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INSTITUTE OF TERRESTRIAL ECOLOGY
(Natural Environment Research Council)

Final Contract Report
Volume 1
EPG 1/3/96

The UN/ECE International Cooperative Programme on Natural Vegetation and Crops

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March 2000

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Background information

By signing the Protocols of the UN/ECE Convention on Long-range Transboundary Air Pollution (LRTAP), the countries of Europe and North America (the ECE region) agree to reduce emissions, to different degrees, by 2010. Most recently, a commitment has been made to reduce the problems associated with acidification, nutrient nitrogen, and ozone by controlling the emissions of sulphur, oxidised and reduced forms of nitrogen, and volatile organic compounds (the Multi-pollutant, Multi-effect Protocol, December, 1999). The negotiations for the Protocols rely on sound scientific knowledge on the effects of pollutants on the environment and health. This information is provided for the Convention by the International Cooperative Programmes (ICPs) of the Working Group on Effects. Each ICP is led by a different country, and specialises in effects on different components of the environment (e.g. forests, rivers and lakes, buildings and cultural heritage). The UK leads the ICP that specialises in the effects of air pollution, primarily ozone, on natural vegetation and crops (ICP Vegetation) by funding a Coordination Centre at the Bangor Research Unit of the Institute of Terrestrial Ecology (ITE-Bangor).

Participants from 38 sites spread over 17 ECE countries participate in the ICP Vegetation by monitoring the impacts of ambient ozone episodes on sensitive species. The data are used to establish and validate critical levels (or thresholds) above which detrimental effects of ozone can be detected in the ECE region. The so-called "level I" long-term critical level for ozone of an AOT40[1] of 3 ppm.h accumulated over three-months was considered to protect against 5% yield reduction in sensitive crops and was included in the negotiations for the Multi-pollutant, Multi-effect Protocol. However, for the future revision of the Protocol anticipated in 2003/04, it was concluded that the modifying influence of several factors such as humidity and soil moisture content on ozone flux and hence effect must be taken into consideration (a "level II" approach). Much of the work of the ICP Vegetation during the last three years has focussed on improving the relevance of the critical levels for ozone by incorporating these level II factors into models for predicting the impact of ozone on vegetation. The ICP has also monitored heavy metal deposition to clover as a contribution to the negotiations for the UN/ECE Heavy Metals Protocol.

[1] AOT40 (Accumulated dose over a threshold of 40 ppb) is 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.

Quantifying the extent of the ozone problem in the ECE region
The long-term critical level was exceeded at over 70% of the ICP Vegetation sites in each of the three years, with the greatest exceedance occurring in 1999 (86% of sites). It was not exceeded at any of the UK sites during the three years of this study (Nottingham in 1997, Ascot in 1998, and Bangor in 1998 and 1999). As expected, ozone concentrations increased with decreasing latitude at rural locations, but local sources of NO_x reduced levels at some semi-urban sites in southern Europe. The highest AOT40 values were recorded in northern Italy and North Carolina (USA) where values were 7 - 11 times the current critical level. As shown by other studies (e.g. EMEP models), no trends with time were found in the ICP Vegetation ozone data from 1990 to 1999 due to a marked year-to-year variation in the frequency of episodes and hence the three-month AOT40.

The ozone climate of Europe caused visible injury to occur on the test species (white clover, *Trifolium repens* cv Regal) at least once at every site in the network in each year (1997-1999). There was a general tendency for the frequency of injury-causing episodes to be higher at sites in the more southern latitudes, although injury was quite prevalent at the northern site of Sweden-Östad, where high humidity increased ozone uptake. Participants in the ICP have also compiled a list of incidences of injury in commercial fields of 9 agricultural crops and 13 horticultural crops. Some of the most important European crops such as wheat, maize, soybean and grapevine are included in the list.

By comparing the biomass of an ozone-sensitive biotype of white clover against that of an ozone-resistant biotype at each site in each year, the participants have shown that ambient ozone pollution regularly reduces growth in southern Europe, with occasional reductions occurring in central and northern Europe. When all of the biomass data were combined together, AOT40 was found to be the parameter with the best fit to the data.

Developing a "level II" model for clover
The data from the clover experiments were used to develop a model of the factors influencing the biomass ratio. Over 240 combinations of up to 21 inputs such as daylight mean temperature, mean NO concentration when the ozone concentration exceeds 40 ppb and the 7h mean ozone concentration, were tested until a model could be selected that performed well for both the data used to develop it and previously unseen data. Multiple linear regression was less good at generalising than non-linear Artificial Neural Networks (ANNs, a form of pattern recognition software). The best performing ANN model had two inputs that described the ozone conditions (AOT40 and the 24h mean ozone concentration, $O_{3\ 24h}$), two temperature inputs (daylight mean and 24h mean), and an NO parameter that was differentiating between rural and semi-urban sites. This model had an r^2 value of 0.84 for the data used to develop it and 0.71 for previously unseen data. It performed considerably better than single ozone factor models that had r^2 values of 0.41 for an ANN and 0.28 for linear regression. The model predicted that the AOT40 required to reduce biomass by 5% over 28 days at an average temperature of 19°C ranged from 0.9 ppm.h in conditions of background ozone close to 40 ppb and low NO (<2 ppb), to 1.65 ppm.h with lower background ozone (18 ppb, $O_{3\ 24h}$) and higher NO (8 ppb).

Predicting wheat yield and clover biomass reductions in Europe

Two contrasting wheat modelling methods predicted that ozone impacts on yield were likely to be highest in northern Italy, most of France, Belgium the Netherlands, Germany and parts of southern Scandinavia. Wheat matures earlier in Spain, Portugal and Greece, and is less likely to be sensitive to ozone at the time when the worst ozone episodes occur in these countries. Both modelling approaches showed that the timing of ozone episodes in relation to growth stage, and the modifying influence of soil moisture and humidity on ozone flux were the main "level II" factors influencing the magnitude of response to ozone. Phenological and soil moisture factors were not considered in the model for clover because it was developed from data from plants that were well watered and had a shorter growth period of 28d. This allowed other level II factors (background ozone, temperature, and proximity to sources of NO_x) to be considered. The zone of highest effect on clover was found to be further south and covered Spain, southern France, Italy, Greece and Slovenia. If such findings are applicable to all ozone-sensitive crops that are usually irrigated in these areas, then effects of ozone could be quite considerable. Indeed, on a local scale, one participant reported that an ozone episode in 1998 caused complete loss of an irrigated lettuce crop in the Acharnes area of Greece.

Effects of ozone on natural vegetation

The impacts of ozone on natural vegetation were much more difficult to consider on a Europe-wide scale due to the rich diversity of ecosystems, species and biotypes. Ambient ozone can cause visible injury and/or growth reductions in sensitive species, yet neither a literature review nor a modelling approach succeeded in completely identifying the plant factors associated with ozone sensitivity. Nevertheless, some patterns were emerging as species with a ruderal or competitor growth strategy, a high relative growth rate, and a high stomatal conductance had the potential to be ozone-sensitive. Further work in this area would eventually lead to the identification of plant communities that have a high proportion of ozone-sensitive species and could be classified as being "at risk" from ozone pollution.

Monitoring heavy metal deposition to clover

Concern over the impacts of heavy metals on the environment and health led to the development of the Heavy Metals Protocol (signed in 1998), which commits countries to a reduction in emissions. Analysis of the lead, cadmium, copper and arsenic content of clover at 18 ICP Vegetation sites showed that concentrations were highest at sites like Italy-Rome and Germany-Cologne where dust deposition was likely to be affected by local traffic and industrial sources. Plants grown at sites in rural areas of Austria, The Netherlands and Germany were away from local influences and thus their mid-range heavy metal contents were more likely to have resulted from longer-range transport, possibly transboundary. Since these sites were in the areas predicted by ESQUAD and EMEP to have relatively high heavy metal deposition, it seems reasonable to accept that the clover system can be used to validate these maps. Additional leaf area measurements planned for the year 2000 sampling season will facilitate calculation of deposition rates for clover.

Output from the ICP Vegetation

The results of the programme have been disseminated in various ways. For the interested public and non-specialist, the work and findings of the programme can be found on the internet (www.nmw.ac.uk/ite/bang/ICPVegetation/home.htm) and in a colour brochure obtainable from the Coordination Centre. Progress reports and technical reports have been presented to the LRTAP Convention each year, and 12 scientific papers have been submitted for publication during the contract.

Future development
The experimental programme will be expanded to include experiments with clover planted directly into commercial fields, and with species/biotypes of natural vegetation. A flux-effect model will be developed for clover from these results and will be used to further improve level II critical level exceedance maps for Europe. Flux modelling for wheat will also be developed further using conductance data collected by ICP Vegetation participants. Experiments with natural vegetation are being developed in preparation for inclusion in the ICP Vegetation network. Work on heavy metals will be expanded considerably by performing a second, more detailed sampling regime in 2000. Results will be compared with those from the European Mosses programme, which will become part of the ICP Vegetation in 2001. The latter involves the sampling of mosses in over 30 countries and analysis of the content of 10 heavy metals.

ACKNOWLEDGEMENTS

We wish to thank the UK Department of the Environment, Transport and the Regions for the continued financial support of the ICP Vegetation (Contract EPG 1/3/96). The support of the Scottish Executive, the National Assembly for Wales and the Department of Environment in Northern Ireland is acknowledged. The contributions of the ICP Vegetation Steering Committee, (Professor Jürg Fuhrer, Dr Ludwig De Temmerman and Dr Ben S Gimeno) and of the ICP Vegetation participants are gratefully acknowledged. We would like to thank Dr Allen Heagle (US Department of Agriculture, Raleigh, North Carolina) for supplying the clover clone material, Dr Lisa Emberson of SEI-Y, and Dr Max Posch of the Coordination Centre for Effects for supplying the maps presented in Section 3 and Dr Dominic Palmer-Brown for his assistance with the modelling work. Finally, Ms Jackie Cooper is thanked for providing clerical support for the programme, and for compiling this report.

ABBREVIATIONS AND DEFINITIONS USED IN TEXT

Abbreviations

ANNs	Artificial neural networks
AOT40	Accumulated over a threshold of 40 parts per billion
CCE	Coordination Centre for Effects
CL	Critical level
EMEP	Cooperative Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe
GR	Global radiation
ICP Vegetation	International Cooperative Programme on effects of air pollution on natural vegetation and crops
ICPs	International Cooperative Programmes
LSR	Least squares regression
LRTAP	Long-Range Transboundary Air Pollution
PAR	Photosynthetically active radiation
ppb	Parts per billion by volume
ppb.h	Parts per billion hours (units of accumulated dose)
%FC	Percentage field capacity
%RH	Percentage relative humidity
%Y	Relative grain yield of wheat
SD	Standard deviation
SE	Standard error
SEI-Y	Stockholm Environment Institute at York
SMD	Soil moisture deficit
TF	Task Force
TFEAAS	Task Force on Economic Aspects of Abatement Strategies
UN/ECE	United Nations/Economic Commission for Europe
VPD	Vapour pressure deficit
WGE	Working Group on Effects

Ozone Exposure Parameters

(i) **7h mean**

Mean ozone concentration for the seven hours in the day with the highest ozone concentration (usually 1000 - 1700h GMT). This parameter is averaged over the growing season/period unless otherwise stated. Units: ppb.

(ii) **Mean daily maximum**

Mean of the daily maximum hourly ozone concentration for each day of the growing season/period. Units: ppb.

(iii) **AOT40**

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 (when the clear sky radiation is greater than 50 Wm^{-2}). Units : ppb.h or ppm.h.

Critical Levels of Ozone for Agricultural Crops

(i) **Critical Level**

Concentration of pollutants in the atmosphere above which adverse effects occur on sensitive receptors, such as plants, ecosystems or materials according to present knowledge.

(ii) **Long-term critical level of ozone for yield reduction in crops**

An AOT40 of 3000 ppb.h accumulated over three months.

(iii) **Short-term critical levels of ozone for injury development**

An AOT40 of 500 ppb.h accumulated over 5 days when mean vapour pressure deficit (9.30 – 16.30 h) exceeds 1.5 kPa.

An AOT40 of 200 ppb.h accumulated over 5 days when mean vapour pressure deficit (9.30 – 16.30 h) is below 1.5 kPa.

1 Introduction

1.1 Objectives of Contract EPG 1/3/96

Contract EPG 1/3/96 covers the coordination and data modelling costs for the UN/ECE ICP Vegetation[2] (formerly ICP Crops). The ICP reports to the Working Group on Effects (WGE) of the Convention on Long-range Transboundary air pollution (LRTAP Convention), on the effects of air pollutants, primarily ozone, on natural vegetation and crops. Participants from 17 countries contribute to the programme by: conducting experiments with ozone-sensitive species; monitoring pollutant and climatic conditions; developing critical levels models for ozone and using them for exceedance mapping; and by measuring the heavy metal content of plants. Experimental data and models are used to understand and quantify the influence of modifying (level II) factors such as humidity and soil moisture deficit on the critical levels for ozone, and contribute to the further development of the recently signed UN/ECE multi-pollutant/multi-effect protocol. Measurements of the heavy metal content of vegetation are being used for the further development of the UN/ECE heavy metals protocol.

The objectives of contract EPG 1/3/96 were:

- To perform the experiments, data processing and administration required as the Coordination Centre for the UN/ECE ICP Vegetation.

- To act as National Focal Centre for the ICP Vegetation and attend all ICP Vegetation Task Force Meetings, Task Force on Mapping, and Working Group on Effects Meetings.

- To use an artificial neural network modelling strategy to interpret the complex relationships between level II factors influencing ozone damage, ozone parameters, and the effects of ozone on crops and natural vegetation in Europe.

- To produce predictive models of minimum complexity that can be used to propose revised definitions for the short-term and long-term critical levels for ozone which include only the most influential level II factors.

- To develop mapping procedures and maps for the stocks at risk from ozone pollution in Europe and to use ICP Vegetation data to illustrate where damage is occurring.

- To address issues arising under the LRTAP Convention which are of relevance to the ICP Vegetation e.g. the potential impact of heavy metals on crops and natural vegetation.

[2] United Nations Economic Commission for Europe International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops.

1.2 Background

In the late 1980s, the International Cooperative Programme on the effects of air pollution on natural vegetation and crops (ICP Vegetation, formerly ICP Crops) was established to consider the underlying science for quantifying damage to plants by ozone and other pollutants. Scientists from the following countries currently participate in the ICP Vegetation: Austria, Belgium, Finland, France, Germany, Greece, Ireland, Italy, Netherlands, Poland, Russian Federation, Slovenia, Spain, Sweden, Switzerland, UK and USA. The programme is led by the UK and coordinated by the Institute of Terrestrial Ecology at Bangor (ITE-Bangor).

The ICP Vegetation is one of several ICPs and Task Forces that report to the WGE of the LRTAP Convention on effects of pollutants on waters, materials, forests, ecosystems, health, and on mapping their effects in the ECE region (Figure 1.1). The protocols of the LRTAP Convention commit countries to reducing pollutant emissions by specific target years. Results from the ICPs are used in both the development of these protocols, and in monitoring their success in reducing the impacts of air pollutants on health and the environment. The multi-pollutant/multi-effect protocol was the most recent (December, 1999), and was designed to address the problems of acidification, nutrient nitrogen, and tropospheric ozone by controlling emissions of sulphur, nitrogen (oxidised and reduced forms) and volatile organic pollutants.

The negotiations concerning ozone for the multi-pollutant/multi-effect protocol were based on exceedance of a so-called level I long-term critical level of ozone for crops and natural vegetation. This value, an AOT40[3] of 3 ppm.h accumulated over three-months was set at the workshop on *Critical Levels for Ozone in Europe: Testing and finalizing the Concept* (Kuopio, Finland, March, 1996) and was considered to be the lowest AOT40 at which significant yield loss due to ozone could be detected, according to current knowledge (UBA, 1996). It was derived from a robust dose-response relationship established from open-top chamber (OTC) exposure experiments with wheat conducted with 10 cultivars, in 6 countries over a period of 10 years. For future revision of the protocol, it was concluded that the level I approach must be expanded to include the modifying influence of climatic conditions, other pollutants, pests, diseases etc. (a level II approach) since these factors will influence the flux of ozone to plants and hence its effect. This is especially important because the current critical level was derived from exposure experiments in OTCs where it is widely acknowledged that ozone flux is enhanced by constant air flow and effects are likely to be over-estimated for a given AOT40 (Pleijel, 1996). Furthermore, the OTC experiments were mainly conducted during the 1980s and often used exposure regimes that were well above the critical level and usually involved daily additions of ozone that did not truly reflect the episodic nature of ozone pollution.

Throughout the three years of contract EPG 1/3/96, ICP Vegetation participants have been working towards a level II approach for ozone critical levels. Data from the ICP Vegetation field experiments have been used in the development of artificial neural network (ANN) methods with the intention of developing a level II model with

[3] 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.

minimum complexity. This was a follow-on from previous modelling work at the Coordination Centre that had shown that ANNs were very useful for detecting patterns in the large "noisy" datasets typically associated with field-based experiments (Balls *et al* 1996, Roadknight *et al* 1997). At the same time, methods for calculating ozone flux for wheat have been developed (funded by DETR contract EPG 1/3/104) as well as a method for inserting modifying factors into the AOT40-yield response function for wheat. The results of these three level II approaches were discussed in detail at the *Critical Levels for Ozone - level II Workshop* (Gerzensee, Switzerland, April 1999), and research needs for the planned revision of the multi-pollutant/multi-effect protocol in 2003/04 were identified (Fuhrer and Achermann, 1999).

By conducting experiments in ambient air, the ICP Vegetation has established a unique database for both developing new critical levels models and for validating the models developed by others. Since 1996, ozone-sensitive (NC-S) and ozone-resistant (NC-R) clones 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 have been 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 clone. The clover clone system was chosen because the forage biomass for both clones was similar in conditions of low ozone, but lower for the NC-S clone 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. The clover clone system replaced the existing experiment in which ethylene diurea (EDU) was used as a protectant against ozone injury on clover (see Ball *et al*, 1998). The new system avoided the uncertainties associated with climatic influences on EDU uptake and provides a better indicator of effects on biomass.

A new development for the ICP Vegetation has occurred during the contract in that we have been asked to monitor heavy metal deposition to vegetation. This request was made because of the lack of biological data to validate the models used in the negotiations for the heavy metals protocol (UBA, 1997). The ICP Vegetation responded by incorporating analysis of lead and cadmium content of the clover material into the experimental programme. These two metals were chosen because of international concern over their environmental and health effects. Two other metals, copper and arsenic were also included in the analysis because they are likely to be considered in the next revision of the protocol.

This report provides an overview of the main findings from the ICP Vegetation for the period April, 1997 to March, 2000. Following an introduction in Section 1, the results of the clover clone experiment and their incorporation into a level II ANN model are described in Section 2. The contribution of the model to mapping level II exceedance is considered in Section 3 along with progress with other level II mapping approaches. The frequency of occurrence of ozone injury in Europe is considered in Section 4 and the evidence for trends in the ozone and biological data is considered in Section 5. Section 6 describes the current status of knowledge on ozone effects on natural vegetation together with an overview of natural vegetation experiments being conducted by ICP Vegetation participants. Heavy metal deposition to clover is considered in Section 7, and the main conclusions are described in Section 8.

Executive Body

- WG Strategies and Review
- EMEP
- WG Effects

International Co-operative Programmes

- Mapping (Germany)
- Integrated Monitoring (Sweden)
- Forests (Germany)
- Freshwaters (Norway)
- Materials (Sweden)
- Vegetation (UK)
- Health (WHO)

Figure 1.1 The Structure of the UN/ECE Convention on Long-range Transboundary Air Pollution

1.3 The Specific Objectives of the ICP Vegetation.

Each year, the ICP Vegetation participants review the objectives of the programme to ensure that current developments within the LRTAP Convention are being addressed. The following objectives were revised at the 13th Task Force Meeting (Semmering, Austria, January 2000) to reflect changes in the structure of the Convention, but with minor changes in wording have held throughout contract EPG 1/3/96:

Long-term objectives

1) To meet the requirements of the UN/ECE Convention on Long-range Transboundary Air Pollution for information on the responses of natural vegetation and crops to atmospheric pollutants.

2) To evaluate experimental data on the responses of natural vegetation and crops to ozone 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-based protocols such as those for heavy metals and POPs, with respect to natural vegetation and crops.

Short- and medium- term objectives

1) To incorporate Level II factors into the long-term critical level of ozone for crops.

2) To produce maps of exceedance of critical levels which incorporate Level II factors (in collaboration with EMEP and the ICP on Mapping).

3) To develop dose-response functions and "stocks at risk" information for use in an economic assessment of crop losses due to ozone.

4) To conduct literature reviews and specific experiments to provide further information on the critical levels of ozone for selected plants, plant communities and biodiversity.

5) To initiate literature reviews and experiments on the accumulation of atmospheric deposition of heavy metals by selected plants.

1.4 Coordination of the programme

1.4.1 Organisation

Contract EPG 1/3/96 was initially let to the Chairperson, Dr G. Mills, at Nottingham Trent University (April, 1997), but was transferred to ITE-Bangor in April 1998 following the appointment of Dr Mills as a Senior Scientific Officer. From April, 1998, the ICP Vegetation Coordination Centre has been at ITE-Bangor where the programme of research has been managed by Dr Mills, with assistance from Ms Felicity Hayes (Scientific Officer). A Data Modelling Centre was established in the Department of Computing, Nottingham Trent University and managed by Dr Graham Ball (Research Fellow) with advice from Dr Dominic Palmer-Brown (Senior Lecturer). The ICP Vegetation also benefits from advice from three international experts (Professor J. Fuhrer (Switzerland, Dr B Gimeno (Spain) and Dr L DeTemmerman (Belgium)), who together with Dr Mills, comprise the Steering Committee of the programme.

