf injury for the ozone-sensitive biotype by weekly recordings. In 2003, the AOT40 for the three-month period ranged from 0.79 ppm h in Sweden to 23.72 ppm h in Switzerland. The long-term critical level for (semi-) natural vegetation and agricultural crops (a three month AOT40 of 3 ppm h) was exceeded at 80% of the biomonitoring sites. However, visible injury was also recorded at sites where the critical level of ozone for yield reduction was not exceeded. The reduction in biomass associated with ozone over the three-month experimental period in the sensitive biotypes relative to that in the resistant biotypes was similar to previous years.

A review of results from the clover biomonitoring experiment from 1996 to 2003 has shown that O_3 injury occurrence is widespread across Europe following O_3 episodes, and occurred every year at all sites except UK-Bangor and Belgium-Tervuren. Ozone data was standardized to canopy height by taking the ozone gradient into account, and AOT40 and AF_{st}5 dose-response relationships have been derived for effects on biomass. Critical levels representing 5% reduction in biomass have been determined from the data as an AOT40 of 2.2 ppm.h and an AF_{st}5 of 1.7 mmol m⁻² PLA, accumulated over three months. A risk assessment for white clover based on mapping of potential growth across Europe and likely exposure to ozone, indicated that the areas at greatest potential risk of damage were central France and Germany, and parts of Italy.

Pilot studies were continued in 2003 on the effects of ozone on (semi-) natural vegetation using ozone-sensitive and ozone-resistant biotypes of *Centaurea jacea* (brown knapweed). Twelve sites participated in the *Centaurea* biomonitoring experiment in 2003, representing all regions of Europe. Ozone-specific injury was observed on the *Centaurea jacea* plants at eight out of the 12 participating sites. The protocol for exposure and assessment of *Centaurea jacea* to ozone in 2004 has been revised to take into account the experiences of the participants in 2003. The production of clonal material is currently being investigated by participants from Switzerland with the aim of reducing the genetic variability between plants.

Revision of critical levels for ozone

During 2003/2004, the ICP Vegetation continued to coordinate the revision of the 'Mapping Critical Levels for Vegetation' chapter of the Mapping Manual (UNECE, 2004). The main change to the previous Mapping Manual (UNECE, 1996) is the addition of flux-based exposure indices to the concentration-based critical levels for ozone that use AOT40 as the exposure index. There is a sound scientific reason for moving towards the use of flux-based exposure indices. Whereas AOT40 is based on the ozone concentration in the air at the top of the plant canopy, ozone flux calculations take into account the influence of climatic, pollutant and plant factors on the amount of ozone that enters the plant through the stomatal pores and reaches the sites of action inside the leaf. For example, for a given ozone concentration, ozone uptake in dry air is much less than that for the same plants exposed to ozone on a humid day because plants reduce the aperture of their stomatal pores in dry air.

Great care has been taken in revising the Critical Levels chapter of the Mapping Manual to scrutinise and peer-review the scientific basis of the ozone flux modelling methods and the decisions made. This has involved many stages, including discussions at an Expert Panel Meeting in Harrogate (June 2002), the UNECE Workshop on 'Establishing Ozone Critical Levels II' in Gothenburg (November 2002), the 16th Task Force Meeting of the ICP Vegetation in Velenje (January 2003), the 19th Task Force Meeting of the ICP Modelling and Mapping in Tartu (May 2003), the 22nd Session of the WGE in Geneva (September 2003), the 17th Task Force Meeting of the ICP Vegetation in Kalamata (February 2004), and finally the 20th Task Force Meeting of the ICP Modelling and Mapping in Laxenburg (May 2004).

Both the concentration-based and flux-based approaches incorporate the concept that the effects of ozone are cumulative and therefore the risk assessments must incorporate a summation of either ozone concentrations or instantaneous fluxes over the growing season of the vegetation. In all cases, a threshold is used and only concentrations or fluxes above this threshold are incorporated into the risk assessment. The time period over which the concentrations or fluxes above a threshold are accumulated varies with the type of vegetation, as does the critical level above which it is deemed that there are changes in growth, biomass or yield. The new Mapping Manual (UNECE, 2004a) 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 new concentration-based critical level has been defined for horticultural crops and a vapour pressure deficit-modified concentrationbased short-term critical level for visible injury has been defined for crops. Values for the flux-based critical levels for ozone have been identified for the crops wheat and potato and provisionally for the tree species, beech and birch. Further studies on the interactions between influencing factors and ozone are required before flux-based critical levels can be established for (semi-) natural vegetation.