1.4.2 Experimental Programme (1997 – 1999)

The experimental programme of the ICP Vegetation has been revised each year to reflect the changing needs of the LRTAP Convention. The activities fell into three groups:

Activity One: The Clover Clone Experiment
Ozone-sensitive and -resistant clones of white clover were grown at approximately 35 sites each year (e.g. Figure 1.2). Every 28 days, the biomass was harvested, and the clones were assessed for visible injury. Artificial neural network (ANN) models have been used to analyse the data in order to identify and quantify the impact of the key factors influencing ozone sensitivity. Stomatal conductance has been measured to assist with the development of flux-effect relationships.

Activity Two: Surveys of Commercial Crops
The phenology of wheat, sugar beet and maize were monitored in commercial fields close to experimental sites. The data is being used to identify the timing of ozone-sensitive growth stages in Europe for use in the development of phenologically-weighted critical level exceedance maps.

Activity Three: Natural Vegetation
A review of the literature on the effects of ozone on natural and semi-natural vegetation was conducted at the Coordination Centre. Initial screening experiments.

Figure 1.2: The ICP Vegetation site at Abergwyngregyn (near Bangor)

were conducted in the temperature-controlled solardomes to identify suitable species for use in a new biomonitoring programme

Activity Four: Heavy Metal Deposition

Clover clone samples were collected in 1998 from each of the sites and sent to Dr Ludwig DeTemmerman, VAR, Belgium, for analysis of lead, cadmium, copper and arsenic content.

Activity Five: Level II modelling and mapping

"Stocks at Risk" maps have been produced for the level I critical level for crops, together with level II exceedance maps for wheat. The latter comprises flux models for wheat being developed by Dr Lisa Emberson (SEI-Y) and Professor Mike Ashmore (University of Bradford, Contract EPG 1/3/104) and maps developed by incorporation of modifying factors into the dose-response function for wheat (in collaboration with Professor J Fuhrer, Switzerland and Dr Max Posch, CCE). Participants have also assisted with mapping procedures by providing information on local irrigation practices. A sensitivity index for crops is being prepared at the Coordination Centre (funded by the French Government).

1.4.3 Participation (1997 – 1999)

Participation in the programme expanded rapidly between 1996 and 1998, resulting in a doubling of the number of experimental sites (Table 1.1). This reflected greater contributions from individual countries rather than a large increase in the number of participating countries. As the success of the ICP Vegetation has become more widely known, several groups have been able to attract national funding for participation in the programme. This is an important new development because, prior to 1997, participation tended to be on a voluntary basis with individuals fitting their experiments in amongst existing research and without dedicated funding. In some countries, e.g. Italy, Slovenia, and Germany, national networks have been established for the ICP Vegetation experiments. The programme has also been used to attract the interest of the public to the ozone problem in Slovenia and smaller-scale experiments to monitor the incidence of ozone injury are now being conducted in the schools in that country. Above all, the increased participation in the programme over the last three years is a reflection of its scientific credibility; all of the leading ozone-effects researchers in Europe are now involved in the programme.

Table 1.1: ICP Vegetation sites 1996-2000

Site	1996	1997	1998	1999	2000
Austria-Seibersdorf	*	*	*	*	*
Belgium-Tervuren	*	*	*	*	*
Denmark	*				
Finland-Jokioinen	*	*		*	*
France-Pau	*		*	*	*
Germany-Braunschweig	*	*	*	*	*
Germany-Cologne		*	*	*	*
Germany-Deuselbach		*	*	*	*
Germany-Essen		*		*	*
Germany-Geisson	*	*	*	*	*
Germany-Trier City		*	*	*	*
Germany-Trier University		*	*	*	*
Greece-Benaki	*				*
Ireland-Dublin				*	
Italy-Isola Serafini		*	*	*	*
Italy-Milan	*				
Italy-Naples			*	*	*
Italy-Pisa		*		*	*
Italy-Rome		*	*	*	*
Netherlands-Wageningen		*	*	*	*
Poland-Kornik			*	*	*
Russia-Moscow				*	*
Slovenia-Iskrba				*	*
Slovenia-Kovk				*	*
Slovenia-Ljubljana		*	*	*	*
Slovenia-Pokljuka				*	*
Slovenia-Rakican			*	*	*
Slovenia-Vnajnarje				*	*
Slovenia-Zalec				*	*
Slovenia-Zavodnije		*		*	*
Spain-Barcelona			*	*	*
Spain-Ebro Delta	*	*	*	*	*
Spain-Madrid			*	*	*
Spain-Navarra			*	*	*
Spain-Valencia			*	*	*
Sweden-Östad	*	*	*	*	*
Switzerland-Cadenazzo	*	*	*	*	
Switzerland-Liebefeld				*	*
UK-Ascot			*	*	
UK-Bangor			*	*	*
UK-Nottingham	*	*			
USA-Raleigh			*	*	*
USA-Long Island				*	*

1.4.4 Task Force Meetings

Each of the ICPs that report to the Working Group on Effects has an annual Task Force Meeting to discuss recent results and plan future activities. ICP Vegetation Task Force Meetings were held in Wageningen, The Netherlands (January, 1998), Beaumaris, near Bangor, UK (January, 1999), and Semmering, Austria (January, 2000). The ICP Vegetation Coordination Centre collates information on participants' requirements for the meeting and arranges the scientific content whilst the host organisation coordinates the domestic arrangements. The Beaumaris meeting in 1999 was both hosted and organised by ITE-Bangor. The meetings attract approximately 40 participants representing most of the countries involved in the programme. In recent years, the Task Force Meeting has started with a welcome reception on a Tuesday evening, followed by a full day of scientific presentations and posters describing results from all facets of the programme. Further results are presented on Thursday, followed by group discussions on areas of future interest for the programme. Typical discussion subjects are flux-modelling methods and methods for studying ozone effects on communities of natural vegetation. On Friday morning, the experimental programme for the coming season is finalised, and other WGE and LRTAP business is considered. Having such a programme has allowed the Task Force Meeting to consider all issues of relevance to the ICP, whilst at the same time functioning as an annual forum for discussion of progress with research on critical levels for ozone.

1.4.5 Participation in the Critical Levels for Ozone – Level II Workshop (Gerzensee, April, 1999)

Preparation for the Gerzensee Workshop was the main focus of data analysis in 1998/99. The results and modelling were presented by Dr G Mills as an invited paper, and have been accepted by Environmental Pollution for inclusion in a Special Issue on the Workshop (Mills et al, in press). Twenty-seven participants in the ICP Vegetation attended the Workshop. Mr J Fuhrer, the organiser, is a member of the Steering Committee, the chairmen of the Crops and Natural Vegetation Working Groups are both members of the ICP Vegetation (Dr H Pleijel and Prof. M Ashmore respectively), and Dr G Mills was the Rapporteur for the Crops Group.

1.4.6 Reporting to the Working Group on Effects

Each year, the ICP Vegetation has presented an Annual Status Report (c. 100 pages) and a Technical Report on progress with critical levels (c. 15 pages) to the annual meeting of the WGE, Palais des Nations, Geneva, Switzerland. Contributions have also been made to joint reports by the ICPs, WGE further development documents, and a report on trends in transboundary air pollution. The ICP Vegetation has contributed papers to UN/ECE Workshops on critical levels for ozone and heavy metals, and participated in Task Force Meetings for the ICP Mapping and the ICP Integrated Monitoring. Dr G Mills has attended twice-yearly Extended Bureau Meetings of the WGE and has thus contributed to discussions on the future work programme for each of the ICPs. Finally, Dr Mills has provided advice to the WGE on ozone and heavy metals issues of relevance to the Convention.

1.4.7 WGE External Review

As part of a general review of activities within the LRTAP Convention, the WGE requested Tom Brydges, Canada, to perform an independent review of the activities of each of the ICPs for the period 1994 - 1998. A package of annual reports, technical reports, experimental protocols, publications by the CC, and a list of publications by participants was sent to Mr Brydges for consideration. The report received by the WGE was very favourable about the ICP Vegetation, and is summarised as follows in EB.AIR/WG.1/1999/3:

" The reviewer noted the excellent progress made by the Programme since the very critical review of its work in 1994. Documents were now well focussed for the policy maker, although executive summaries could be even stronger. The objectives of the programme were clear, but did not indicate how they related to the effect-oriented activities in general. It was agreed that:

(a) Economic aspects of crops were important and should be addressed in future work;
(b) The planned natural vegetation pilot studies could be an important part of the programme in future;
(c) Further work on critical levels was needed;
(d) The effective use of artificial neural networks for developing predictive models from available data should be explained clearly, with examples, to other scientists;
(e) There were possible opportunities for linking the activities with the world climate programme provided it was consistent with the objectives of the Convention."

The recommendations of the review have been considered by the ICP Vegetation Steering Committee, and points (a) to (d) have already been incorporated into the programme. Point (e) will be considered in the near future.

1.5 Deliverables and Reporting for Contract EPG 1/3/96

A list of reports and publications is presented in Annex 1, and Journal and Conference Proceedings papers are collated as Volume 2 of this report.

The deliverables are considered in the order in which they were listed in Contract EPG 1/3/96:

1. Annual Reports have been submitted in March, 1998, March, 1999, and this final report has been prepared for March, 2000. Quarterly Reports were submitted when appropriate.

2. As agreed with the Nominated Officer, the focus of the ICP Vegetation has moved away from the short-term critical level for ozone with the change in international emphasis on to the long-term critical level. Thus, a report outlining the revised definition for the short-term critical level has not been prepared, although a summary of progress is presented in Section 4 of this report. This deliverable was replaced in year 3 by a *Review of the Effects of Ozone on Natural Vegetation* that included the research recommendations of the Gerzensee Critical Levels Workshop (see Section 6 of this report).

3. A Report was submitted in May 1999 that described the main conclusions and recommendations of the Gerzensee Critical Levels Workshop.

4. Maps of "Stocks at Risk" from ozone pollution were incorporated into the Annual Reports of years 1 and 2, and are included in revised form in Section 3 of this report.

5. The minutes of the Task Force Meeting of the ICP Vegetation, notes on the TF-Mapping meetings, and reports of the Working Group on Effects meetings have been submitted to DETR.

6. Status Reports (length 100+ pages) have been submitted to the Annual Meeting of the Working Group on Effects in 1997, 1998 and 1999. Technical Reports on progress with critical levels, and contributions to joint reports of the ICPs, including a report on Trends in the data have also been produced.

7. A review of trends in the ICP Vegetation data has been included as Section 5 of this report. A synopsis of the review was also included in a WGE publication on Trends in Impacts of Long-Range Transboundary Air Pollution (UN/ECE, 1999).

8. A colour brochure describing the ICP Vegetation was produced in 1999 and distributed at the Gerzensee Workshop. An ICP Vegetation web-site has been established at *http://mwnta.nmw.ac.uk/ITE/bang/ICPVegetation/home*, and a CD-ROM of pollutant and climatic data is available for use within the Convention.

9. Seven papers have been submitted to Scientific Journals and five papers have been published in Conference Proceedings since April, 1997 (see Appendix 1). These are included as Volume 2 of this report.

2 Measuring and modelling ozone-induced reductions in clover biomass in Europe.

2.1 Aims

Monitoring the environmental impacts of pollutants forms an integral part of the work-plan of each of the ICPs that report to the WGE. For the ICP Vegetation, the main emphasis has been to monitor the impacts of ambient ozone on sensitive and resistant clones of white clover (*Trifolium repens* cv Regal). By determining the biomass ratio at each site in each year, we have been able to build a large database on the impacts of ambient ozone on this species. This data has been used to develop PROBE, a predictive model of the influence of climatic conditions and other pollutants on the biomass response of white clover to ozone. The use of the model to map ozone effects across Europe is presented in Section 3.

The aims were:

- To monitor the impacts of ambient ozone on a sensitive species at sites across Europe.
- To develop a high quality database of the climatic conditions, pollutant conditions, and biological responses at each of the sites.
- To use the database to develop and test a parsimonious model of the impacts of level II factors on the biomass response to ozone.
- To use the model as an interpretative tool for investigating the influence of level II factors.

2.2 Introduction

Open-top chambers (OTCs) have been one of the most popular exposure systems used in recent decades to determine the effects of ambient and near-ambient concentrations of ozone on vegetation (e.g. Jäger *et al*, 1992). However, constant air movement within the chambers and other changes in microclimate (Sanders *et al*, 1991) may alter pollutant flux to the vegetation, leading to errors in concentration-effect relationships derived using this exposure method (Pleijel, 1996). To overcome these problems, the UN/ECE ICP Vegetation sought to quantify the effects of ambient ozone over the broad range of climatic conditions that exist within Europe using an inexpensive biomonitoring system. Following a pilot study in 1995 with ozone-sensitive (NC-S) and ozone-resistant (NC-R) clones of white clover (*Trifolium repens* cv Regal) selected by Heagle *et al* (1995), a programme of experiments was conducted in 1996, 1997, 1998 and 1999. The clover clone system was chosen because the forage biomass for both clones was similar in conditions of low ozone, but lower for the NC-S clone at high ozone concentrations (12h mean > 40-50 ppb, Heagle *et al*, 1995). Furthermore, analysis of the data would contribute to the current debate in Europe on critical levels for ozone in which the importance of modifying (so-called level II factors) is being considered in preparation for a revision of the currently used level I critical level for crops (UBA, 1996).

Multivariate statistical analysis is usually used to identify and quantify the influence of modifying factors from data from multiple site comparisons (e.g. Chevone *et al*, 1998). However, these methods rely on linear relationships between parameters and might be less reliable where co-variables are non-linearly related, as in the case of temperature and ozone. Roadknight *et al* (1997) and Ball *et al* (1998) have developed the use of artificial neural networks (ANNs) for analysis of such non-linear plant-environment interactions. Each ANN comprises an input layer (the causal variables), a hidden layer (feature detectors) and an output layer (the effect e.g. biomass change); the mathematical weightings of the interconnections between these layers are repeatedly modified until the outputs match those predicted from the inputs. An ANN is trained using a large proportion of the database and tested for generality using the remaining, previously unseen proportion of the database (for further details, see Appendix 2).

The development of ANN modelling methods has been an on-going part of the work performed under contract EPG 1/3/96 by Dr Graham Ball (Research Fellow) and Dr Dominic Palmer-Brown (Senior Lecturer) at The Nottingham Trent University. Methods were initially developed using the data from the experiments conducted under the previous contract (EPG 1/3/13) in which ethylene diurea (EDU) was used as a protectant against ozone effects on white clover (*T. repens* L.cv Menna). Inclusion of level II factors (vapour pressure deficit (VPD), temperature, altitude, and latitude) in an ANN produced a model with an r^2 of 0.79 compared to 0.16 for a linear regression of AOT40 against biomass ratio (Ball *et al*, 1998). Graham Ball developed the methods further when more data became available from the clover clone experiment (Ball *et al*, in press). By using repeated randomly selected sub-sets of the data, Ball *et al* (in press) overcame the problem of having a relatively small database for the modelling. Climatic conditions were found to be more important modifiers of the response to ozone than "geographical factors" such as latitude and altitude. For further information on this part of the ANN modelling, please read Ball *et al* (1998) and Ball *et al* (in press) presented in Volume 2 of this report.

The modelling presented in this report was selected for inclusion in the Special Edition of Environmental Pollution on the *Critical Levels for Ozone – Level II Workshop* (Gerzensee, April, 1999) and will be published later this year (Mills *et al*, in press). Data from the 1996 – 1998 experiments were used to develop and test the model, and data from 1999 have subsequently been used to check how well the model generalised. To increase the amount of data available for modelling, and hence the reliability of the model, data from each 28d harvest interval were used. Over 240 combinations of inputs were tested until a reliable parsimonious model named PROBE (PRedicting Ozone impacts on Biomass in Europe) had been developed. This model was then used to predict the influence of level II factors on the biomass response to AOT40.

2.3 The ICP Vegetation Clover Clone Experiment

Clover clone experiments were conducted at ICP Vegetation sites between 1996 and 1999 according to a standard protocol distributed by the Coordination Centre (e.g. UN/ECE, 1998). The experimental sites were in open locations, at least 50m from buildings, and 200m from main roads (e.g. Cadenazzo, Switzerland, Figure 2.1).

Thirty cuttings each of NC-S and NC-R clones of white clover (*Trifolium repens* L. cv Regal) were supplied to each participant by the Coordination Centre from stock plants grown from cuttings supplied by A. Heagle (USDA, North Carolina, USA). On receipt by the participants, each cutting was planted in horticultural compost in a 1litre pot, and maintained in a greenhouse. Ten days later, a 25ml soil drench containing 5g/l of *Rhizobium* (supplied by A. Heagle) was applied to each pot. After a further 18 days, the cuttings were transplanted to 15 litre pots containing growing medium plus 60g of a 9-month slow-release fertiliser (13N:13P:13K). Choice of growth media was left to individual participants to ensure that the medium was appropriate for local climatic conditions. The plants were maintained in a well-watered state by use of a wick and water reservoir system (fibre-glass wicks supplied by the Coordination Centre), and the pots and water reservoirs were covered with reflective aluminised bubble-wrap to avoid overheating. After transplanting, 20 plants per clone were transferred to the ambient air plots for the duration of the experimental season (usually May-September). The clover plants were arranged at each plot in four rows of ten plants, with NC-S clones alternating with NC-R clones. Pesticides were applied whenever necessary, although the use of any fungicides known to interact with ozone was prohibited.

The clones were assessed every 28d for the incidence of leaf damage due to ozone, pests or diseases. The foliage and stems were then excised to a height of 7cm above the surface of the growth medium; all harvested material per pot was dried to constant weight at 70°C, and the mean biomass per clone was calculated. Four or five harvests were conducted at each site in each year; data from the first harvest were discarded as this represented a period when the plants were establishing outdoors.

Figure 2.1: The clover clone experiment at Cadenazzo, Switzerland

2.4 Assessment of data quality, and data preparation

For each year and site, participants sent an Excel spreadsheet of hourly measurements for temperature, VPD, solar radiation, ozone, sulphur dioxide, and oxides of nitrogen for the experimental period (June to September) to the Coordination Centre. For each 28d harvest interval, the mean pollutant and climatic parameters were calculated from the summary table, and three-month values were calculated for the interval between harvests 1 and 4. The participants also provided the mean biomass of each clone at each harvest together with information on the incidence of visible ozone injury, pests, diseases and physiological abnormalities.

Before confidence could be placed in the analyses and accurate models developed, an assessment of the quality of the data had to be conducted. Exclusion rules for the pollutant and climatic data were developed based on the extent of missing data in the harvest period. A two-stage assessment was carried out. In the first stage the hourly data was assessed. If, for a given day, there were more than 4 consecutive hourly means missing for the daylight period then the data from that day were considered "unusable". Next, the daily summaries were calculated. If more than two consecutive days for AOT40 data or more than four consecutive days of climatic data or data for other pollutants were missing then the data for that harvest were also rejected. Where occasional hourly means were missing, surrogate values were calculated from the mean of that hour both two days before and two days after the day with the missing value. The rules for ozone data were more stringent than those for other parameters as the cumulative AOT40 value was used. Thus, the errors associated with missing values for AOT40 would also be cumulative over the harvest interval. Missing daily means for all parameters were calculated as the average of the two days before and after the missing values.

Once the quality of the data from an individual site had been assessed, the hourly data were used to calculate a range of pollutant and climatic parameters. These were:

- For all variables:
  The daylight mean (notation $_{day}$)
  The mean when daylight (solar radiation ≥ 50 Wm^{-2}) ozone concentrations were greater than 40ppb (notation $_{>40}$).

- For temperature:
  The 7h mean (notation $_{7h}$), the 24h mean (notation $_{24h}$) and the mean daily maximum (notation $_{max}$).

- For ozone:
  The 7h mean (notation $_{7h}$), the 24h mean (notation $_{24h}$) and the mean daily maximum (notation $_{max}$), the AOT40 and AOT30 during daylight hours.

- For all pollutants:
  The mean pollutant concentrations for hours when minimum and maximum values commonly occurred. These times were 0700, 1700 and 1500 for the minimum concentrations and 1600, 0800 and 0700 for the maximum concentrations of O_3, NO and NO_2 respectively and are represented in the text by subscripted times.

In the second stage, the quality of the biomass data was also assessed based on the extent of damage to the clover clones caused by biotic and abiotic factors other than ozone. Data for a particular harvest were rejected if a pest or disease affected one of the clones more than the other. If the biomass of either or both clones was less than the 25th percentile for that harvest (all data combined from 1995-1998) then the possibility of rejection was considered. Data for the whole season was rejected for an individual site when the clones were exhibiting uncharacteristically slow growth at both the first and second harvests. Slow growth was defined as a percentage increase in growth between harvests 1 and 2 that was less than the 10th percentile for the data set, that occurred at an AOT40 lower than the 75th percentile for that harvest interval. In other words, the possibility of ozone causing the slow growth was only considered where the AOT40 exceeded the 75th percentile.

This rigorous analysis of the quality of the data resulted in the rejection of approximately one third of the data. The main cause of data rejection was gaps in the ozone data.

2.5 The physical and pollution climate in Europe (1997 – 1999)

The sites in the ICP-Vegetation represent a broad range of pollutant and climatic conditions (Table 2.1 and Figures 2.2 – 2.4). The three-month AOT40 values ranged from 0 ppm.h to 34.9 ppm.h (Italy-Isola Serafini in 1998) and thus were up to eleven times the current level I critical level for ozone (Figure 2.2). More usually, the AOT40 was in the range 2 - 6 ppm.h. The long-term critical level of 3 ppm.h was exceeded at 77% of sites in 1997, 71% of sites in 1998 and 86% of sites in 1999. The concentrations of ozone tended to be higher in the southern half of Europe in 1998 than in the other two years, but interestingly were lowest in the northern half of Europe in the same year (Table 2.1 and Figure 2.2). In general, the AOT40s in countries like Switzerland, Italy and Austria were more than double those measured in the more northern countries like Belgium, the UK, Sweden and Finland. Superimposed on the north-south trend in ozone concentration was the effect of proximity to sources of other pollutants. The sites ranged from rural locations (e.g. Austria-Seibersdorf) where NO and NO_2 daylight means were below 4 ppb to semi-urban sites where these pollutants had daylight mean concentrations in excess of 20 ppb (e.g. Italy-Milan, Table 2.1). Sites with high NO_x had different proportions of NO and NO_2 according to local sources (Figure 2.3). For example, a major motorway passes through the rural area of Italy-Isola Serafini resulting in an NO_x profile dominated by NO_2. In contrast, the site at Germany-Trier City is close to the city centre and experiences a higher proportion of NO in its NO_x pollution.

Temperature profiles fitted the expected north-south pattern with the lowest three-month T_{24h} being recorded at Sweden-Östad and UK-Bangor and highest recorded in Italy, Spain and Switzerland (Figure 2.4 (a)). The site at USA-Raleigh was the hottest included in the network. Over three months, the 24h mean VPD tended to be below 0.8 kPa at most of the sites with the exception of Finland-Jokioinen, Germany-Trier City, Italy-Isola Serafini, Spain-Navarra, and USA-Raleigh (Figure 2.4(b)). The range and mean daylight temperature and VPD for the sites selected for modelling are included in Table 2.1.

Further details on the pollution and physical climate at the ICP Vegetation sites can be found in the analysis of trends (1990 to 1999) presented in Section 5.

2.6 Single-factor modelling: AOT40-biomass response relationships

The NC-S clone of white clover proved to be sensitive to ozone within the range of three-month AOT40s experienced at the ICP Vegetation sites (Figure 2.5), with biomass reductions as high as 40% (relative to NC-R) recorded at the most polluted site at Italy-Isola-Serafini. When all of the data were combined for the years 1996 – 1999, the linear relationship had an r^2 of 0.402. No significant differences were found between the slopes of the lines for each year of the experiment (figure not presented). For modelling purposes, the data were separated into the individual 28d harvest intervals (excluding harvest intervals 0 – 1); the r^2 for the combined data set (harvests 2 – 4) was 0.325 (Figure 2.6). Statistical analysis revealed that although the slope of the regression for harvest 4 was steeper than that for harvest 2 the difference was not significant ($p = 0.071$, Figure 2.7).

Table 2.1: A summary of the ICP Vegetation data used in this analysis. The data are the mean and range (in brackets) of 28d values for each parameter.

Country - site	Latitude	Longitude	Altitude (m.a.s.l.)	Years	Repli-Cates	AOT40 (ppm.h)	NO_{day} (ppb)	$NO_{2\,day}$ (ppb)	T_{day} (°C)	VPD_{day} (kPa)
A-S	47°59'N	16°31'E	190	1998	3	2.29 (2.2-3.3)	1.4 (0.7-2.1)	2.9 (2.1-3.9)	20.5 (17.1-25.7)	1.13 (0.73-1.79)
B-T	50°49'N	4°31'E	80	1996- 1998	9	0.71 (0.0-2.1)	2.5 (0.7-4.7)	8.0 (6.7-10.6)	18.6 (16.4-22.5)	0.70 (0.41-0.99)
FIN-J	60°47'N	23°28'E	100	1996- 1997	3	0.64 (0.2-1.2)	0.8 (0.2-1.2)	0.8 (0.2-1.5)	18.9 (16.5-20.6)	0.78 (0.62-0.92)
D-B	52°15'N	10°30'E	85	1997	3	1.57 (0.5 - 3.5)	1.9 (1.2-2.5)	2.7 (1.3-4.2)	20.3 (17.4-23.7)	0.88 (0.63-1.30)
D-C	50°55'N	6°36'E	n.a.	1997	2	0.97 (0.0-1.9)	10.0 (8.7-11.2)	14.7 (12.6-16.8)	20.0 (17.6-22.3)	0.88 (0.64-1.13)
D-D	49°45'N	7°03'E	480	1997- 1998	6	2.65 (0.2-3.5)	0.5 (0.4-0.6)	4.0 (3.0-5.6)	16.3 (13.6-21.8)	0.79 (0.46-1.14)
D-E	51°22'N	6°55'E	60	1997	2	0.38 (0.1-0.6)	9.9 (9.9-9.9)	14.5 (14.1-14.9)	18.8 (17.3-20.3)	0.71 (0.56-0.85)
D-G	50°35'N	8°42'E	190	1998	3	2.93 (1.2-4.0)	4.7 (3.7-5.4)	7.1 (6.1-8.6)	19.0 (17.9-20.2)	0.84 (0.66-1.00)
D-T	49°46'N	6°39'E	255	1997- 1998	6	0.43 (0.0–1.4)	37.7 (27.3-49.8)	25.5 (23.5-26.9)	19.7 (15.2 21.6)	1.04 (0.64-1.44)
I-IS	45°06'N	9 °53'E	40	1997- 1998	4	10.04 (7.4-12.0)	3.2 (1.3-5.8)	6.0 (0.7-12.4)	25.6 (24.2-26.8)	1.49 (1.16-1.72)
I-M	45°28'N	9°12'E	120	1996	2	2.71 (0.3-5.2)	22.8 (5.7-39.9)	25.7 (11.0-40.4)	21.9 (19.1-24.6)	1.22 (0.95-1.49)
SI-Z	45°26'N	15°01'E	800	1997	1	3.21	0.84	3.9	16.9	0.79
ES-N	42°48'N	1°39'W	440	1998	4	1.66 (0.4-2.5)	4.2 (1.96-9.62)	9.7 (7.6-11.7)	23.6 (18.0-26.8)	1.34 (0.53-2.02)
CH-C	46°10'N	8°56'E	200	1996- 1998	5	6.49 (3.6-9.0)	0.4 (0.1-1.05)	4.0 (0.7-9.3)	23.4 (21.2-25.8)	1.19 (1.08-1.33)

Key: A-S: Austria-Seibersdorf; B-T: Belgium-Tervuren; FIN-J: Finland-Jokioinen; D-B: Germany-Braunschweig; D-C: Germany-Cologne; D-D: Germany-Deuselbach; D-E: Germany-Essen; D-G: Germany-Giessen; D-T: Germany-Trier; I-IS: Italy-Isola Serafini; I-M: Italy-Milan; SI-Z: Slovenia-Zavodnije; ES-N: Spain-Navarra; CH-C: Switzerland -Cadenazzo. m.a.s.l : metres above sea level; n.a. data not available.

Note: This analysis was only performed for those sites at which NOx concentrations were recorded.

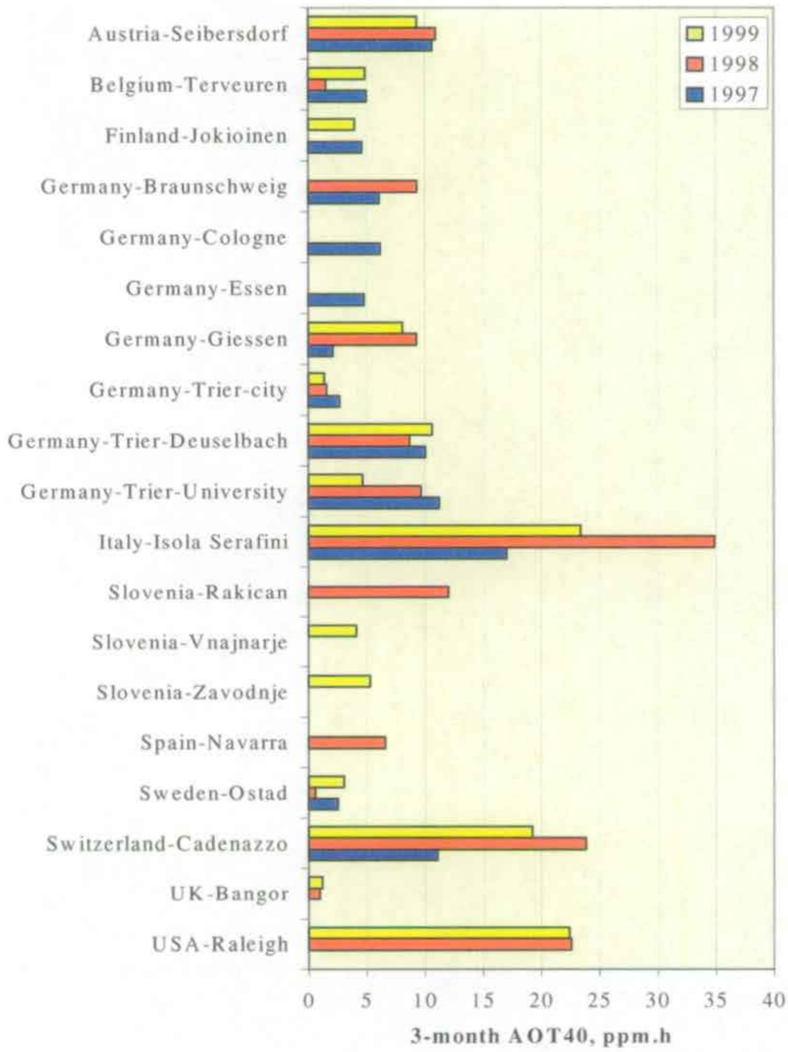

Figure 2.2: The three-month AOT40 at ICP Vegetation sites (1997 – 1999).

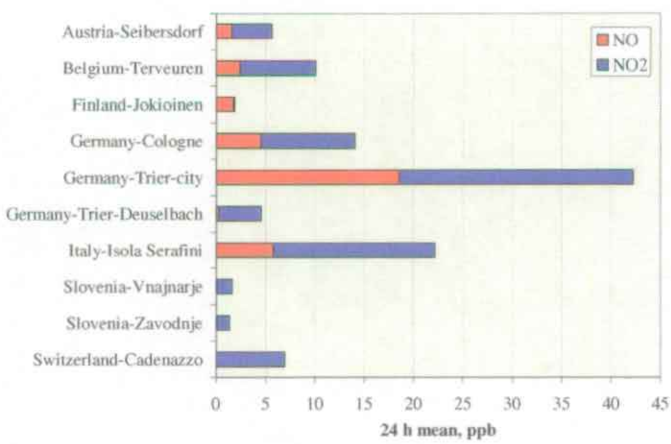

Figure 2.3: The 24h mean NO and NO_2 concentrations at selected sites in 1999.

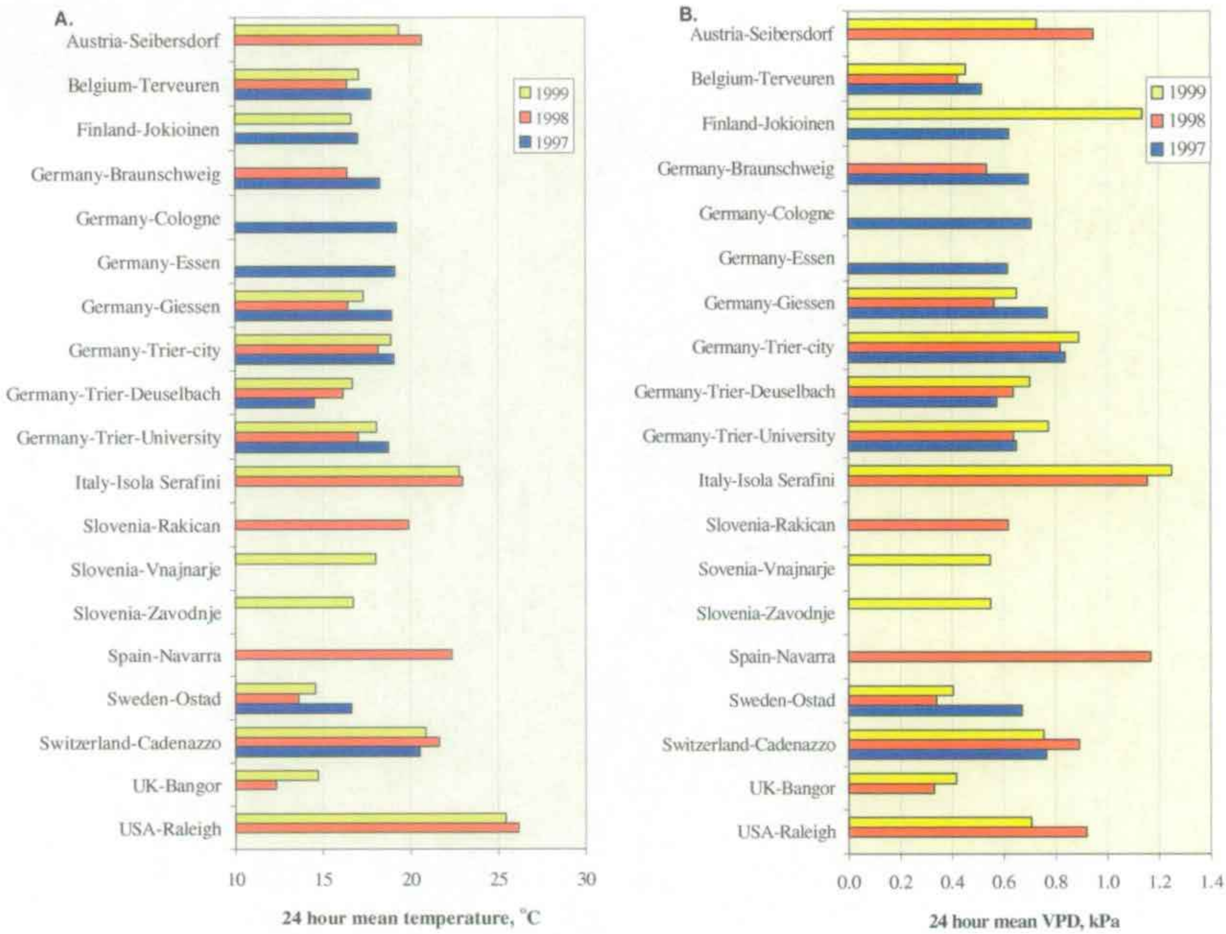

Figure 2.4: The three-month 24h mean temperature and VPD at ICP Vegetation sites (1997 - 1999).

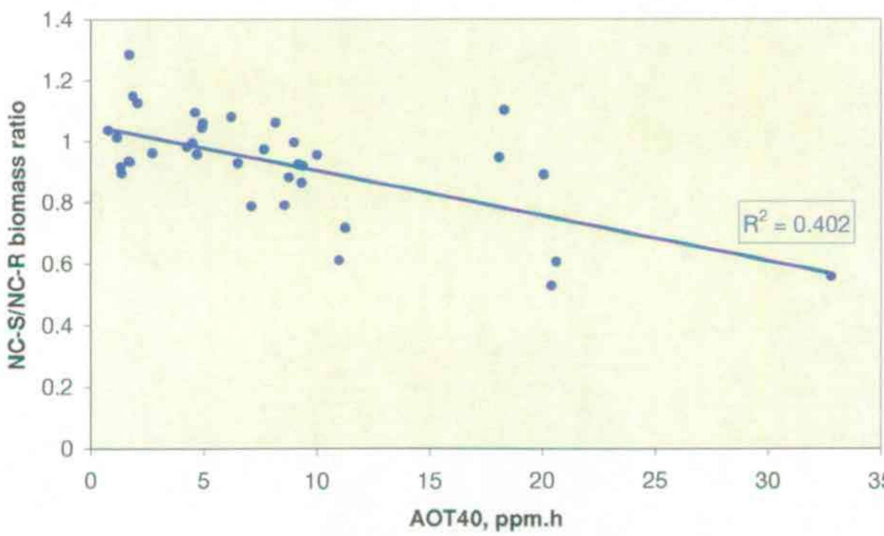

Figure 2.5: The effect of AOT40 on the NC-S/NC-R biomass ratio of white clover: the data accumulated over three months (1996 – 1999).

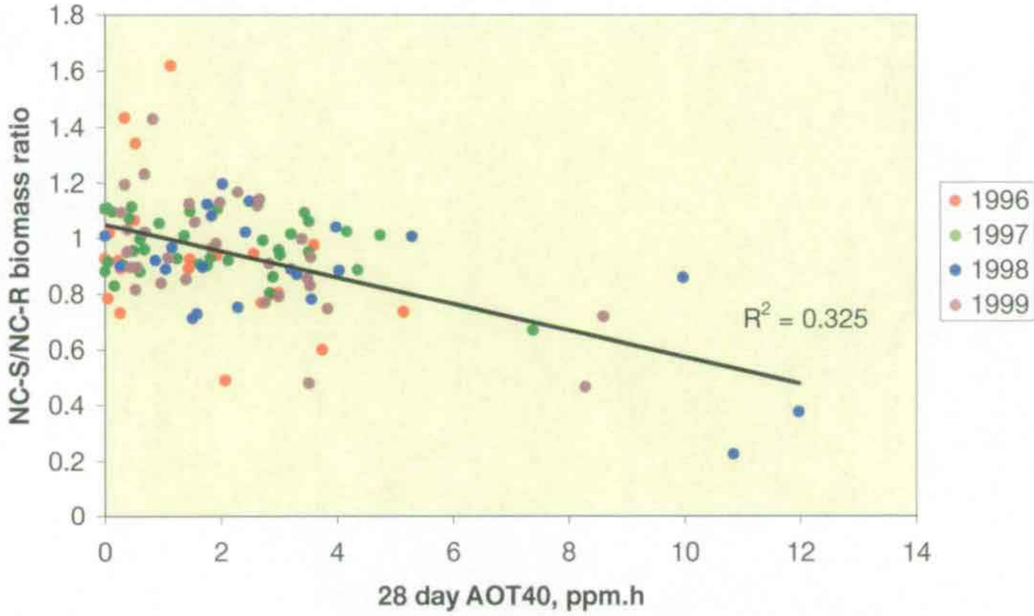

Figure 2.6: The effect of AOT40 on the NC-S/NC-R biomass ratio of white clover: data for individual 28 d harvests (harvests 2 – 4, 1996 – 1999).

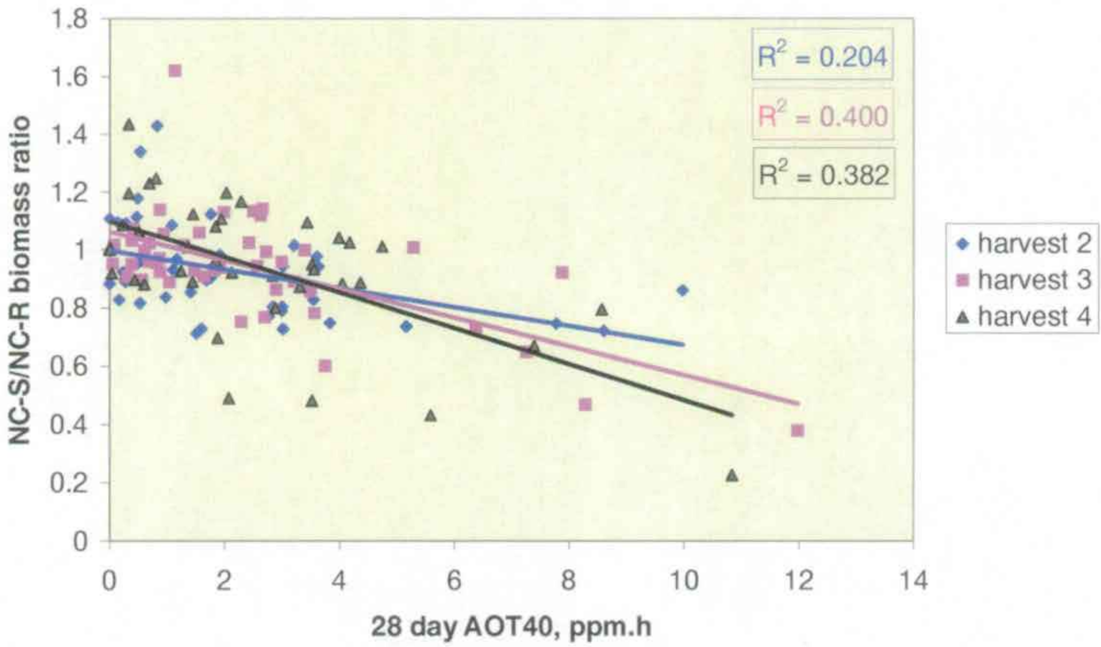

Figure 2.7: As Figure 2.6, but separated into individual harvests.

The large reductions in biomass ratio at the most polluted sites showed that the clover clone system was working well within Europe. However, all of the regression coefficients were below 0.5 indicating that there was a lot of scatter within the data. Multi-factor modelling methods were used in all subsequent analysis in order to improve the fit to the data by including level II (modifying) factors.

2.7 Development of PROBE, a multi-factor model for predicting biomass.

2.7.1 Selection of the best performing model

The modelling included in this section was performed for the 28d data for the individual harvest intervals (excluding harvest interval 0 – 1) for the data from 1996 - 1998. The analysis was performed for a sub-set of the data from 14 sites in eight countries where participants measured NOx as well as ozone concentrations (Table 2.1). Data from the 1999 season were used for validation of PROBE (Section 2.9).

Before modelling commenced, a test data set, comprising 25% of the database, was randomly extracted and set aside for testing the ability of the linear and ANN models to make generalised predictions for unseen data. The remaining 75% of the data (training data) was analysed using Minitab-11 for regression analysis and Neuroshell-2 (Ward Systems Ltd.) for development of ANNs using three-layer multi-layer perceptrons, with back-propagation algorithms. Training was run to convergence, the point at which the error for unseen test data could not be reduced further (for details, see Appendix 2).