Heavy metal deposition to vegetation

Two approaches to monitoring heavy metal deposition to vegetation are adopted by the ICP Vegetation, one using experimental clover plants from the ozone impacts experiments (see above) and the other a long-term, European-wide survey of the concentration of heavy metals in naturally growing mosses. Whilst the experimental protocol for monitoring the deposition of heavy metals to clover was improved in 2003/4, no experiments were conducted during that period. However, coordinated experiments are being conducted again in 2004 under the supervision of Ludwig de Temmerman (Belgium) using the improved experimental protocol

(UNECE, 2004b). Results of the 2000/2001 European heavy metals in mosses survey were published last year (Buse *et al.*, 2003b). Since then, data received from Iceland have been included in the moss database and in additional publications regarding the 2000/2001 moss survey (Harmens *et al.*, *in press*); preparations have started for the next European moss survey in 2005.

Pollutant mixtures

It is likely that the responses of vegetation to one pollutant are modified by the responses to others. In a pilot study, five of the European sites participating in the ozone biomonitoring study with *Centaurea jacea* exposed the plants to three levels of nitrogen. There was a suggestion that the extent of ozone-specific visible injury on *Centaurea jacea* plants was decreased with increased levels of nitrogen fertilisation, however, not enough sites, particularly those with high ambient ozone conditions participated to draw definite conclusions. Regarding plant biomass production, none of the sites observed an increased sensitivity to ozone with increasing nitrogen fertilisation. However, it is unclear whether increasing nitrogen fertilisation decreased sensitivity of *Centaurea jacea* to ozone or had no effect. The study will be repeated in 2004 with more sites participating, including sites with high ambient ozone conditions and using an improved protocol (UNECE, 2004b).

Future work

Ozone

The ICP Vegetation will continue to monitor the extent of ozone damage to vegetation by conducting standardized experiments with ozone-sensitive species of crops and (semi-) natural vegetation. The formulation and parameterisation of the stomatal conductance model for white clover will be further improved and data will be collected to help to scale up stomatal conductance models from single leaves to the whole canopy. The aim is to produce a canopy flux-effect model for clover, and to use it to develop canopy flux-effect relationships for both ozone injury and biomass reduction. Ultimately, a comparison of the predictive abilities of the single leaf and canopy flux-effect relationships will determine which approach will be the most appropriate to use in future assessments of ozone damage to clover at the pan-European scale. In addition, work will focus on the validation of flux-based critical levels for additional agricultural crop and tree species by reviewing the literature on effects of ozone together with data on stomatal conductance-environmental parameter relationships for these species. Biomonitoring of the impact of ozone on semi-natural vegetation, using *Centaurea jacea* as a model species, will continue with the ultimate aim of completing a flux-effect model for this species.

Work will continue on the ICP Vegetation database on ozone effects on (semi-) natural vegetation, with the aim of mapping (semi-) natural communities at risk from ozone damage, including the link to flux-effect modelling. Maps of the exceedance of the revised critical levels will be produced in collaboration with EMEP/MSC-W. ICP Vegetation encourages the development of a harmonized land cover data set for application under the Convention of Long-range Transboundary Air Pollution, as was agreed at an *ad hoc* expert meeting on 10 March 2004 in Laxenburg, Austria. A new data set will be produced which merges CORINE and SEI (Stockholm Environment Institute) land cover data.

Further work will be carried out on the economic losses associated with exceedance of the new concentration-based and flux-based critical levels of ozone for crops. Analysis of the

uncertainties in concentration- and flux-based methodologies and in the economic analysis will also be made. Further development of the economic assessment of ozone damage to crops will include a refinement of the model structure and considerations of approaches for dealing with impacts that cannot be described in quantitative terms (e.g. visible injury). Regarding the economic assessment, close links will continue to be maintained with the EU programme CAFE (Clear Air For Europe).