Over 240 input combinations were considered during the selection of a model that had the best performance for the previously unseen test data (Table 2.2). For each input combination considered, the following three processes were employed:

Performance analysis

The accuracy of predictions for each model was determined separately for the training and test data by calculating the r^2 values for the actual versus predicted values.

Optimisation

For each combination of inputs, 15 separate models with between 1 and 15 hidden nodes were trained. The one with the highest r^2 for both the training and test data was considered to be the "optimised" model.

Weightings analysis

The extent of influence of each input on the predictions of the model was determined by summing the absolute weights of the connections leading from each input to the model (Balls *et al*, 1996).

For comparison with ANN analysis, multiple linear regression (MLR) was performed for each combination of inputs used in the development of ANN Model 3 and ANN Model 4. At each stage, the performance of the MLR equation was tested by regression of the actual versus the predicted NC-S/NC-R biomass ratio for both the training and the test data (Table 2.2).

Using all possible inputs, ANN Model 1 had an r^2 of 0.660 for the training data and 0.473 for the test data (Table 2.2). AOT40 had the largest influence of all of the inputs on the model, with 8 of the 10 highest influencing factors being either O_3 or NO_x parameters and T_{day} and VPD_{40} being the remaining influences (for details see Mills *et al*, in press, volume 2). Retraining the ANN model without the correlated inputs reduced the errors in the model (ANN Model 2, Table 2.2). Further improvements in the performance of the ANN models occurred as the weakest influencing inputs were removed until only AOT40, $O_{3\ 24h}$, NO_{1700} and T_{day} remained (ANN Model 3, $r^2 = 0.776$ for the training data and 0.673 for the test data). In every case, the MLR equation performed less well for both the training data and the test data.

Adding each of the remaining inputs in turn to ANN Model 3 led to improvements in the performance for the training data when $O_{3\ 0700}$, $NO_{2\ >40}$, T_{24h}, T_{max}, VPD_{day} and $VPD_{>40}$ were added (data not presented). Inclusion of T_{24h} caused the greatest improvement, resulting in an r^2 of 0.840 for the training data and 0.710 for the test data (ANN Model 4). No further improvement was produced by the addition of any of the other inputs to Model 4 (data not presented).

To assist with interpretation of the model structure and performance, three alternative structures were considered for ANN Model 4. When NO_{1700} was replaced by $O_{3\ 1700}$, the performance decreased to an r^2 of 0.708 for training data and 0.509 for test data. Adding harvest interval to ANN Model 4 decreased the performance to an r^2 of 0.614 for the training data and 0.438 for the test data, and thus increased the error in the model. Finally, $VPD_{>40}$ was added to ANN Model 4 because of the importance of VPD in determining stomatal conductance. Again, the performance decreased, resulting in an r^2 of 0.77 for the training data and 0.69 for the test data. Thus, $O_{3\ 1700}$, harvest interval, and $VPD_{>40}$, were excluded from the model used for predictive modelling (ANN Model 4). Model development was then considered to have been completed. ANN Model 4, with AOT40, T_{day}, $O_{3\ 24h}$, T_{24h}, and NO_{1700} as inputs was renamed PROBE (PRediciting Ozone impacts on Biomass in Europe), and used to predict the influence of level II factors on the biomass response to ozone (Section 2.8).

2.7.2 Consideration of the structure and performance of PROBE

The inputs to PROBE could be ranked in the following order of importance: NO_{1700} > AOT40 > T_{day} > $O_{3\ 24h}$ > T_{24h} (data not presented). Of these inputs, only AOT40 (Figure 2.6) and $O_{3\ 24h}$ had any notable influence on the NC-S/NC-R biomass ratio when considered individually (Figure 2.8). Where interactions occurred between the inputs, they were non-linear and were best represented by a second-order polynomial function (Table 2.3).

As a first investigation of the model, the actual and predicted values were compared for all of the data by linear regression to see if the model predictions were offset from the ideal intercept of 0 or skewed away from a gradient of 1. Confidence was gained from the low offset of 0.02, the gradient of 1.03, and the r^2 of 0.732 for the combined

Table 2.2: The development and performance of Artificial Neural Network and multiple Linear Regression Models from the clover-clone data.

Model Development			Artificial Neural Network		Multiple Linear Regression	
Model Number	Approach used to find the optimum model structure	Inputs used in the model with the best performance.	R^2 for the training data	r^2 for the test data	r^2 for the training data	r^2 for the test data
Single factor	AOT40 only	AOT40	0.59	0.41	0.53	0.28
1	All available inputs were used for ozone, temperature, VPD, NO and NO_2.	All 21 inputs	0.66	0.47	0.75	0.10
2	The lowest ranking of pairs of correlated inputs (r^2 values > 0.81) were removed. The model was re-trained using the remaining 13 inputs.	AOT40, AOT30, $O_{3\ 24h}$, $O_{3\ 0700}$, $O_{3\ 1600}$, $NO_{>40}$, NO_{0800}, NO_{1700}, $NO_{2\ day}$, $NO_{2\ 1500}$, T_{day}, $T_{>40}$, $VPD_{>40}$.	0.68	0.59	0.66	0.28
3	The inputs to model 2 were sequentially removed until AOT40 was the only input. The best performing input combination was selected	AOT40, $O_{3\ 24h}$, NO_{1700}, T_{day}.	0.78	0.67	0.59	0.36
4 "PROBE"	All remaining inputs were repeatedly added in turn (including the correlated inputs excluded during the development of model 2) until no further improvement in performance occurred.	AOT40, $O_{3\ 24h}$, NO_{1700}, T_{day}, T_{24h}	0.84	0.71	0.42	0.20

Table 2.3: Correlation models used to produce co-varying values for the inputs of PROBE when used to predict biomass ratio for a range of realistic conditions (see Figure 2.10). The second order polynomials presented provided better fit for each pair of parameters than linear or exponential relationships (data not presented).

Pair	R^2	Equation
AOT40 vs $O_{3\ 24h}$	0.734	$AOT40 = 0.0042(O_{3\ 24h})^2 - 0.0549\ O_{3\ 24h} + 0.1486$
AOT40 vs T_{24h}	0.362	$AOT40 = 0.053\ (T_{24h})^2 - 1.520\ T_{24h} + 11.75$
AOT40 vs T_{day}	0.429	$AOT40 = 0.061\ (T_{day})^2 - 1.994 T_{day} + 17.17$
NO_{1700} vs $O_{3\ 24h}$	0.253	$NO_{1700} = 0.024\ (O_{3\ 24h})^2 - 1.818\ O_{3\ 24h} + 33.37$
T_{24h} vs $O_{3\ 24h}$	0.239	$T_{24h} = -0.004\ (O_{3\ 24h})^2 + 0.347\ O_{3\ 24h} + 11.61$
T_{day} vs $O_{3\ 24h}$	0.282	$T_{day} = -0.001\ (O_{3\ 24h})^2 + 0.202\ O_{3\ 24h} + 15.49$
T_{24h} vs T_{day}	0.824	$T_{24h} = 0.014\ (O_{3\ 24h})^2 + 0.375\ O_{3\ 24h} + 4.60$

Note: No relationship was found between AOT40 and NO_{1700}, T_{24h} and NO_{1700}, T_{day} and NO_{1700} (r^2<0.2); median values for NO_{1700} were used in these instances.

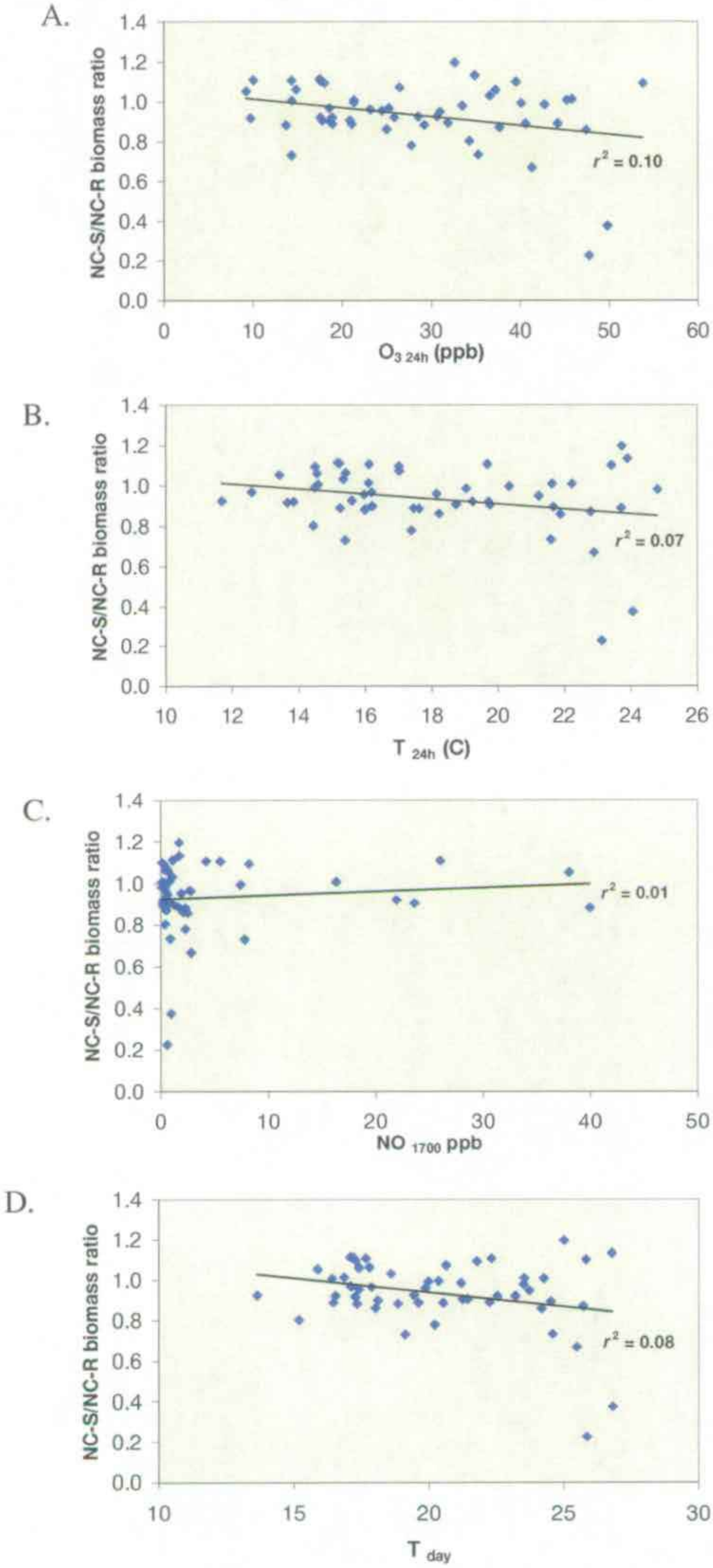

Figure 2.8: The influence of (A) $O_{3\ 24h}$, (B) T_{24h}, (c) NO_{1700}, and (D) T_{day} on NC-S/NC-R biomass ratio.

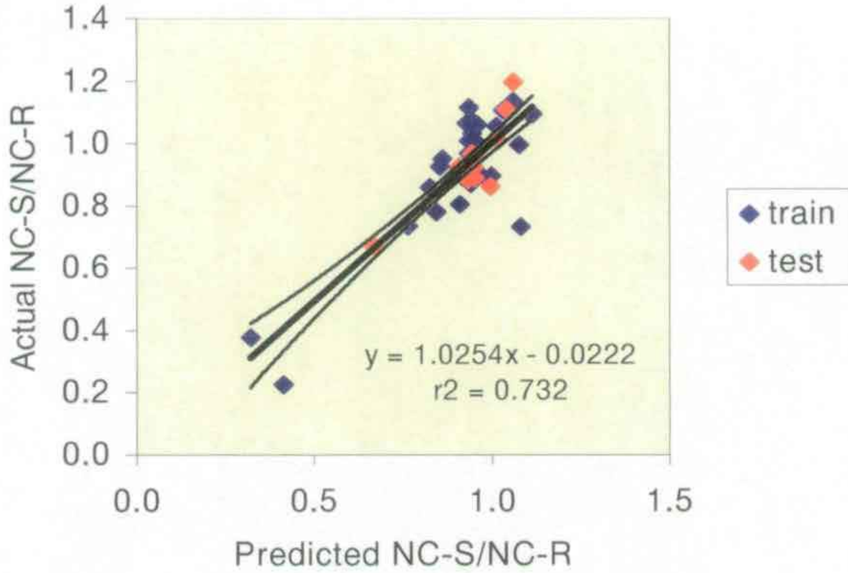

Figure 2.9: The actual versus predicted values produced by ANN Model 4 for the combined training and test data including the 95% confidence intervals.

test and training data (Figure 2.9). The confidence intervals for the regression line were then determined using the Statistical package Unistat.

2.8 Predictive modelling using PROBE

PROBE (ANN Model 4), using AOT40, $O_{3\ 24h}$, NO_{1700}, T_{day} and T_{24h} as inputs, was used to predict the NC-S/NC-R biomass response to AOT40 for a range of conditions for the other inputs. To develop realistic sets of conditions for these inputs for use in the predictive model, the strongest relationship between each pair of inputs was first determined by regression analysis of the whole data set, using linear, exponential and second-order polynomial functions (Table 2.3). The equations were used to calculate co-varying data for the median and quartile values of each input variable, and then used to develop an AOT40 dose-response curve for each group of conditions. From the predicted response relationships, the AOT40 values required to produce a 5% change in the NC-S/NC-R biomass ratio were determined, and the conditions listed. The 95% confidence intervals were plotted for each predicted response curve by applying the confidence intervals determined from plots of the actual verses predicted data.

The model predicted that for T_{day} (Figure 2.10) and T_{24h} (data not presented), the response to AOT40 became more pronounced as temperature increased. For $O_{3\ 24h}$, the offset at 0 ppm.h AOT40 was higher for the 1st quartile (18 ppb) than for the 2nd and 3rd quartiles (31.3 and 35.2 ppb respectively); the lines converged at an AOT40 of 6 ppm.h. NO_{1700} had little discernible effect on the AOT40 response relationship within the range of the 1st to 3rd quartiles (0.4 to 2.7 ppb), but reduced the response to ozone at 10 ppb in the range 0 – 2.5 ppm.h. Above 2.5 ppm.h, the response to AOT40 increased at a more pronounced rate than predicted for NO_{1700} values of 2.7 ppb and below.

The 28d AOT40s associated with a 5% reduction in NC-S/NC-R biomass ratio were calculated from PROBE. They ranged from 0.9 ± 0.28 for conditions of 24 – 31 ppb $O_{3\ 24h}$, 0.1 – 1.1 ppb $NO_{\ 1700}$, and 19.4 – 20.9 °C $T_{\ day}$, to 1.65 ± 0.40 ppm.h for 18 ppb $O_{3\ 24h}$, 8.4 ppb $NO_{\ 1700}$, and 18.9 °C $T_{\ day}$.

2.9 Retraining of PROBE by including the 1999 data in the inputs

As a general rule, ANNs train better with larger data sets than smaller ones. For this reason, PROBE has recently been retrained using 1999 data in both the training and test datasets. This led to an improvement in the performance of the model for the test data from an r^2 of 0.71 for the original model to an r^2 of 0.84 for the new model ($PROBE_{99}$). A small decline in the r^2 value for the training data from 0.84 to 0.77 occurred.

Table 2.4. Results of the optimisation of the input set to the model using 1999 data using a stepwise approach.

Step	Number of hidden nodes	r^2 for the training data	r^2 for the test data
AOT40 + T_{day}	9	0.590	0.635
$O_{3\ 24h}$ added	15	0.671	0.766
$NO_{\ day}$ added	15	0.689	0.829
+ T_{max} added	14	0.719	0.834
+T_{mean} added	15	0.847	0.852

The model containing the 1999 data was re-parameterised using a stepwise approach to determine the optimum set of inputs for the expanded dataset. Initially, a number of models were trained each using AOT40 plus one of the available inputs. The model with the input combination producing the best performance was then rerun adding all additional inputs singly from the available input set. Again the model input combination having the best performance was selected for the next step. This process was repeated until no further improvement in performance occurred. The model with the best performance had inputs for AOT40, $O_{3\ 24h}$, $T_{\ day}$, $T_{\ max}$, $T_{\ 24h}$, and $NO_{\ day}$ (Table 2.4). Thus the same type of parameters featured as important when the dataset had been expanded to include the 1999 data. The only changes from PROBE were an additional temperature input ($T_{\ max}$) and $NO_{\ 1700}$ was replaced by $NO_{\ day}$. $PROBE_{99}$ performed slightly better than PROBE having an r^2 for training data of 0.85 (compared to 0.84 for PROBE) and an r^2 of 0.852 for the test data (compared to 0.71 for PROBE).

2.10 Discussion

The ICP Vegetation clover clone experiment has shown that ambient ozone concentrations in some parts of Europe can cause substantial reductions in the biomass of a well-watered ozone-sensitive species. These effects have been detected in the open-air and without the confounding influence of any chamber system on ozone flux. The detail of the ICP Vegetation database allowed a model to be developed (named PROBE) that could be used to predict level II critical levels for ozone in a range of climatic and pollutant conditions typically associated with Europe. By meticulously testing numerous input combinations, it has been possible to select a parsimonious ANN model that had an r^2 value for the training data of 0.840 and 0.710

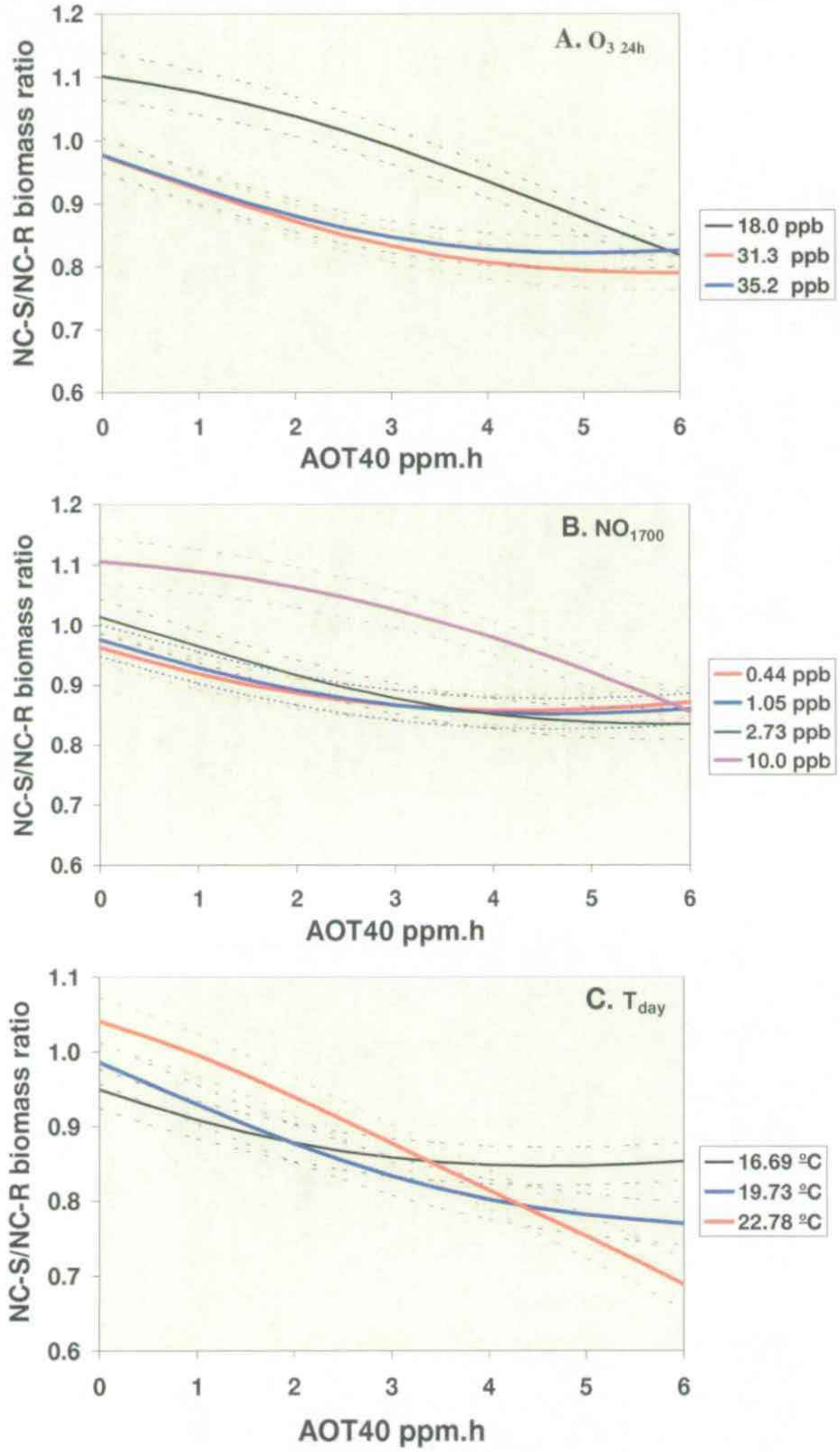

Figure 2.10: Predicted AOT40-biomass ratio responses for (A) $O_{3\ 24h}$, (B) NO_{1700} and (C) T_{day}. The lines indicate the relationship for the 1st, 2nd, and 3rd quartile values of each input. Other input variables were co-varied using the equations shown in Table 2.2.

for the test data, and thus fitted the data far better than a single factor (AOT40) model (r^2 of 0.59 for the training data and 0.41 for the test data). Using a similar approach, the best multiple linear regression model had twice as many inputs, and had a much poorer performance for the test data. Furthermore, where direct comparisons were made, the non-linear ANN models performed better than MLR in every case. This was especially apparent when the models were tested with previously unseen data, and confirms other findings from comparisons of the two methods. Using both MLR and ANN analysis, the inclusion of additional pollutant parameters in the model (eg $O_{3\ 24h}$, $NO_{\ 1700}$) seemed more important than the inclusion of climatic conditions such as temperature and VPD.

2.10.1 Sources of error in the model

Before the results of this modelling exercise can be considered further, it is important to take account of possible sources of errors in the results. A frequent criticism of the NC-S/NC-R clone system is that it was developed in North Carolina, USA, and is not suitable for use in cool northern European climates. By inference, critics assume that the response to temperature must override the response to AOT40. However, no relationship was found between biomass ratio and T_{24h} or T_{day} (r^2 = 0.07 and 0.08 respectively, Figure 2.8), and both MLR and ANN analysis indicated that temperature was less important than AOT40 in determining the biomass ratio. Two data replicates (Italy-Isola Serafini, harvest 3 and 4, 1998) may have had a relatively large influence on model development as the NC-S/NC-R ratios were 0.38 at an AOT40 of 11.98 ppm.h and 0.23 at an AOT40 of 10.84 ppm.h respectively. There was no justification for removing these data during the quality assessment exercise as there were no pest or disease problems, nor any problems with the establishment of either clone. Indeed, the biomass ratios were in the same range as those experienced at Riverside, California, in 1993 and 1994 (Heagle and Stefanski, 2000) where three month ratios of 0.31 and 0.41 were associated with three-month AOT40s of 45.8 and 47.2 ppm.h respectively (hourly means accumulated over 12h rather than daylight hours).

The decision to use different soil media at the different sites might have introduced some error into the modelling process. Ideally, the soil substrate should be fixed by the experimental protocol, however, experience from previous ICP Vegetation experiments has shown that a growth medium suitable for a cool – wet site such as UK-Bangor is inappropriate for hot-dry climates typical of southern Europe (unpublished). An artificial substrate could have been used, but this would have reduced the relevance of the findings to field conditions. Other sources of error, such as differences in water availability and harvesting procedure, were avoided by the use of a common experimental protocol. Whatever the source, the errors in PROBE were relatively small as the r^2 value for predicted versus actual values was 0.84 for the training data set and 0.71 for the test data. These high r^2 values gave us the confidence to use the output of the model, as well as features of its development, for consideration of critical levels for the clover clone system.

2.10.2 Model structure and predictions

Several exposure indices for ozone were considered in the analysis. In each type of analysis, AOT40 was found to be the most important ozone parameter. This is in agreement with previous analysis of ozone-effect data for cereals (see review by Fuhrer *et al*, 1997), and confirms that this parameter is suitable for use with non-cereal crops. $O_{3\ 24h}$ was the next most important ozone parameter included in both the "best subsets" MLR (data not presented) and the development of ANN Model 3. When combined with AOT40, these two parameters provide a better description of the ozone conditions at any one site by providing information on the "peakiness" of the ozone. For example, Germany-Giessen, a rural site at an altitude of 190m had an AOT40 of 4.04 ppm.h with an O_3 $_{24h}$ mean of 29.2 ppb (harvest 4, 1998), whereas at 480m, the rural site of Germany-Deuselbach had a similar O_3 $_{24h}$ of 28.6 ppb but a much lower AOT40 of 0.2 ppm.h. (harvest 5, 1998). Thus, by including both parameters, the model can predict responses for sites such as that at Germany-Deuselbach where background concentrations are relatively high but peaks above 40 ppb are relatively rare.

An intriguing feature of PROBE was the high weighting given to NO $_{1700}$ as an input. Values for NO $_{1700}$ were mainly below 3ppb, but were in the range 8 - 38 ppb at the Germany-Trier and Germany-Essen sites where AOT40 was below 1.5 ppm.h. Other NO_x parameters were included in the best performing MLR model, and NO_2 $_{day}$ and NO $_{>40}$ were included in the 5- and 6-input ANN models developed during the selective reduction of inputs to ANN Model 2. The possibility that NO $_{1700}$ was being used in the model to predict low ozone concentrations was excluded when the model performed less well when the NO $_{1700}$ input was swapped for O_3 $_{1700}$. It is more likely that NO $_{1700}$ was being used as surrogate for the mixture of pollutant and climatic conditions associated with urban and semi-urban sites as data points from such sites were grouped together by cluster analysis (data not presented). In addition, the NC-S clone of white clover may be sensitive to NO and NO_2 as well as ozone, since Murray *et al* 1994 have shown that the shoot biomass of white clover cv Haifa was reduced by half after a 149d exposure to 29 ppb NO and NO_2 (ratio 3:1). Whether such possible effects were additive (e.g. Ashenden and Edge 1995) or synergistic (e.g. Bender *et al*, 1991) could not be determined from the data. However, PROBE predicts that the NO $_{1700}$ values experienced at 75% of the sites (up to 2.7 ppb) had little discernible effect on the response to ozone. It was also predicted that response to NO $_{1700}$ was only important at "high" values (e.g. 10 ppb) with a positive effect at 0 AOT40, and an increasing response to ozone at AOT40s above 2.5 ppm.h. Thus, PROBE appeared to simulate the increased sensitivity of the NC-S clone at the high AOT40/high NO_x site of Italy-Isola Serafini, whilst also showing that this parameter was not important in the range of AOT40s associated with a critical level for biomass reduction.

Both temperature and VPD are widely recognised as factors influencing the flux of ozone into the leaf, and therefore are expected to be important components of a level II model for ozone (e.g. Grünhage *et al*, 1999). However, several of the climatic inputs were removed from the modelling process at an early stage because of cross-correlation. Of those that were left, T $_{day}$ was more important in the MLR and ANNs than T $_{>40}$ and VPD $_{>40}$. When the correlated inputs were re-considered in the development of PROBE, inclusion of T $_{24h}$ led to the best performing model. Models with T $_{max}$, VPD $_{day}$, or VPD $_{>40}$ as additional inputs had high r^2 values for the training data, but did less well for the test data. The models presented in this section indicate that temperature during daylight hours, and the 24h mean temperature, were more important than either temperature or VPD at the times when ozone concentrations exceeded 40 ppb. It is possible that temperature effects on growth as well as on conductance may have been contributing to the response to ozone since Werner and Büker (1999), using controlled environment exposures of NC-S and NC-R clover clones, have shown that VPD and radiation were stronger influences on conductance than temperature. Several authors have suggested that wind speed and soil moisture deficit (SMD) are more important than VPD and temperature in influencing the flux of ozone to wheat (e.g. Grünhage *et al*, 1999) through their influence on atmospheric conductivity of ozone and stomatal aperture respectively. These factors were not included in the current analysis because use of the wick system ensured that soil moisture content was not limiting, and there were insufficient sites where hourly wind speed records were available. An experimental programme using soil-grown plants and incorporating measurements of wind-speed is planned for future years.

There was some evidence of an increase in the sensitivity of the clover clones to ozone between the second and the fourth harvest. Such effects were also noted for the clover clones by Chevone *et al* (1998), and for white clover as a component of pasture (Fuhrer *et al*, 1994). However, inclusion of harvest number in PROBE reduced the performance considerably. This might have been because harvest interval was included as an integer of range 2-6 and not as a continuous variable like temperature, or it may have been due to the relatively low number of replicates per harvest making modelling less accurate.

Recent tests of the model by incorporating new data from the 1999 season have further increased our confidence in its use. Using the same structure as PROBE, the new model performed slightly better after retraining. Although not as exhaustive as the development of PROBE given the limited time available between receipt of the 1999 data and the production of this report, the step-wise selection procedure employed led to a model with very similar structure to that of PROBE that generalised better for unseen data. Two inputs for ozone, three for temperature, and one for NO were used in the best performing model. The prevalence of temperature inputs suggests that when soil moisture is not limiting, and phenological influences on sensitivity are equal at all sites, temperature is an important level II factor.

2.10.3 Final model and it application to Critical Levels

For comparison with the current level I critical level for wheat, the AOT40 predicted to cause a 5% reduction in biomass was calculated from PROBE as 0.9 ± 0.28 ppm.h. The r^2 values for both the wheat and the clover models were similar at 0.84 and 0.88 (Fuhrer *et al*, 1997) respectively. A benefit of the clover model is that other factors are included, allowing the key conditions associated with a 5% biomass reduction to be identified. For example, the average environmental conditions associated with an AOT40 of 0.9 ppm.h were 26.4 ppb $O_{3\ 24h}$, 0.7 ppb $NO_{\ 1700}$, 20.0 °C T_{day} and 17.7 °C T_{24h}, indicating high background ozone with low and infrequent peaks, low NO, and medium-range temperature. The clover model can also be used to predict effects over a shorter time interval of one month rather than the three months used for wheat. Retraining PROBE after addition of the 1999 data to the dataset led to an improvement in the performance of the model for unseen data, indicating that the five inputs (AOT40, $T_{\ day}$, $O_{3\ 24h}$, $T_{\ 24h}$, and $NO_{\ 1700}$) were indeed good predictors of biomass change in white clover. The application of PROBE to critical level exceedance mapping is described in Section 3.

3 ICP Vegetation Contributions to Level II Mapping for Ozone

3.1 Aims

During the first year of this contract EPG 1/3/96, the ICP Vegetation began to coordinate research in Europe on critical levels of ozone for crops and natural vegetation because several research groups were conducting closely-related research. This opened up new communication channels between the groups and has led to a more coherent approach. The research groups report their results annually to ICP Vegetation Task Force Meetings and a summary report is presented to each WGE meeting. The aims were:

- To coordinate "critical levels for ozone" research in Europe and report recent developments to the WGE.
- To develop methods for extracting equations from ANNs.
- To use the equations extracted from PROBE to produce a map of clover biomass ratio in Europe (in collaboration with Dr M Posch, CCE).
- To develop methods for incorporating level II factors into the dose-response function for wheat, and to use these methods to produce level II maps for wheat yield in Europe (in collaboration with Dr M Posch, CCE and Prof. J Fuhrer, Switzerland).
- To develop ozone flux models for wheat (in collaboration with Dr L Emberson (SEI-Y) and Prof. M Ashmore (University of Bradford, funded by contract EPG 1/3/104).

3.2 Introduction

The level-I critical level for ozone using the AOT40 index is considered a minimum requirement for mapping exceedance (UBA, 1996). Local factors, such as crop species and cultivar, phenological stage, soil moisture deficit (SMD), co-occurrence with other pollutants and climatic factors (vapour pressure deficit (VPD) and wind speed) should be taken into account when calculating site-specific critical levels for ozone. However, there was insufficient information available at that time of the Kuopio Workshop (March, 1996) for these so-called level II factors to be included in the revised definition for the long-term critical level. Thus, the (level I) critical level was set at an AOT40 of 3 ppm.h accumulated during daylight hours (>50 Wm^{-2}) over three months where AOT40 is the sum of hourly mean concentrations above 40 ppb (UBA, 1996). This critical level was reviewed and retained at the *Critical Levels for Ozone – Level II Workshop* (Gerzensee, Switzerland, April, 1999). The value was used in the development of the UN/ECE Multi-pollutant/Multi-effect protocol (signed December, 1999) and will be used subsequently to map exceedance in Europe. However, it was concluded that the accuracy of the critical level of ozone for crops would be substantially improved if future research was focussed on a level II approach (Fuhrer and Achermann, 1999).

During the course of this contract, three level II approaches have been developed by ICP Vegetation participants. Firstly, the ICP Vegetation Data Modelling Centre has developed a parsimonious model (named PROBE) from the results of the clover clone experiment that allows biomass responses to ozone to be predicted in climatic and pollutant conditions typical of Europe (Section 2 of this report). Equations extracted from the model have been used to map ozone effects across Europe. In the second approach, a function for the modifying effect of SMD on crop yield has been incorporated into the dose-response function for wheat enabling reduced effects of ozone in areas where soil moisture is limiting to be incorporated into exceedance maps. The maps have also been improved by altering the timing of the three-month window to the time in which the crop is actively growing in each area of Europe. Finally, a method for estimating the flux of ozone from the maximum potential stomatal conductance of wheat has been developed by Dr L Emberson and Prof. M Ashmore and used to develop maps of ozone fluxes for Europe. The progress made with each of these three methods is summarised in this Section.

3.3 Coordination of Critical Levels Research in Europe.

The ICP Vegetation agreed at the 16th Session of the WGE (August, 1997) to coordinate critical levels research in Europe to ensure efficient use of resources. An Ozone Mapping Committee was formed, and the first meeting was held in Nottingham, UK, on 11th December, 1997. The meeting was attended by Prof. K Bull (Chairman, WGE), Dr G Mills (Chairperson, ICP Vegetation), Dr G Ball (ICP Vegetation Data Modelling Centre), Prof. J Fuhrer (ICP Vegetation Steering Committee), Dr M Posch (CCE), Dr D Simpson (EMEP), Dr L Emberson (then ICCET), UK), Mr J Kuylenstierna and Mr H Cambridge (SEI-Y)). Apologies were received from Dr M Ashmore (then ICCET) and Dr D Palmer-Brown (ICP Vegetation Data Modelling Centre). Participants described their most recent work, and identified areas where rapid progress could be made. Work programmes were considered in preparation for the Ozone Critical Levels Workshop hosted by Switzerland in April, 1999. Closer collaboration including exchange of data, methods and vegetation maps was agreed. Since then, the group have met at each subsequent ICP Vegetation Task Force Meeting as well as at the Gerzensee Critical Levels Workshop.

3.4 Mapping biomass reduction in clover

3.4.1 Extraction of an equation from PROBE

PROBE, the ANN model for predicting clover clone biomass could only be run using Neuroshell2 software and thus could not be linked to a GIS to produce maps of predicted biomass ratio for Europe. To overcome this problem, an empirical equation was extracted from the weight of PROBE by a further development of the methods of Roadknight *et al* (1997). This made the model portable and allowed it to be used by a wider group including the CCE in conjunction with EMEP. The inputs used (AOT40, T_{day}, $O_{3\ 24h}$, T_{24h}, and NO_{1700}) could all be calculated from the EMEP database.

The first step in equation extraction involved determination of the form of the equation from the weights. This was based on the function:

$$H_n = f((I_1.Whn_1 + I_2.Whn_2 + I_3.Whn_3 \ldots\ldots\ldots + I_m.Whn_{n,m}) + Bhn)$$

and

$$Output = f((H_1.Wout_1 + H_2.Wout_2 + H_3.Wout_3.......... + H_n.Wout_n)+Bout)$$

Where H_n = output from hidden node n
I_m = scaled input value for input m
$Wh_{n,m}$ = weight to from input m to hidden node n.
$Wout_n$ = weight from hidden node n to output.
Bhn = bias connection to the hidden node n.
$Bout$ = bias connection to the output.
f = a sigmoidal function representing the connections of the network (transfer functions), defined as:

$$f(x)=1/1+EXP(-x).$$

This produced an equation for values scaled between -1 and +1. The next stage was to unscale the input and output terms, and hence the predictions. This was achieved for each input using the following term:

Unscaled value = (2(scaled value - minimum value /range of values))-1

The final equation (PROBE-equation) took the form:

$$H1 = 1/(1+(EXP(-(((O3_{24h}-9.21)/13.759)+((AOT40)/25.274)+((NO_{1700}-0.2)/-124.907)+((T_{24h}-11.69)/-4.546)+((T_{day}-13.64)/-2.395)+2.632))))$$

$$H2=1/(1+(EXP(-(((O3_{24h}-9.21)/-384.052)+((AOT40)/-16.547)+((NO_{1700}-0.2)/20.904)+((T_{24h}-11.69)/226.035)+((T_{day}-13.64)/-63.932)-0.946))))$$

$$H3=1/(1+(EXP(-(((O3_{24h}-9.21)/-84.375)+((AOT40)/20.871)+((NO_{1700}-0.2)/98.578)+((T_{24h}-11.69)/72.033)+((T_{day}-13.64)/51.850)-1.317))))$$

H4....

H15....

$$NC\text{-}S/NC\text{-}R = ((1/(1+(EXP(-((1.809*H1) - (0.549*H2) + (0.08*H3) - (0.312*H4) + (3.439*H5) + (2.472*H6) - (0.509*H7) - (0.509*H8) - (1.005*H9) - (0.299*H10) - (0.431*H11) - (2.75*H12) - (0.79*H13) - (0.171*H14) - (0.348*H15) - 0.65))))+0.23)*1.03).$$

The predicitions of $PROBE_{equation}$ were validated by linear regression (Figure 3.1). This approach showed that $PROBE_{equation}$ suffered no loss of accuracy producing an r^2 value of 0.731 for all data compared to 0.732 for the original ANN model, PROBE. However, a slightly steeper slope and a lower intercept were produced indicating some loss of accuracy.

3.4.2 Using $PROBE_{equation}$ to develop maps of biomass ratio.

$PROBE_{equation}$ was used by Dr M Posch of the CCE to predict the biomass ratio from the EMEP/MSC-W model for 1990 for each 150km x 150km grid square. These predictions were then incorporated into a map based on the EMEP grid (Figure 3.2).

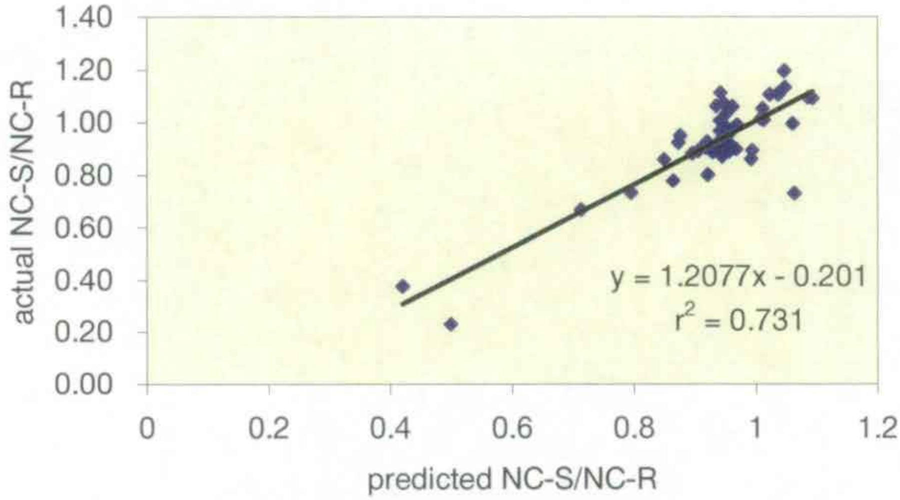

Figure 3.1: A comparison of the actual NC-S/NC-R biomass ratio with that predicted by $PROBE_{equation}$.

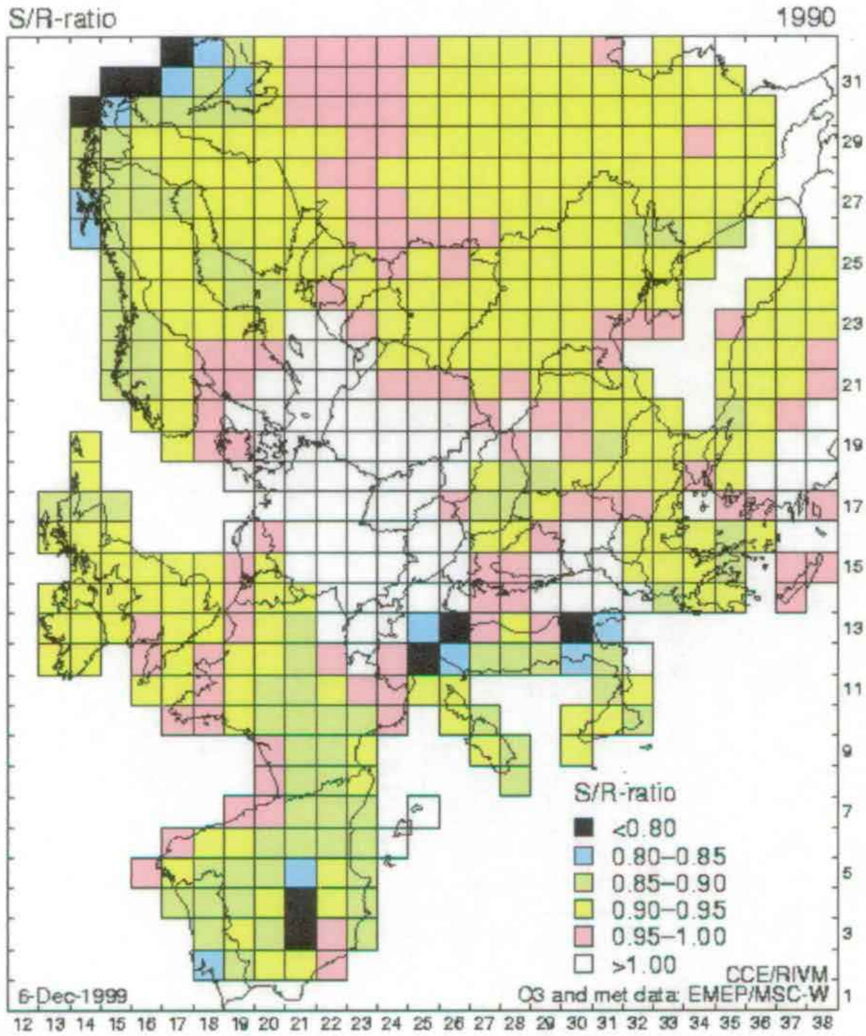

Figure 3.2: Map of the NC-S/NC-R biomass ratio developed by applying $PROBE_{equation}$ to the EMEP/MSC-W data for 1990.