Heavy metals

Preparations are already being made for the next heavy metals in mosses survey in 2005. The experimental protocol for 2005 will be revised in consultation with all participants. Reference samples of mosses will be distributed among participants for quality assurance; these samples will be the same as those used in the 1995 moss survey. Analysis of the temporal trends in the existing data set, extending back to 1980, will be undertaken. These trends will be compared with trends in EMEP heavy metal deposition data. In addition, the concentration of heavy metals in mosses in the 2000/2001 survey will be compared with EMEP heavy metal deposition data for validation purposes. Coordinated experiments will be conducted again in 2004 to establish the atmospheric deposition of heavy metals on white clover, using an improved experimental protocol.

Nutrient nitrogen

In a pilot study, spatial and temporal trends in nitrogen deposition to vegetation will be studied for selected European countries by analysing (1) the nitrogen concentration of moss samples from previous moss surveys (dating from 1977 to 2001) and (2) the nitrogen concentration in mosses from herbaria.

Pollutant mixtures

ICP Vegetation will continue to monitor the interacting effects of ozone and nitrogen on *Centaurea jacea* using an improved experimental protocol. In addition, a literature review will be conducted on the modifying effect of other pollutants on the response of crops and (semi-) natural vegetation to ozone.

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Annex 1. Objectives of the ICP Vegetation

Agreed at the 17^{th} meeting of the Programme Task Force, Kalamata, Greece, $10^{\text{th}} - 13^{\text{th}}$ February 2004.

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 experimental data on the responses of (semi-) natural vegetation and crops to air pollutants to validate the critical levels 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 monitor the impacts of ambient ozone on crops and (semi-) natural vegetation.
- 2. To complete the revision of the critical levels of ozone for crops, (semi-) natural vegetation and trees.
- 3. To produce maps of exceedance of the revised critical levels (in collaboration with the ICP Forests, EMEP and the ICP on Modelling and Mapping).
- 4. To further develop ozone flux-response relationships for trees, semi-natural vegetation and additional crop species.
- 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 of 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 the TF Health).
- 8. To prepare for and conduct the 2005 survey of heavy metal concentrations in mosses in Europe.
- 9. To investigate methods for estimating and mapping heavy metal deposition from the heavy metal concentration in mosses data.
- 10. To study the temporal trends in the atmospheric deposition of nitrogen by analysing the nitrogen content of mosses.
- 11. To review the literature on, and conduct studies of, the interactions between ozone and nitrogen.
- 12. To review the literature on the effects of ozone in a changing climate and to consider the possibility of including experimental and modelling work within the programme.

Annex 2. Participation in the ICP Vegetation (2003-2004)

Those participants named in bold are members of the Steering Committee of the ICP Vegetation. In many countries, several other scientists (too numerous to include here) also contribute to the biomonitoring programmes, analysis and modelling procedure that comprise the work programme of the ICP Vegetation.

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			Ozone	Heavy metals	Nitrogen
Austria					
Gerhard Soja	ARC Seibersdorf Research Department of Environmental Research / ULU A-2444 Seibersdorf	gerhard.soja@arcs.ac.at	~		
Alarich Riss	Dept. Terrestrial Ecology Federal Environment Agency Spittelauer Laende 5 A-1090 Vienna	riss@ubavie.gv.at		~	
Harald G. Zechmeister	Institute of Ecology and Conservation Biology University of Vienna Althanstraße 14 A 1090 Vienna	Harald.Zechmeister@univie.ac.at		~	
Belgium					
Ludwig De Temmerman	Veterinary and Agrochemical Research Centre VAR_CODA_CERVA Leuvensesteenweg 17 B-3080 Tervuren	ludet@var.fgov.be	V	~	
Bulgaria					
Lilyana Yurukova	Institute of Botany Bulgarian Academy of Sciences Acad. G.Bonchev Str., Block 23 1113 BG, Sofia	yur7lild@bio.bas.bg		•	
Jordan Stamenov	INRNE Bulgarian Academy of Sciences 72 Tzarigradsko chaussee Blvd. 1784 Sofia	jstamen@inrne.bas.bg		•	
The Czech Republic					
Ivan Suchara and Julie Sucharová	Silva Tarouca Research Institute for Landscape and Ornamental Gardening Kvetnove namesti 391 CZ-252 43 Pruhonice	suchara@vukoz.cz sucharova@vukoz.cz		~	
Denmark					
Martin M Larsen	National Environmental Research Institute Department of Environmental Chemistry P O Box 358 DK-4000 Roskilde	MML@dmu.dk		~	
Estonia					
Siiri Liiv	Tallinn Botanic Garden Kloostrimetsa tee 52 11913 Tallinn	<u>siiri@tba.ee</u>		~	