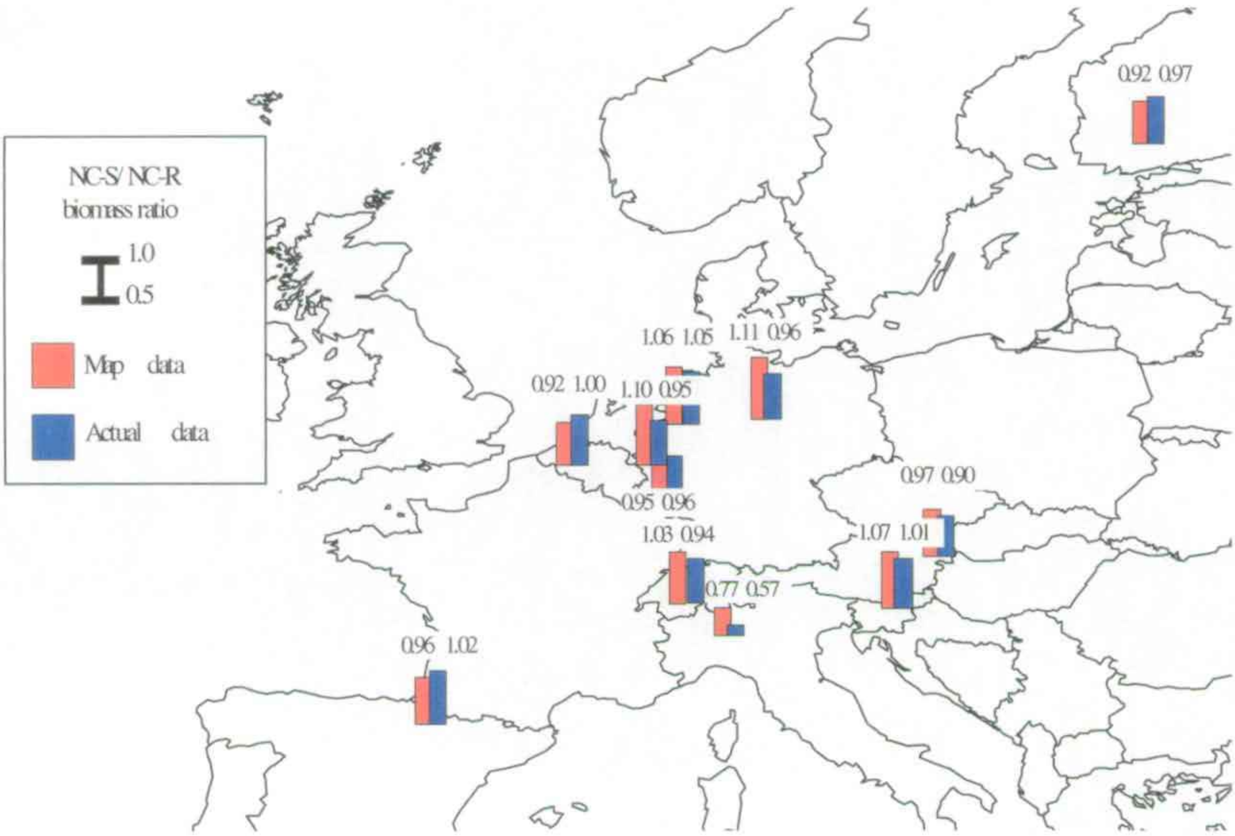

Figure 3.3: Map showing a comparison between the EMEP map data (1990) and the actual data (mean of 1996 to 1999) for the ICP Vegetation sites used in the development of PROBE.

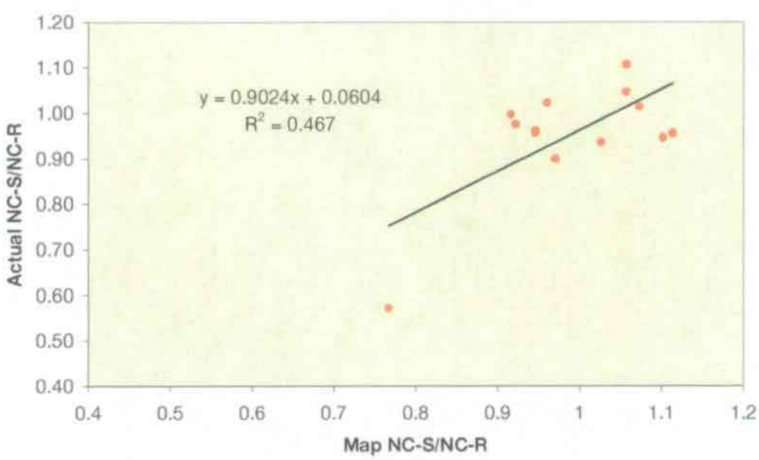

Figure 3.4: A comparison of the predicted NC-S/NC-R biomass ratio values (based on 1990 data) with actual ICP-Vegetation NC-S/NC-R values (mean of 1996-1999) for sites in the corresponding grid square.

The map showed the expected reductions in the NC-S/NC-R biomass ratio in the southern areas such as Spain and Italy, with higher ratios in central and southern areas. On a finer scale, the predictions of biomass ratio for the squares incorporating the ICP Vegetation sites used in the development of the model have been compared with the actual values meaned over the 1996 – 1998 seasons (Figure 3.3). Applying $PROBE_{equation}$ to the 1990 data caused an over-prediction of damage for the sites at Germany-Braunschweig and Germany-Essen (low-medium ozone, mid-range temperature) and an under prediction of damage at Italy-Isola Serafini (high temperature, high ozone). The predicted value for 1990 was within 10% of the actual data (meaned from 1996 – 1998) for the remaining ten sites. Overall, the regression of the mapped values versus the meaned actual values had an r^2 of 0.47 (Figure 3.4).

3.5 Mapping yield reduction in wheat

3.5.1 Using level II factors to modify the wheat yield response function

In collaboration with Prof. J Fuhrer (IUL, Switzerland) and Dr M Posch (CEC).

The critical level of ozone for yield reduction was derived from experimental data from open-top chamber experiments performed with 10 cultivars of wheat in 6 countries over 10 seasons (r^2 of 0.88). This equation has been used by Dr M Posch (CCE) to produce an exceedance map for Europe showing AOT40 values of more than three-times the critical level in most of France, Italy, Germany and Belgium. As a first stage in the movement towards a level II critical level for ozone, weighting factors for phenology and SMD were incorporated into the dose response function of Fuhrer *et al.* (1997).

Maps of critical level exceedance were first refined by adjusting the period for the calculation of AOT40 according to plant development or phenology because wheat sensitivity to ozone varies throughout the life cycle (Soja *et al*, in press). The period of grain fill has been identified as a particularly sensitive growth stage. ICP Vegetation participants monitored the development of commercial fields of winter wheat at 13 sites in Europe during 1997 and 1998 (Figure 3.5). The most rapid development occurred at Spain-Catalonia where anthesis was reported on 2nd May (day 122, Table 3.1), and the least rapid development occurred at Finland-Jokioinen, where anthesis occurred on the 3rd July (day 184).

Using temperature data from EMEP/MSC-W and the ICP Vegetation data on the timing of anthesis, phenology has been incorporated into ozone mapping procedures by adjusting the three-month window for accumulating AOT40 to two months before and one month after anthesis. The day of anthesis of wheat was assumed to be the day when the growing degree days (GDD), i.e. the sum of mean daily temperatures above 0°C, reached a value of 1150. Using 6-hourly temperature data for 1990 (2m above ground) for the 150km×150km EMEP grid provided by EMEP/MSC-W, the Julian day for the anthesis of wheat was calculated (Figure 3.6). As expected, the predicted day of anthesis was earliest in the south and latest in the north.

The three-month AOT40 for each grid cell was calculated using modelled 6-hourly ozone data for April-September 1990 provided by EMEP/MSC-W (Simpson *et al.* 1997). In Figure 3.7 the exceedance of this modified AOT40, i.e. AOT40 minus 3 ppm-h, is compared with the exceedance of the level-I AOT40 for crops, i.e. the AOT40

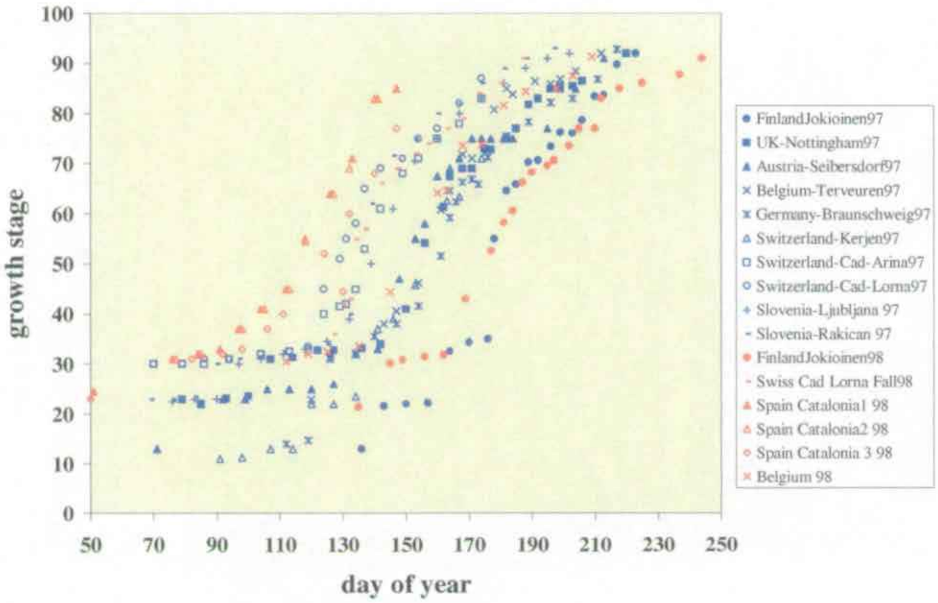

Figure 3.5: The development of winter-wheat at European sites in 1997 and 1998. Anthesis takes place at growth stage 60. For details on other growth stages see Tottman and Broad (1987) or the experimental protocols for 1997 and 1998.

computed for the fixed 3-months growing season May-July. Note, that for grid cells with an anthesis before 15 May (shaded dark grey in Figure 3.7) the modified AOT40could not be calculated, since no ozone data was available for days before 1 April. Comparing those grid cells for which both types of AOT40 can be computed shows that taking anthesis into account results in slightly lower overall exceedances. To get a complete picture, however, ozone values for the months before April are also required.

The next stage in the consideration of phenology was to incorporate weighting factors for the individual growth phases of wheat using the methods developed by Soja *et al.* (in press). Multiplication factors of 0.402 and 1.062 applied to months 1 and 2 (before anthesis) and 1.536 applied to the month after anthesis were applied to the AOT40s for each month of the three-month period, thus allowing the change in sensitivity during the life-cycle of the crop to be taken into account. Applying this calculation to the map for 1994 caused a shift eastwards and northwards in the areas where the AOT40 exceeded 6 ppm.h. In the south-west areas the crop was in the latter stages of development when exceedence of the critical level occurred and was therefore less sensitive to ozone (Figure 3.8 (b)). Thus, by incorporating phenological factors in these ways, the areas where critical level exceedance might be causing effects on yield can be more clearly identified.

This level II approach was developed further by consideration of SMD. This level II parameter was selected because SMD is sufficiently low in some areas of Europe to reduce the impact of ozone on wheat yield by reducing stomatal conductance and hence the flux of ozone into the plant. Using data from field experiments in which wheat was grown with or without water stress, Fuhrer (1996) derived a function, f_{SMD}, for the modifying effect of SMD on the yield response of wheat to ozone:

$$f_{SMD} = min\ \{max\{0.1 - 1.8.\ swc - 0.198\},1.\}$$

Where *swc* is the mean relative soil water content during the three-month growth period.

This function was incorporated into the AOT40 yield-response function by Dr M. Posch (CCE) by calculating *swc* according to a model that was essentially the same as that used in the IMAGE Global Change Model (Leemans and van den Born, 1994) and follows the approach by Prentice *et al* (1993). This method reduced the amount of exceedance further in drier countries such as Greece and Spain (Figure 3.8(c)).

In the final stage conducted so far, the phenological and SMD weightings were combined (Figure 3.8(d)). This showed that the highest AOT40 values, and hence expected effects on yield were predicted for large areas of France, Belgium, The Netherlands, Italy and Germany. The potential effects of ozone in Greece and Spain, and to a lesser extent Italy, are considerably lower than suggested by the level I map (Figure 3.8(a)).

3.5.2 Modelling ozone flux to wheat

In collaboration with Prof. M Ashmore (University of Bradford), Dr L Emberson, Dr H Cambridge and Dr J Kuylenstierna (SEI-Y, UK), and Dr D Simpson (EMEP).

The level II factors already discussed modify the plants response to a specific ozone concentration by determining the uptake or flux of the gas. An alternative Level II approach has been developed by Prof. M. Ashmore and Dr. L. Emberson in which published data has been used to model and map ozone fluxes to wheat (DETR Contract EPG 1/3/104). The model calculates atmospheric (R_a) and boundary layer (R_b) resistances as a function of meteorology and vegetation surface. Stomatal resistance is calculated according to species-specific maximum stomatal conductance, its variation over the growing season, and relationships with irradiance, temperature, VPD and SMD, all parameters having been derived empirically. Further inputs to the model describe modelled ozone concentration fields and meteorological data across Europe at a 6 hourly temporal and 150 x 150 km spatial resolution. These data were obtained for a single year, 1994.

Figure 3.9 shows the modelled mean monthly ozone flux to wheat for June during 1994 and the corresponding AOT40 data. Comparing these two maps shows that the highest AOT40 values are not necessarily associated with the highest ozone fluxes. Analysis of the 6 hourly data reveals that the main reason for these lower fluxes are the associated environmental conditions which co-occur with high ozone concentrations. Overall, the most important of these is VPD, which tends to increase in parallel with increasing ozone concentrations and limits stomatal conductance and hence ozone uptake. This modelling approach also indicated that temperature is likely to limit ozone flux towards the beginning and end of the growing season, i.e. when ozone concentrations are usually lower.

Figure 3.9 also shows the impact that growing season has on flux. The banding of flux classes across Europe is a result of the growth period falling earlier in the year in the more southerly latitudes. By the end of June, senescence has begun in the Mediterranean region, and ozone uptake is subsequently reduced.

Four EMEP grid squares with contrasting ozone exposure regimes were selected from Figure 3.9 for further study (Table 3.2). The modelled cumulative flux to wheat for the UK and central European squares were similar even though the AOT40 for the UK was approximately half of that for the central European square. When a flux threshold of 1.5 nmol m^{-2} s^{-1} was introduced, the cumulative flux for the UK square was higher than that in central Europe.

3.6 Discussion

Table 3.1: The timing of anthesis of commercial winter wheat in fields near ICP Vegetation sites.

Country	*1997* Day number (Date) of anthesis	*1998* Day number (Date) of anthesis
Austria-Seibersdorf	157 (6th June)	-
Belgium-Terveuren	161 (10th June)	158 (7th June)
Finland-Jokioinen	181 (30th June)	184 (3rd July)
Germany-Braunschweig	165 (14th June)	-
Slovenia-Rakian	145 (25th May)	-
Slovenia-Ljubljana	137 (17th May)	-
Spain-Catalonia 1	-	122 (2nd May)
Spain-Catalonia 2	-	122 (2nd May)
Spain-Catalonia 3	-	132 (12th May)
Switzerland Cadenazzo cv Arina	142 (22nd May)	-
Switzerland Cadenazzo cv Lorna	135 (15th May)	-
Switzerland-Cadenazzo cv Lorna (Autumn sowing)	-	139 (19th May)
Switzerland-Cadenazzo cv Lorna (Spring sowing)	-	153 (2nd June)
Switzerland-Kerjen	157 (6th June)	-
UK-Nottingham	161 (10th June)	-

3.6.1 The uncertainties associated with level II mapping

Over the last three years, the ICP Vegetation has explored three different approaches to the on-going problem of incorporating level II factors into the long-term critical level of ozone for crops. Nevertheless, common features are appearing in the results such as the prediction that level II factors reduce the impact of relatively high ozone levels in southern countries of Europe. Before these features are considered in detail,

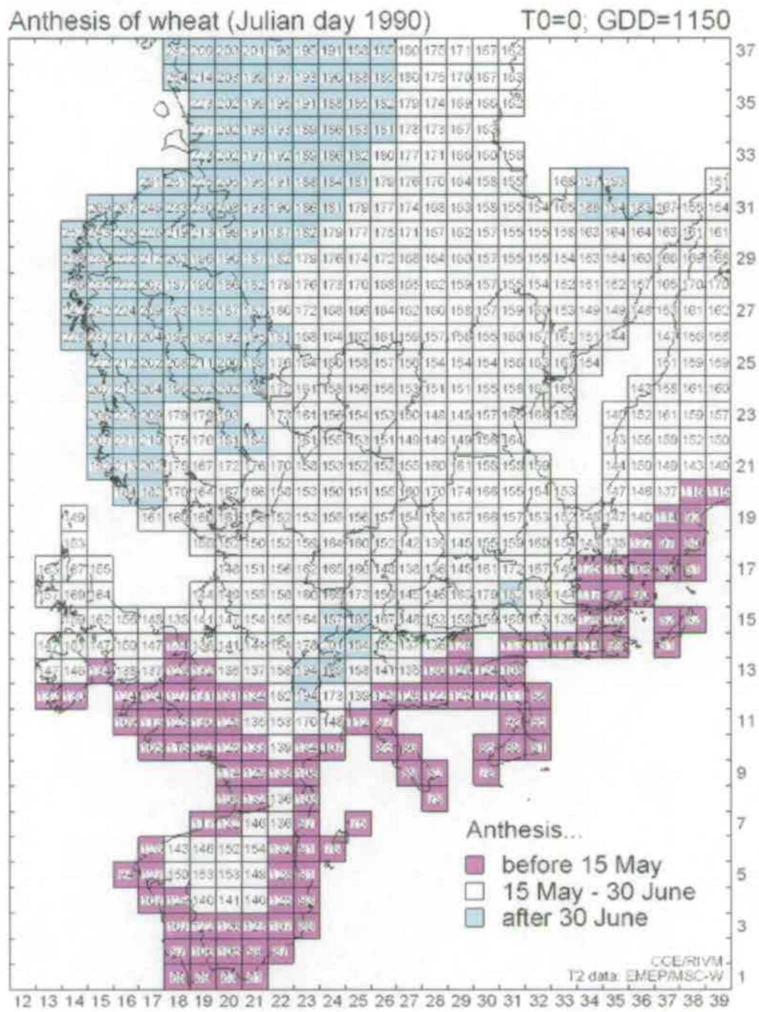

Figure 3.6: Julian date of the start of anthesis of wheat defined as GDD=1150 with a threshold of 0°C.

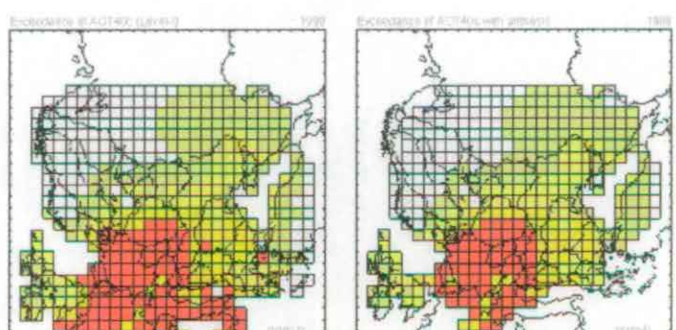

Figure 3.7: Exceedance of the critical level for crops with constant growing season May-July (Level I; left) and with a 3-month growing season dependant on the day of anthesis as shown in Figure 5 (right).

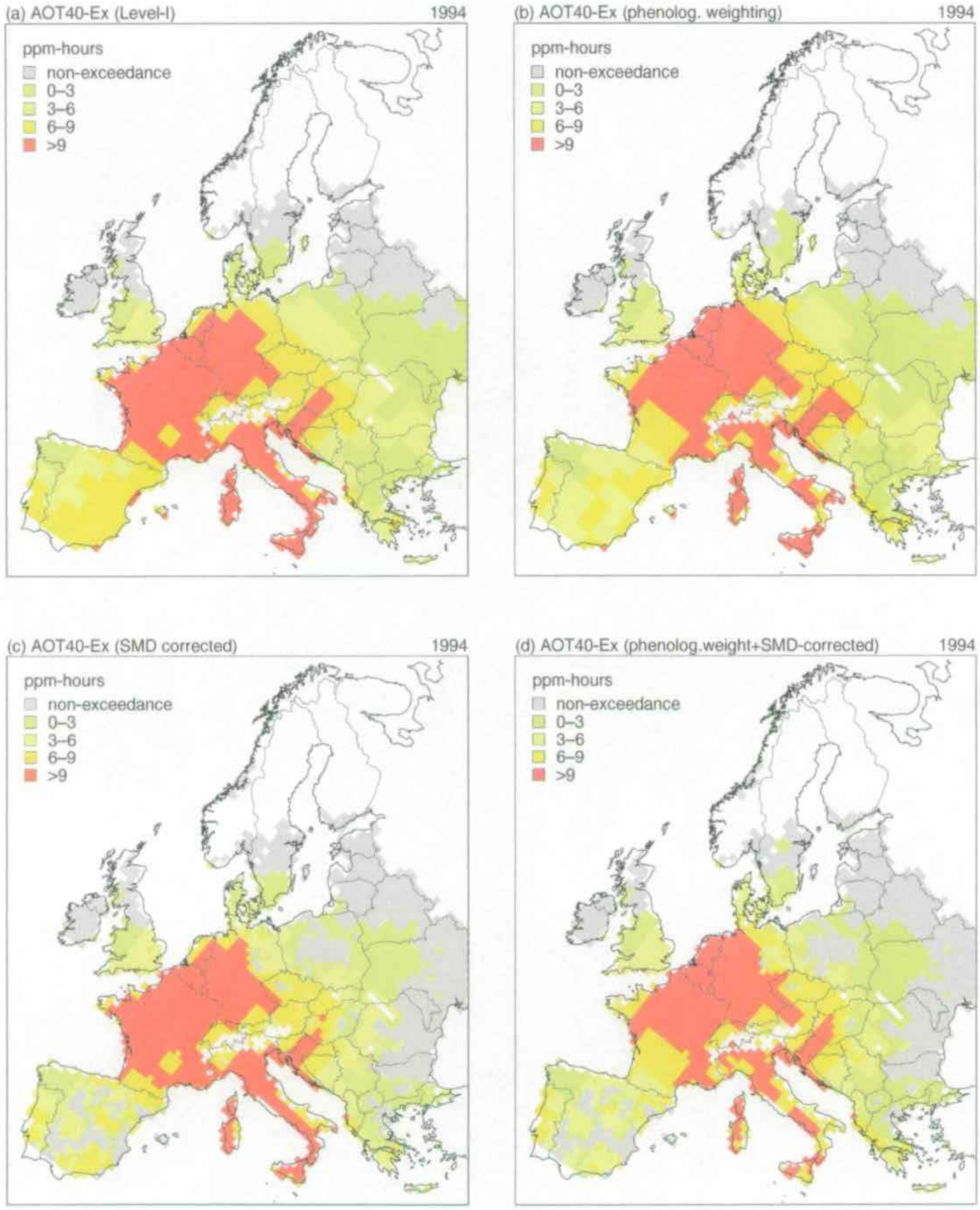

Figure 3.8: Exceedance of the critical level (an AOT40 of 3 ppm.h) for crops: (a) Level-I (May-July), (b) AOT40 computed with phenological weighting according to Soja et al. (in press), (c) Exceedance taking into account soil moisture deficit, (d) combination of (b) and (c).

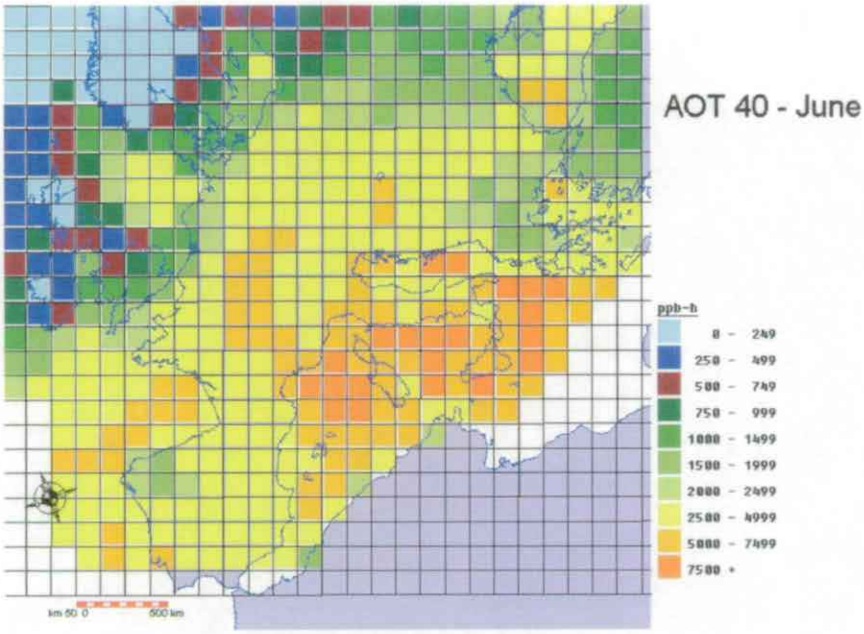

Figure 3.9 (a). Cumulative AOT40 (ppb.h.) for the month of June 1994.

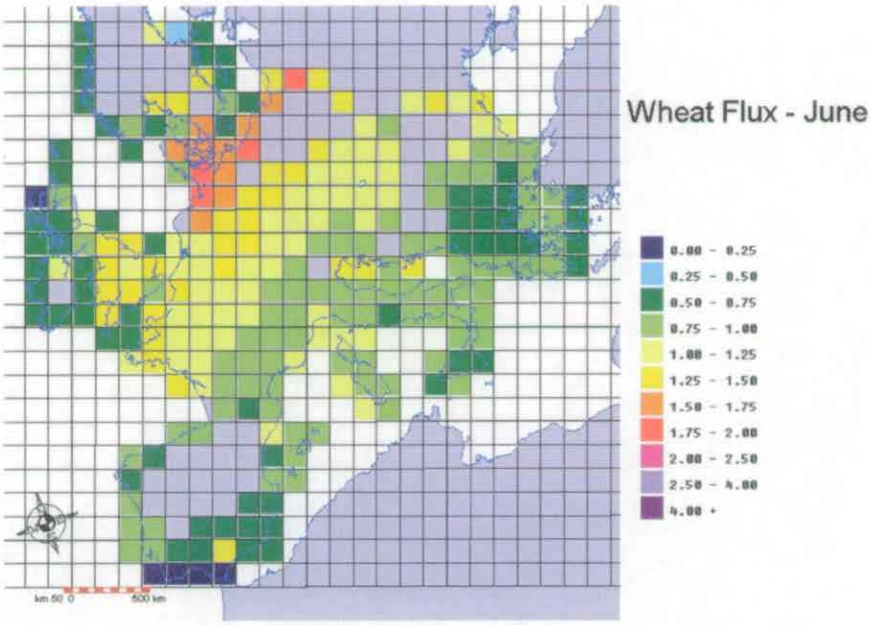

Figure 3.9 (b): Mean monthly ozone flux (nmol m^{-2} s^{-1}) to wheat in June during 1994.

Table 3.2: Calculated values for wheat for 1994 for total ozone flux (CFO_3 and CFO_3 > 1.5 nmol m^{-2} s^{-1} in mmol m^{-2}) and AOT40 in ppb.h. From Emberson *et al*, 1999.

	CFO_3	CFO_3 (> 1.5 nmol m^{-2} s^{-1})	AOT40
Sweden	7.35	3.33	4714
UK	6.54	3.23	4407
Central Europe	6.43	2.44	8730
Spain	6.65	4.48	2283

the uncertainties associated with each method and their common data sources for mapping should be reviewed. Each method relied on EMEP/MSC-W ozone and climate data for the production of European maps. The relatively coarse spatial and temporal resolution of the data meant that local variations in climate such as those found with increasing elevation could not be incorporated in the models (Ashmore and Emberson, 1999). Over the time-course used in the models (one to three months), errors associated with temporal variations during the course of each day would accumulate when either AOT40 or flux is calculated from the 6-hourly mean data available from EMEP. Furthermore, assumptions have to made when applying the EMEP data for 3m height to a low-growing crop canopy such as that of clover or a 0.8 - 1.0m canopy of wheat. These factors all contribute to the uncertainties associated with the maps presented in this section of the report.

In the first level II modelling approach, data from the ICP Vegetation clover clone experiments covering sites as far apart as Spain-Navarra and Finland-Jokoinen were used to develop a predictive model, PROBE using ANN methods (see Section 2). Using a method developed at the ICP Vegetation Data Modelling Centre, an empirical equation was extracted from PROBE (Roadknight *et al*, 1997, Ball *et al*, in press). This allowed PROBE to become "transparent" and usable by others. In so doing, a small loss in accuracy occurred as the regression equation for the relationship between actual and predicted values became skewed away from the 1:1 relationship established for PROBE. This meant that $PROBE_{equation}$ tended to over-estimate effects at low AOT40 and under-estimate effects at high AOT40. This suggests that some of the assumptions used in the extraction procedure (e.g. in the use of sigmoidal transfer functions and scaling of the inputs/outputs) may need reconsidering. When the equation was applied to the EMEP ozone data, a map of the NC-S/NC-R biomass ratio was generated for 1990. Predictions were within 10% of the actual value for over 75% of the sites and were within the accuracy expected given that the data were meaned for each site for the 3-4 harvests per year between 1996 and 1998. This modelling approach does have the advantage that it has been developed from "real" data from the exposure of plants to "real" ozone episodes in a variety of climatic conditions and without the confounding effect of any chamber system on ozone flux.

Two approaches were used for wheat. The first method involved the incorporation of modifying factors into the existing dose-response function and thus represented a logical development of the level I approach. ICP Vegetation data from assessments of the development of wheat at 13 sites across Europe were used to select a more appropriate three-month window for calculating the accumulated AOT40 for each EMEP square. The phenological weightings developed by Soja *et al* (in press) were based on good physiological assumptions but were derived from only two experiments in which ozone was applied at different growth phases during the development of the crop (Soja, 1996, Pleijel *et al*, 1998). Similarly, the SMD function was derived from relatively few experiments (Fuhrer, 1996). In the second wheat method, Emberson and colleagues have modelled the flux of ozone to the youngest fully developed leaf at the top of the canopy (usually the flag leaf for wheat). The model was developed from boundary layer analysis of pooled data from the literature on ozone experiments in open-top chambers allowing the maximum potential stomatal conductance to be calculated from functions for SMD, temperature, VPD and irradiance. The disadvantage of this approach is that the variation associated with differences in cultivars and combinations of climatic conditions are not incorporated into the model and thus it is not possible to apply confidence intervals to the modelled flux values. Furthermore, the flux-effect modelling developed so far assumes that flux equals effect and does not take account of ozone detoxification in the apoplast. Clearly, the assumptions made for both modelling approaches for wheat require validation by conductance measurements in ambient air, and by conducting purpose-designed exposure experiments.

3.6.2 An overview of the main results of the models

Taking all of the above uncertainties into account it is very encouraging that two quite different modelling approaches for wheat are producing similar results. The flux models of Dr L Emberson and colleagues have suggested that the ozone flux in June is high in Europe in the northern half of France, most of Germany, The Netherlands and Belgium. For most of these areas, anthesis falls in early-mid June; high ozone fluxes in June would thus be occurring at the time when the crop was most sensitive to ozone and would be likely to have a large impact on the final yield (Soja *et al*, in press). The same areas of Europe were identified by Dr M. Posch and colleagues as having the highest "modified AOT40" when modifying factors for phenology and SMD were incorporated into the AOT40 response function for wheat. It was less appropriate to compare the two models for more southern countries such as Spain, Italy and Greece since ICP Vegetation participants have shown that anthesis occurs in Spain (and possibly Greece and Italy) in early May. Thus, wheat would be senescing for a large part of June, and ozone flux would, as predicted by Emberson and colleagues, be significantly reduced. By shifting the timing of the three-month window according to phenological considerations, the method employed by Posch and colleagues allows the "modified AOT40" to be established for an appropriate time period for these countries. The modelling suggests that crops grown in Italy (and Slovenia) are more at risk from ozone than those grown in Greece and Spain.

Incorporation of SMD factors into exceedance mapping had little effect on the AOT40 map for northern European countries such as the UK, Denmark and Sweden because the soil moisture content is rarely limiting in these countries. However, inclusion of phenological factors resulted in an increase in the "modified AOT40" for the southern areas of Scandinavian countries due to the later timing of anthesis (late June/early July in Finland). Emberson and colleagues also identified these areas as having a high potential for ozone effects on yield as the highest fluxes predicted for June for all of Europe were found there. This was primarily because the low VPDs experienced in southern Scandinavia were highly conducive to ozone uptake.

The two models for wheat were derived from data from ozone exposure experiments conducted in open-top chambers. The constant air speed and turbulence in the chambers, the warming effect of the transparent walls, and the constant exposure to ozone (usually 7h d^{-1} for 90d) will have influenced the flux of ozone to the plant (Pleijel *et al*, 1998, Sanders *et al*, 1991) and hence the level II models developed from the data. As an alternative approach, the ICP Vegetation model PROBE has been developed from biomass changes in plants exposed to a diverse range of ambient pollutant and climatic conditions. The model has been developed from data from well-watered plants (i.e. SMD was not limiting) and is for a shorter time interval of 28d. The benefit of using this species and approach is that the phenological and SMD components are removed from the model allowing other level II factors to be considered. Two factors describing the ozone exposure (AOT40 and $O_{3\ 24h}$), two factors describing temperature (T_{day} and T_{24h}) and NO_{1700} were found to have the strongest influence; VPD parameters were not found to be important (see Section 2). $PROBE_{equation}$ predicted that reductions in biomass ratio were highest in parts of Italy and central Spain, with reductions of 10% or more predicted for most of Portugal, Spain, France, Italy and Greece. This zone of highest effect is generally further south than that predicted for wheat (see above) suggesting that when growth stage and SMD are not limiting, significant impacts of ozone can occur in these countries.

3.6.3 Conclusions from the Gerzensee Critical Levels Workshop

The work presented in this Section formed an integral part of the discussions at the *Critical Levels for Ozone – Level II Workshop* (Gerzensee, Switzerland, April, 1999, Fuhrer and Achermann, 1999). The workshop concluded that the flux-response approach described in Section 3.5 would ultimately be the way forwards. In the meantime, important progress could be made using the modified AOT40 approach described in Section 3.4. The results from all three methods were used to compile the following list of factors that are considered to modify the sensitivity of crops to ozone:

Soil factors
Soil moisture deficit if the crop is not well watered
Irrigation (especially important for southern areas)

Plant development factors
Relevant time interval
Variation in sensitivity during different growth phases

Factors influencing instantaneous ozone uptake by plants
Temperature
Vapour pressure deficit
Global radiation
Wind speed

Sources of data for further development of level II models were considered. Concern was raised about reliance on the data from open-top chamber experiments due to the influence of the chamber system on ozone uptake (see above). Data from the ICP Vegetation clover clone experiments were considered an invaluable source of information for level II modelling as the database is the only one in existence for plants grown in such a wide range of ambient conditions. The flux-effect modelling proposed for the next ICP Vegetation contract (see Section 8) will utilise existing and new data for stomatal conductance and will hence facilitate further progress with level II mapping. Furthermore, the proposed experiments with soil-grown clover clones was flagged as important for validation of the level II maps.

4 The occurrence of ozone injury on vegetation in the ECE area

4.1 Aims

Recording of the timing of visible injury was an important component of the work programme of the ICP Crops (former name of the ICP Vegetation) during the previous contract (EPG 1/3/13, 1994 – 1997). At the time of the change-over from contract EPG 1/3/13 to the present one, there was a lot of international interest in the so-called short-term critical level of ozone for visible injury. Interest declined during the current contract, with more emphasis being placed on the long-term critical level for yield/biomass reduction. Nevertheless, monitoring the frequency of injury-causing ozone episodes has remained an integral component of the activities of the ICP Vegetation. The aims were:

- To monitor the geographical areas in each year in which injury-causing ozone episodes occurred.
- To survey commercial fields for the presence of ozone injury after an injury-causing episode has occurred.
- To validate the short-term critical levels derived from the 1995 data of the ICP Vegetation.

4.2 Introduction

The presence of ozone injury on the leaves of sensitive plants provides a reliable bioindicator of a recent ozone episode. By monitoring the frequency of injury occurrence at the ICP Vegetation sites we have provided conclusive evidence of the extent of the ozone problem in the ECE region. Our surveys of commercial fields have shown that a range of crops can develop ozone injury in response to an episode. For some crops, the injury may only occur on one or a few leaves, and thus might not lead to yield reduction. However, when injury occurs on the leaves of crops grown for their foliage such as spinach and lettuce, the market value may be severely affected. For example, Velissariou (1999) reported 100% crop loss for lettuce and chicory in the Achames area of Greece (12km north of Athens) following an ozone episode in mid-October, 1998. The extensive reddening and necrotic symptoms meant that these two horticultural crops could not be marketed with financial losses as high as 12,500 ECU reported for a single one-acre glasshouse of "Butterhead" lettuce. Even if visible ozone injury is not associated with commercial losses, it does represent a demonstrable effect of ozone that can be used to show the public that ozone can have a damaging effect on vegetation.

Ozone injury symptoms are characteristically seen as small flecks or stipples on the interveinal areas of the upper surface of leaves (Figure 4.1). The flecks may be white, red, black or bronze depending on species and may coalesce to form extensive areas of chlorosis as the leaf ages. Ozone enters plants via the stomatal pores in the leaf surface. It subsequently reacts with cell wall and membrane components (Kangasjärvi *et al*, 1994) resulting in the formation of reduced oxygen species such as hydroxyl and superoxide radicals and hydrogen peroxide which are highly reactive with biological molecules. Consequently, membrane integrity is disturbed, thus modifying cell permeability (Heath, 1987) and osmotic pressure, membrane potentials and the activity of membrane-bound enzymes such as ATPases are affected (Dominy and Heath, 1985). Membrane disruption, leading to cell death causes chlorotic flecking, necrosis and bronzing to appear on the foliage.

Two short-term critical levels of ozone for visible injury were set at the *UN/ECE Workshop on Critical Levels for Ozone in Europe-Testing and Finalising the Concepts* (Kuopio, Finland, 1996) following analysis of the 1995 ICP Vegetation experimental data (Benton *et al*, 1996). These were:

- An AOT40 of 200 ppb.h accumulated over five days when mean VPD (0930 – 1630h) was less than 1.5 kPa.

- An AOT40 of 500 ppb.h when mean VPD VPD (0930 –1630h) exceeded 1.5 kPa.

Vapour pressure deficit was included in these definitions because it is one of the main factors influencing stomatal conductance and thus the flux of ozone into the plant. For example, when VPD is high, conductance is reduced (Grantz and Meinzer, 1990) and the flux of ozone into the leaf is restricted. In these conditions, higher ambient ozone concentrations (or a higher AOT40) are required before an adequate dose is absorbed to cause injury. However, when VPD is lower, stomatal conductance increases which boosts the flux of ozone into the leaf. Thus, it is possible that the absorbed dose is sufficient to cause injury even though ambient ozone concentrations are low. Indeed, it has been suggested that higher yield reductions or injury levels occur when ozone concentrations are moderate (Grünhage and Jager, 1994; Krupa *et al*, 1995) because high ozone concentrations often coincide with conditions which are not conducive to ozone uptake, for example, high VPD. Gimeno *et al* (1995) described how ozone injury on tobacco cultivars in Spain was more prevalent in coastal areas but decreased at sites further inland. It was suggested that high relative humidity at the coastal sites could favour ozone phytotoxicity. Similarly, Balls *et al* (1996) reported how the level of injury observed on subterranean clover increased as VPD decreased and attributed this to an influence on stomatal conductance.

Figure 4.1: Ozone injury on white clover following on ozone episode in Italy (Photo: I. Fumagalli).

4.3 Surveys of ozone injury on crops

In each year of the contract, some of the participants have surveyed commercial fields for the presence of ozone injury on agricultural and horticultural crops. The surveys were made on the days following an incidence of injury on the experimental clover plants. Photographs of ozone injury and of injury caused by pests/diseases/ environmental stress that could be mistaken for ozone injury were used to confirm the causal agent of the symptoms.

Ozone injury was mainly detected on crops growing in the Mediterranean areas (Fumagalli *et al*, in press), however, previous surveys in Belgium, France and Switzerland have also revealed ozone injury on bean, maize, potato, soybean, and wheat (Benton *et al*, 2000). The ICP Vegetation keeps a record of all reported incidences of injury and has compiled a list of commercial crops which have developed ozone injury after ambient episodes (Table 4.1).

Table 4.1: Commercial agricultural and horticultural crops injured by ambient ozone episodes.

Agricultural crops		Horticultural crops	
Bean	*Phaseolus vulgaris*	Courgette	*Cucurbita pepo*
Clover	*Trifolium repens*	Chicory	*Chicorium endiva*
Corn	*Zea mays*	Lettuce	*Lactuca sativa*
Grape-vine	*Vitis vinifera*	Muskmelon	*Cucumis melo*
Peanut	*Arachis hypogea*	Onion	*Allium cepa*
Potato	*Solanum tuberosum*	Parsley	*Petroselinum sativum*
Soybean	*Glycine maxima*	Peach	*Prunus persica*
Tobacco	*Nicotiana tabacum*	Pepper	*Capiscum anuum*
Wheat	*Triticum aestivum*	Radish	*Raphanus sativus*
	Triticum durum	Red beetroot	*Beta vulgaris*
		Spinach	*Spinacea oleracea*
		Tomato	*Lycopersicon esculentum*
		Watermelon	*Citrullus lanatus*

4.4 Incidences of ozone injury on clover species

Recording the presence and, in recent years, the extent of ozone injury for each of the harvest intervals in the clover clone experiment has been an integral part of the ICP Vegetation experiments conducted at the 35+ sites across Europe and the USA. Ozone injury was detected on the NC-S clone of white clover at all of the experimental sites in each year (1997-1999, Figure 4.2). Injury occurred each year at 75% or more of the harvests at the following sites: Sweden-Östad, Italy-Isola Serafini (not monitored 1994-1996), Italy-Milan (not monitored 1997-1999), Italy-Rome, Germany-Trier, Slovenia-Ljubljana (not monitored in 1994 or 1997) and Switzerland-Cadenazzo. There was a general tendency for the frequency of injury-causing episodes to increase in the more southern latitudes, although injury was quite prevalent at the northern site of Sweden-Östad.