			Ozone	Heavy metals	Nitrogen
Faroe Islands					
Maria Dam	Food, Veterinary and Environmental Agency Falkavegur 6 FO-100 Tórshavn	mariad@hfs.fo		>	
Finland					
Katinka Ojanpera and Marja-Liisa Vieraankivi	MTT AgriFood Research Finland FIN-31600, Jokioinen	<u>Katinka.Ojanpera@mtt.fi</u>	~		✓
Ahti Mäkinen	Department of Ecology and Systematics P.O. Box 65 (Viikinkaari 1) FIN-00014 University of Helsinki	<u>ahti.makinen@helsinki.fi</u>		✓	
Eero Kubin	Finnish Forest Research Institute Muhos Research Station Kirkkosaarentie 7 FIN-91500 Muhos	<u>Eero.Kubin@metla.fi</u>		~	
Mikael Pihlström	Syst. Biologian Osasto PB7 (Unioninkatu 44) 00014 Hgin Yliopisto	mikael.pihlstrom@helsinki.fi		~	
France					
Guy Landrieu, Regis Farret and Shailendra Mudgal	INERIS Division des Risques Chroniquws BP2, Parc Alata F-60550 Verneuil-en-Halatee	guy.landrieu@ineris.fr	~		
Sandrine Gombert,	Laboratoire de Cryptogamie	sgombert@mnhn.fr		✓	
Catherine Rausch de Traubenberg	Muséum National d'Histoire Naturelle 12 rue Buffon 75231 Paris cedex 05	<u>crausch@mnhn.fr</u>			
Laurence Galsomiès	ADEME, Deptartment Air 27 rue Louis Vicat 75737 Paris Cedex 15	laurence.galsomies@ademe.fr		~	
Germany					
Willy Werner	FBVI - Dept. of Geobotany Faculty of Geography and Geosciences Trier University Universitaetsring 15 D-54286 Trier	werner@uni-trier.de	V		✓
Jürgen Bender, Elke Bergmann and Hans-Joachim Weigel	Institute of Agroecology Federal Research Centre of Agriculture (FAL) Bundesallee 50 D-38116 Braunschweig	juergen.bender@fal.de hans.weigel@fal.de	V		
Andreas Fangmeier, Andreas Klumpp and Jürgen Franzaring	Universität Hohenheim Institut für Landschafts- und Pflanzenökologie (320) Fg. Pflanzenökologie und Ökotoxikologie Schloss Mittelbau (West) 70599 Stuttgart-Hohenheim	afangm@uni-hohenheim.de aklumpp@uni-hohenheim.de franzari@uni-hohenheim.de	~		✓

			Ozone	Heavy metals	Nitrogen
Barbara Köllner and Georg Krause	State Environmental Agency North-Rhine Westphalia Wallneyer Str. 6, D-45133 Essen	Barbara.Koellner@lua.nrw.de georg.krause@lua.nrw.de	~		
Christoph Schlüter	Federal Environment Agency Task Force on Mapping UBA, Bismarck pl. 1 D-14191 BERLIN	christoph.schlueter@uba.de		✓	
Winfried Schröder and Roland Pesch	Hochschule Vechta Institute für Umweltwissenschaften Postfach 1553 D-49364 Vechta	wschroeder@iuw.uni-vechta.de rpesch@iuw.uni-vechta.de		~	
Greece					
Dimitris Velissariou	Technological Educational Institute of Kalamata Antikalamos 241 00 Kalamata	d.velissariou@teikal.gr	~		
Pavlina Drogoudi	Pomology Institute National Agricultural Foundation PO Box 122 59200 Naoussa	pdrogoudi@alfanet.gr	~		
Costas Saitanis	Agricultural University of Athens Laboratory of Ecology & Environmental Sciences Iera Odos 75 Botanikos 11855, Athens	saitanis@aua.gr	~		
Hungary					
Gábor Turcsanyi	Institute of Environmental Management Szent Istvan University Godollo	tgabor@fau.gau.hu		✓	
Iceland					<u> </u>
Sigurður Magnússon	Icelandic Institute of Natural History Hlemmur 3 125 Reykjavík	sigurdur@ni.is		~	
Ireland Alison Donelly	Centre for the Environment Department of Botany University of Dublin Trinity College, Dublin 2, Dublin	Alison.Donnelly@tcd.ie	~		
Italy					
Armando Buffoni	Instituto di Ricerche Ambiente Italia Via Carlo Poerio 39 20124 Milano	<u>ab-mi@libero.it</u>		✓	
Giovanna Martignon	CESI SpA Via Reggio Emilia, 39 20090 Segrate (MI)	martignon@cesi.it	~		

			Ozone	Heavy metals	Nitrogen
Luigi Postiglione and Massimo Fagnano	Dip. di Ingegneria agraria ed Agronomia del Territorio Università degli studi di Napoli Federico II Via Università 100 80055 Portici (Naples)	postigli@unina.it fagnano@unina.it	V		
Cristina Nali, Giacomo Lorenzini and Chiara Pucciariello	Dipartimento Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi" Via del Borghetto 80 56124 Pisa	<u>cnali@agr.unipi.it</u>	~		
Fausto Manes,	Dipartimento di Biologia Vegetale Università di Roma "La Sapienza" P.le Aldo Moro, 5 I-00185 Rome	fausto.manes@uniroma1.it	×		
Renate Alber	Environmental Agency Biological Laboratory Bolzano via Sottomonte 2 I-39055 Laives	Renate.Alber@provinz.bz.it		~	
Francesca Fornasier, Roberto Caracciolo	ANPA Via Vitaliano Brancati 48, 00144 Rome	ffornasier@inwind.it		~	
Latvia Olgerts Nikodemus	Faculty of Geography and Earth Sciences University of Latvia 19 Raina blvd, Riga, LV - 1586	nikodemu@latnet.lv		~	
Guntis Brumelis and Guntis Tabors	Faculty of Biology University of Latvia 4 Kronvalda blvd Riga, LV – 1842	<u>moss@latnet.lv</u> <u>guntis@lanet.lv</u>		~	
Lithuania					
Kestutis Kvietkus	Institute of Physics Gostauto 12 2600 Vilnius	kvietkus@ktl.mii.lt		~	
Darius Ceburnis	Department of Experimental Physics University College Galway Galway, Ireland	darius.ceburnis@nuigalway.ie		~	
The Netherlands					
Tom Dueck	Plant Research International for Agrobiology and Soil Fertility Bornsesteeg 65 PO BOX 14 6700 AA Wageningen	<u>th.a.dueck@plant.wag-ur.nl</u>	V		
Bert Wolterbeek	Interfaculty Reactor Institute Delft University of Technology Mekelweg 15 NL-2629 JB Delft	H.T.Wolterbeek@iri.tudelft.nl		~	
Norway					
Eiliv Steinnes	Department of Chemistry Norwegian University of Science and Technology NO-7491 Trondheim	Eiliv.Steinnes@chem.ntnu.no		~	

			Ozone	Heavy metals	Nitrogen
Linn Bryhn Jacobsen	Norwegian Pollution Control Authority P.O.Box 8100 Dep N-0032 Oslo	linn.bryhn-jacobsen@sft.no		↓	
Poland					
Krystyna Grodzińska and Grażyna Szarek- Łukaszewska	Institute of Botany Polish Academy of Sciences Lubicz 46 31-512 Krakow	grodzin@ib-pan.krakow.pl		~	
Klaudine Borowiak	Department of Ecology and Environmental Protection August Cieszkowski Agricultural University of Poznan Ul. Piatkowska 94C 61-691 Poznan	<u>klaudine@owl.au.poznal.pl</u>	V		
Portugal					
Rui Figueira	Jardim Botânico da Universidade de Lisboa R. Escola Politécnica , 58 1250-102 Lisboa	pcrfigeria@alfa.ist.utl.pt		✓	
Romania					
Adriana Lucaciu	National Institute of Physics and Nuclear Engineering Horia Hulubei Atomistilor 407, MG-6 76900 Bucharest	lucaciu@ifin.nipne.ro		~	
Eugen Lörinczi	Str. COZIA nr7 1900 Timisoara	consultim@mail.dnttm.ro		~	
Russian Federation					
Marina Frontasyeva	Frank Laboratory of Neutron Physics Joint Institute for Nuclear Research, Joliot Curie 6 141980 Dubna Moscow Region	<u>marina@nf.jinr.ru</u>		~	
Natalia Fedorets and Olga Bakhmet	Forest Research Institute of Karelian Research Centre of Russian Academy of Sciences Pushkinskaya st., 11 185610 Petrozavodsk	fedorets@krc.karelia.ru bakhmet@krc.karelia.ru		~	
Natalia Goltsova	Biological Research Institute St.Petersburg State University Oranienbaumskoe schosse 2 Petrodvoretz, St. Petersburg RUS-198903	Natalia.Goltsova@pobox.spbu.ru corina@mail.dux.ru		~	
Serbia and Montenegro					
Marko Saboljevic	Department of Plant Ecology and Phytogeography Institute of Botany Faculty of Biology University of Belgrade Takovska 43 YU-11000 Belgrade	marko@bfbot.bg.ac.yu		~	

			Ozone	Heavy metals	Nitrogen
Slovakia					Ĺ
Blanka Maňkovská	Forest Research Institute T.G.Masaryka str.22 960 92 Zvolen	mankov@fris.sk		~	
Matej Florek	Deparment of Nuclear Physics Comenius University Mlynská Dolina, F1 842 15 Bratislava	florek@fmph.uniba.sk		~	
Slovenia					
Franc Batic, and Boris Turk	University of Ljubljana, Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, 1000 Ljubljana	franc.batic@bf.uni-lj.si boris.turk@uni-lj.si	~		
Nataša Kopušar	ERICO Velenje Ecological Research & Industrial Cooperation Koroška 58, 3320 Velenje	natasa.kopusar@erico.si	~		
Zvonka Jeran	Jožef Stefan Institute Department of Environmental Sciences, Jamova 39 1000 Ljubljana	zvonka.jeran@ijs.si		~	
Spain					
J. Angel Fernández Escribano and Alejo Carballeira Ocaña	Ecologia Facultad De Biologia Univ. Santiago de Compostela	<u>bfjafe@usc.es</u> <u>bfalejo@usc.es</u>		~	
Ben Gimeno , Victoria Bermejo and Daniel de la Torre	15782 Santiago de CompostelaDepartamento de ImpactoAmbiental de la EnergíaCIEMAT, Ed 70Avda. Complutense 2228040 Madrid	benjamin.gimeno@ciemat.es	~		~
Jose Luis Porcuna and Carmen Ocón	Servicio de Sanidad Vegetal Apartado 125 46460 Silla (Valencia)	porcuna_jos@gva.es	√		
Josep Penuelas Angela Ribas	CSIC-CREAF (Center for Ecological Research and Forestry Applications) Facultat de Ciències Universitat Autònoma de Barcelona 08193 Bellaterra (Barcelona)	Josep.Penuelas@uab.es	~		
Jesus Santamaria, Juan Jose Irigoyen and Raúl Bermejo-Orduna	Departmento de Quimica y Edafologia Universidad de Navarra Facultad de Ciencias Irunlarrea s/n 31080 Pamplona	<u>chusmi@unav.es</u>	V		
Sweden					
Per-Erik Karlsson, Gunilla Pihl Karlsson and Helena Danielsson	IVL Swedish Environmental Research Institute PO Box 47086, S-402 58 Göteborg	pererik.karlsson@ivl.se	~		
Håkan Pleijel	Applied Environmental Science Göteborg University, Box 464 S-40530 Göteborg	hakan.pleijel@miljo.gu.se	~		
Åke Rühling		ake.ruhling@telia.com		\checkmark	

			Ozone	Heavy metals	Nitrogen
Switzerland					
Jürg Fuhrer , Franziska Keller and Seraina Bassin	Federal Research Station for Agroecology and Agriculture (FAL) Reckenholzstr. 155 CH-8046 Zurich	juerg.fuhrer@fal.admin.ch franziska.keller@fal.admin.ch <u>seraina.bassin@fal.admin.ch</u>	~		
Lotti Thöni	FUB-Research Group for Environmental Monitoring Untere Bahnhofstr.30 Postfach 1645 CH-8640 Rapperswil	lotti.thoeni@fub-ag.ch		~	
United Kingdom					
Harry Harmens (Chairperson of ICP Vegetation), Gina Mills, Felicity Hayes and Phil Williams	CEH Bangor Orton Building Deiniol Road Bangor Gwynedd LL57 2UP	<u>hh@ceh.ac.uk</u> <u>gmi@ceh.ac.uk</u> <u>fhay@ceh.ac.uk</u> <u>pdwi@ceh.ac.uk</u>	V	~	✓
Lisa Emberson, Steve Cinderby, Howard Cambridge and Patrick Büker	Stockholm Environment Institute at York University of York Box 373 York YO1 5YW	l.emberson@york.ac.uk	V		
Sally Power, Emma Green and Sally Gadsdon	Department of Environmental Science and Technology, Imperial College, Silwood Park, Ascot, Berkshire SL5 7PY	<u>s.power@imperial.ac.uk</u>	V		~
Mike Ashmore, Andrew Terry, Ben Haworth, Khalid Choudhury and Laura Shotbolt	University of Bradford Department of Environmental Science West Yorkshire BD7 1DP	<u>m.r.ashmore@bradford.ac.uk</u> <u>a.c.terry@bradford.ac.uk</u>	V	~	~
Mike Holland	EMRC 2 New Buildings Whitchurch Hill Reading RG8 7PW	mike.holland@emrc.co.uk	✓		
Susan Parry	Department of Environmental Science and Technology Imperial College of Science, Technology and Medicine Silwood Manor, Silwood Park Ascot, Berks SL5 7PY	<u>s.parry@ic.ac.uk</u>		~	
Ukraine					
Oleg Blum	M.M. Grishko National Botanical Garden National Academy of Sciences of Ukraine Timirazevska Str. 1 Kyiv-01014	<u>blum@cbg.freenet.kiev.ua</u>		~	

			Ozone	Heavy metals	Nitrogen
USA					
Filzgerald Booker and Kent Burkey	US Department of Agriculture ARS N.C. State University 3908 Inwood Road Raleigh North Carolina 27603	fbooker@mindspring.com	~		
Margaret Tuttle McGrath	Department of Plant Pathology Cornell University Long Island Horticultural Research and Extension Center 3059 Sound Avenue Riverhead, NY 11901-1098	mtm3@cornell.edu	V		

Air Pollution and Vegetation UNECE ICP Vegetation Annual Report 2003/2004

This report describes the recent work of the International Cooperative Programme on effects of air pollution on natural vegetation and crops (ICP Vegetation), a research programme conducted in 32 countries of Europe plus the USA. Reporting to the Working Group on Effects of the Convention on Long-range Transboundary Air Pollution, the ICP Vegetation is providing information for the revision of international protocols to reduce air pollution problems caused by, for example, tropospheric ozone, nitrogen and heavy metals. Progress and recent results from the following activities are reported:

- Biomonitoring programmes that indicate the geographical extent of ozone damage on sensitive vegetation, including crops and (semi-) natural species.
- Analysis of trends in ozone biomonitoring data (1996 to 2003).
- Studies into establishing flux-based critical levels of ozone for crops, trees and (semi-) natural vegetation.
- Monitoring heavy metal deposition to crops and mosses.

For further information or copies contact: Harry Harmens CEH Bangor Orton Building Deiniol Road Bangor Gwynedd LL57 2UP United Kingdom Tel: +44 (0) 1248 370045 Fax: +44 (0) 1248 355365



Centre for Ecology & Hydrology

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