In 1998 and 1999, the NC-S and NC-R clones were scored for ozone injury at each harvest according to the following key: 1: first evidence of symptoms; 2: 1- 5% of leaves injured; 3: 5 – 25% injured; 4: 25 –90% injured; 5: 90 –100% injured. As expected the NC-S clone was far more sensitive to ozone than the NC-R clone, with

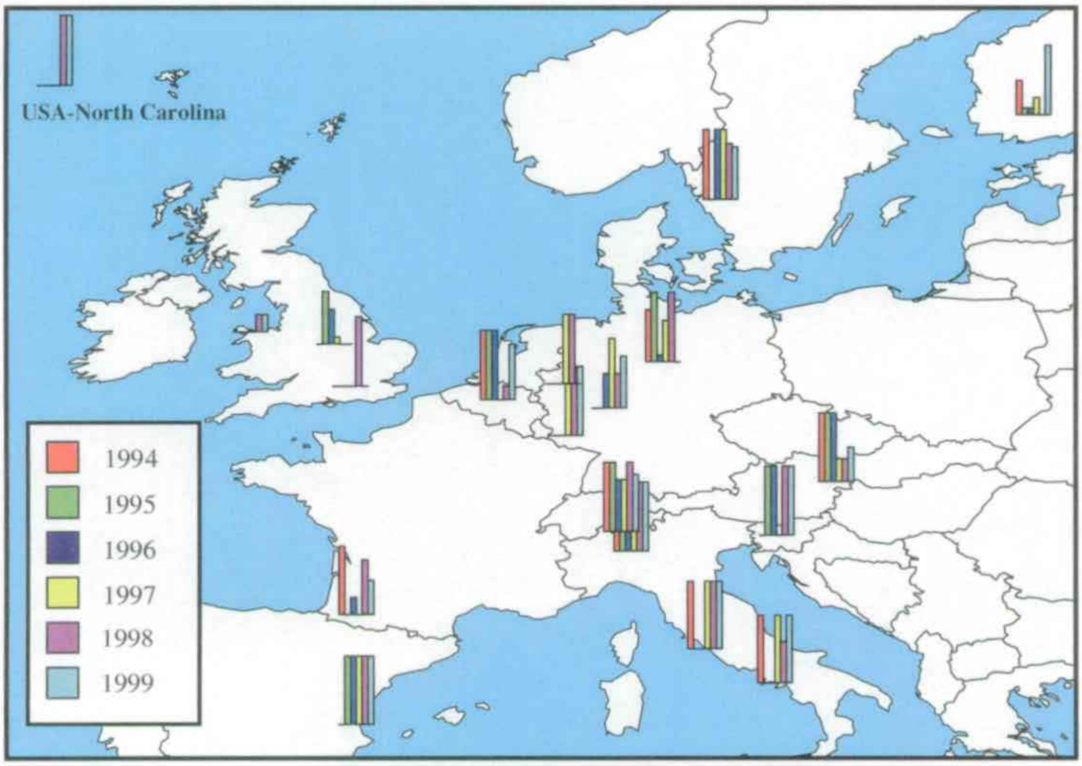

Figure 4.2: – Map of ICP Vegetation sites showing the percentage of harvests with injury in each year.

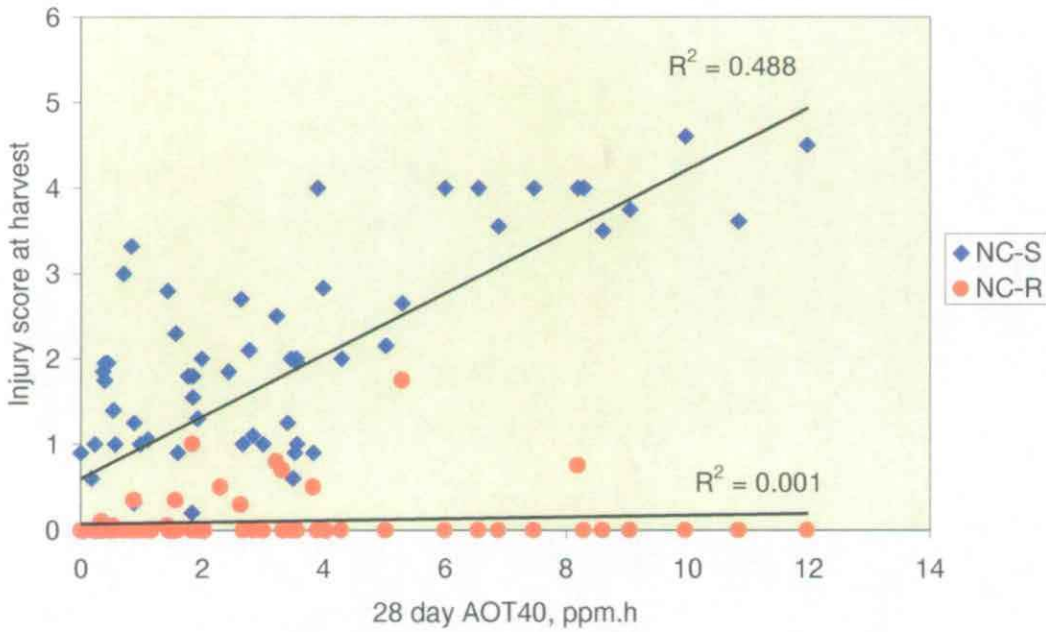

Figure 4.3: The mean injury score on the NC-S (ozone-sensitive) and NC-R (ozone-resistant) white clover clones at each harvest plotted against the 28d AOT40 for the harvest interval. Data from the 1998 and 1999 experiments.

the mean injury score at harvest ranging from 0.6 at 180 ppb.h AOT40 to 4.6 at an AOT40 of 11,976 ppb.h (Figure 4.3). Further detail is provided for the 1999 season in Figure 4.4 where the mean injury score for the NC-S clone at each harvest is presented for selected rural sites. Mean injury scores of two and above were recorded at Sweden-Östad, Netherlands-Wageningen, Germany-Deuselbach, France-Pau, Italy-Pisa, and Italy-Isola Serafini. Comparable mean injury scores were recorded for harvest 2 at Italy-Isola Serafini (3.5) and Sweden-Östad (3.0) where the 28d AOT40s were markedly different (8.60 and 0.83 ppm.h respectively). This disparity is an indication of the importance of level II factors in determining the likelihood of ozone injury.

In summary, the ICP Vegetation experiments have indicated that the occurrence of ozone injury in response to the ambient episodes is widespread in Europe.

4.5 Validation of the Short-term critical levels for injury

The short-term critical levels for injury were established from the 1995 data of the ICP Vegetation (Figure 4.5). In that year, injury only occurred at 5d AOT40s below 500 pbb.h when AOT40s as low as 200 ppb.h coincided with low VPD (i.e. high relative humidity). Thus, the two critical levels defined in Section 4.2 seemed appropriate. In 1996, VPDs were generally lower across Europe, and the two critical levels held for 83% of incidences of injury. The timing of injury was also noted in 1997 and 1999 as part of the current contract. The higher ozone threshold of 500 ppb.h held well, but the VPD threshold of 1.5 kPa was repeatedly encroached. A lower VPD threshold of 1.0 kPa is suggested (dotted line on Figure 4.5). Using this new threshold, the proportion of outliers where the AOT40 is in excess of 200 ppb.h would be reduced to 4%. However, in 1997 and 1999, injury frequently occurred when the AOT40 was below 200 ppb.h regardless of the VPD. These results suggest that there is a need for further revision of the short-term critical levels.

4.6 Discussion

The ICP Vegetation experiments have shown that the ozone concentrations in 1997, 1998, and 1999 were sufficient to induce visible injury on white clover at the sites in southern and central Europe, and at almost all of those in northern Europe. Over 20 commercially-grown crops have also been shown to develop ozone injury in response to ambient ozone episodes. Although ozone injury is undoubtedly widespread, the factors influencing the development of injury have not yet been fully documented.

The existing critical levels were appropriate for the 1995 data from which they were set, and to a lesser extent for the 1996 data, but data from 1997 and 1999 indicated that further revision is necessary. It may be that the 5d period for accumulation of AOT40 needs revising as plants may be responding to more immediate conditions or alternatively to the conditions accumulated over a longer time-period. In a closed chamber study Pihl-Karlsson *et al* (1995b) showed that injury to subterranean clover was greater following a short period with high ozone than following a longer period with lower ozone concentration. However, Amiro *et al* (1984) found that ozone concentration and length of ozone exposure were not sufficient to explain the onset of injury on *P. vulgaris*.

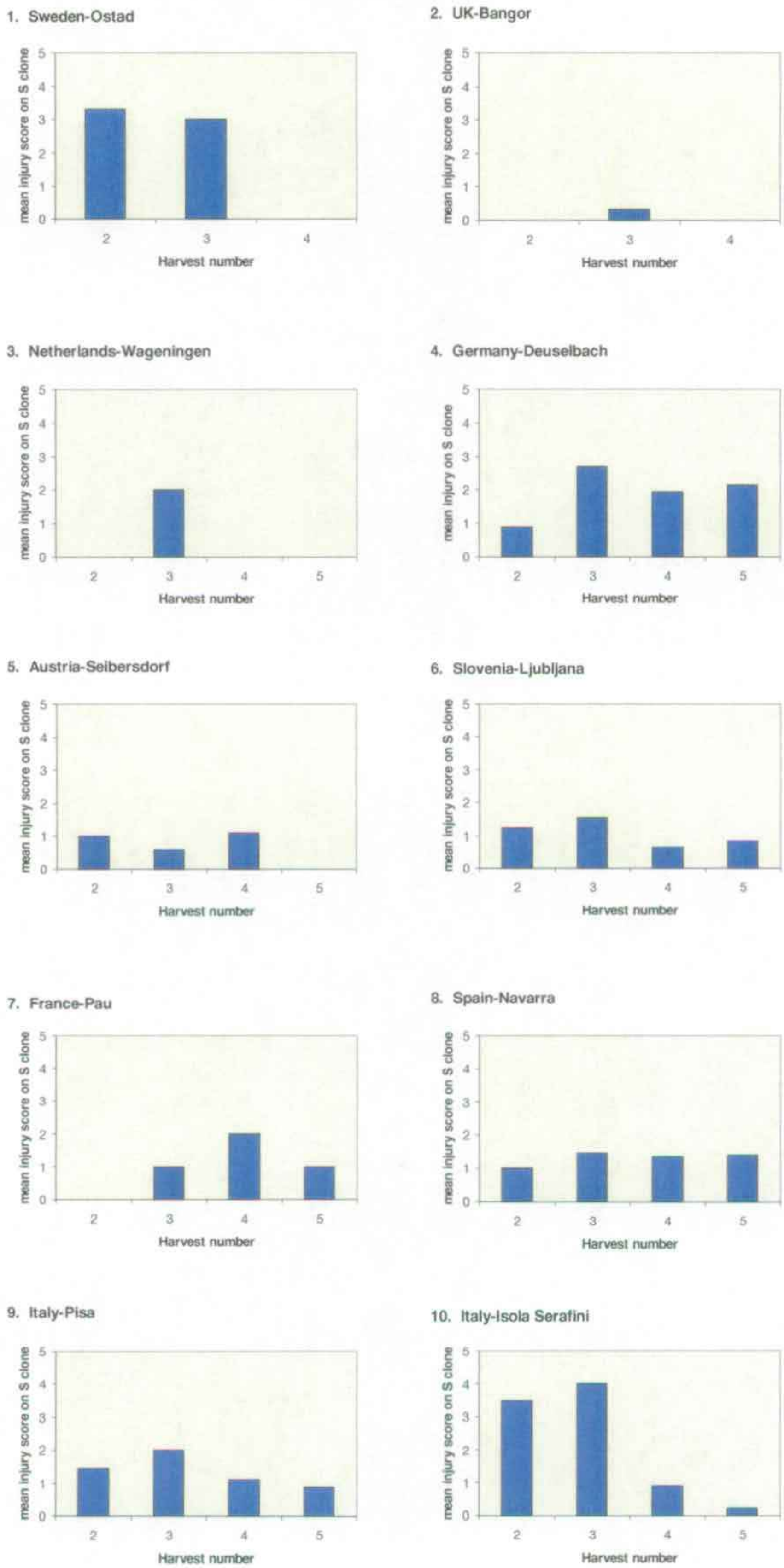

Figure 4.4: The mean injury score on the NC-S (ozone-sensitive) clone at harvests 2–5 for selected sites in 1999. The sites are ordered by latitude.

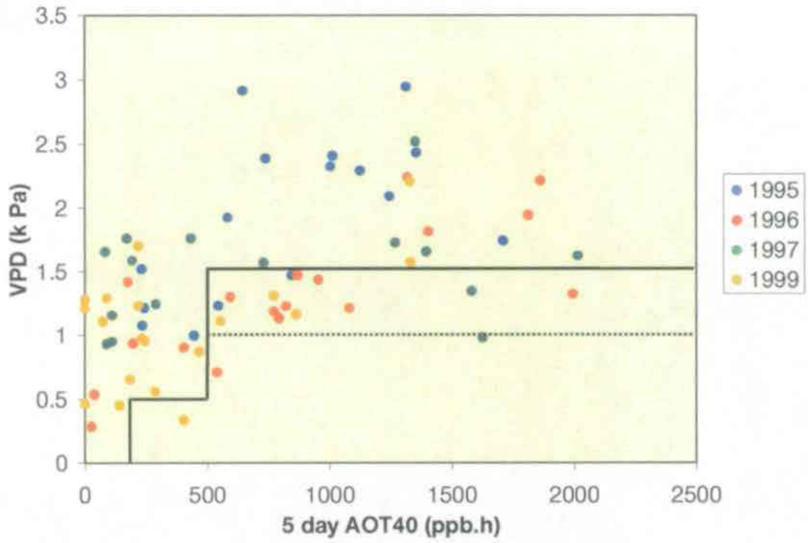

Figure 4.5: The AOT40 and mean VPD (09.30 to 16.30) for the five days preceding the occurrence of ozone injury on white clover.

The two critical levels for visible injury incorporate the humidity factor, VPD, because it is one of the main factors influencing stomatal conductance and thus the flux of ozone into the plant. When VPD is low, stomatal conductance and hence ozone flux is high and the likelihood of injury is increased; the converse is also true. The influence of VPD may explain the apparent similarity in the extent of injury at two contrasting sites, Italy Isola-Serafini and Sweden-Östad. For harvest two of 1999, the mean injury score at each site was the same at 3.2. However, injury first occurred during the harvest interval 1 – 2 following a 5d AOT40 of 1330 ppb.h and VPD of 1.57 kPa at the Italian site, and after a much lower 5d AOT40 of 405 ppb.h at the more humid Swedish site where the corresponding VPD was 0.33 kPa. Thus, the same amount of injury was caused by one third as much ozone at the more humid site. The lowest AOT40 at which injury has been reported was 27 ppb.h (in 1996) at the very low VPD of 0.28. In an allied study, Tonneijck and van Dijk (1997) reported similar findings for subterranean clover.

The high number of incidences of injury to white clover at AOT40s below 200 ppb also indicates that it may be necessary to refine these critical levels to address the occurrence of injury when AOT40 and VPD are small. A lower dose threshold might be necessary (e.g. 30 ppb), or possibly the importance of mean daily maximum ozone concentration should be re-considered. Other climatic factors that modify the outcome of ozone exposure may become important in these conditions. For example, temperature and solar radiation also influence stomatal conductance, and windspeed, through an influence on the laminar boundary layer, and atmospheric resistance determines the actual ozone dose available for absorption by the plant (Grünhage and Jager, 1994; Grünhage and Jäger, 1996).

Due to a change in policy within the LRTAP Convention, the emphasis of the ICP Vegetation has changed towards the long-term critical level for yield/biomass reduction, and also towards studying natural vegetation. Thus, the data presented in this section were not analysed in any detail. Should policy change, the database could be re-analysed since detailed hourly climatic and pollutant data are available for the days preceding the development of ozone injury. Conductance measurements are also available for some sites allowing a critical flux for ozone injury to be developed from the ICP Vegetation database.

5 Trends in the ICP Vegetation database

5.1 Aims

The ICP Vegetation (formerly ICP Crops) has been in existence since 1988, with reliable data being collected from 1990 onwards. Along with the other ICPs reporting the WGE, a substantial database on effects of pollutants on the environment has been established. At the 16th Session of the WGE (August 1997), a study of the trends in the ICP databases was initiated. The ICP Vegetation aimed to do the following as a contribution to the WGE study:

- To transfer the early data from the 1989 – 1993 experiments into an electronic format.
- To examine the data for trends in ozone concentrations and biological effects.
- To contribute to the WGE report on *Trends in Impacts of Long-range Transboundary Air Pollution.*
- To update the data in the WGE report by inclusion of results from 1998 and 1999.

5.2 Introduction

Several of the ICPs were in operation during the period of negotiation and ratification of protocols for control of the emission of pollutants such as S, N, and VOCs. Collection of environmental data by the ICPs during this period has allowed any changes in environmental condition to be related to reductions in emissions (UN/ECE, 1999). The data may also provide the means for modelling trends in effects with a view to predicting future changes in the environment. For the acidifying pollutants, reductions in emissions over the last 20 years have been linked to observed recovery of the chemistry and biology of freshwaters and the decrease in the rate of corrosion in countries in the ECE region. It is more difficult to detect changes in effects of ozone due to the year to year variation in concentration.

Nine ICP Vegetation sites were selected for a detailed analysis of trends based on their geographical distribution and the extent of data availability. These sites were Austria-Seibersdorf, Belgium-Terveuren, Finland-Jokioinen, France-Pau, Germany-Giessen, Italy-Milan, Sweden-Östad, Switzerland-Cadenazzo and UK-Nottingham. More sites were included in the analysis of trends in the biological data in order to present a wider picture of the geographical extent of effects.

5.3 The ICP Vegetation database (1990 – 1999)

The ICP Vegetation biomonitoring experiments have been running for 10 experimental seasons. In the early stages of the programme (1989-1993), the data was submitted to the Coordination Centre as paper summaries. The first stage in the analysis of trends was to convert the pollutant data into an electronic form. Biological data were easily transferred to an Excel Spreadsheet, but the pollutant data was more difficult. Although hourly records were made at the selected sites, the data was recorded as weekly 7 hour mean ozone concentrations i.e. the mean concentration between 0900 and 1600 (GMT) over the period of a week. In order to calculate the AOT40 for inclusion in this report a relationship between the AOT40 and 7-hour mean concentration was required. This relationship was determined for years and countries where both the weekly 7-hour mean and weekly AOT40 data were available. The best-fit relationship between the two variables was produced using a second order polynomial function (Figure 5.1). A high r^2 of 0.89 indicated a good fit to the data, and justified the used of the equation to convert weekly 7h mean values into three-month AOT40 values. From 1994 onwards, a more comprehensive database has been collected comprising of hourly ozone concentration, temperature, humidity, solar radiation and rainfall measurements for sites covering a wide area of Europe.

Data on the frequency of occurrence of visible injury have been collated for 23 sites in 18 countries, for the period 1992 to 1999. To determine trends in effects on biomass, the data from two phases of ICP Vegetation experiments have been combined. These were the 1994 – 1996 experiments in which ethylene diurea (EDU) was used as a protectant of white clover against ozone injury, and the data from the 1997 – 1999 clover clone experiments.

5.4 Trends in Ozone Concentration at the ICP Vegetation sites

5.4.1 Diurnal profiles.

Trends in the diurnal profile are a good indicator of changes in the pollutant climate at a individual site. The ozone concentration at a given time of day is strongly influenced by the concentrations of other pollutants such as NO and NO_2 in the area. Daily ozone profiles (for June to August) for sites with characteristics ranging from urban to rural at a range of latitudes are presented in Figure 5.2. Figure 5.2(a) shows the profile for Switzerland-Cadenazzo a southern rural site; 5.2(b) was for Sweden-Östad a northern rural site; 5.2(c) was for Belgium-Tervuren a semi-urban site and 5.2(d) was for Germany-Trier City an urban site. The characteristics of these

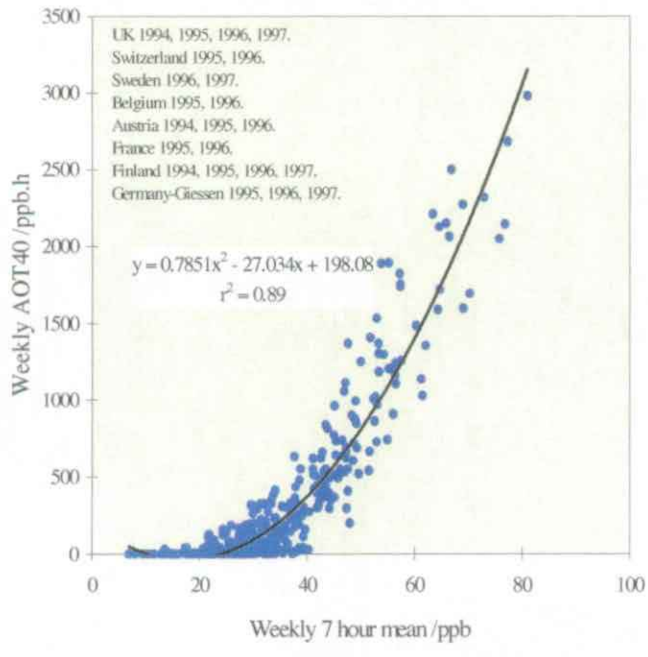

Figure 5.1: Relationship between weekly AOT40 and weekly 7h mean ozone concentration for a range of countries showing the fitted 2[nd] order polynomial trend line.

locations was reflected in the profiles of each site. The more northern and rural sites had lower peak concentrations than the southern sites. During the night, the urban sites had lower ozone concentrations than the rural sites.

Over the range of years for each site the ozone profiles were very similar at a give site and no clear trends were apparent. The only exception to this was 1998, which showed lower peak concentrations in the northern and urban sites investigated and higher concentrations in Switzerland-Cadenazzo, the southern rural site. In each case, the 1998 profile differed from that of all other years.

5.4.2 Frequency of ozone episodes.

Further information about the ozone profile at a site is contained in the frequency of episodic concentrations above a set threshold. The ozone profile is known to influence the extent of damage to the plant, with more 'peaky' dose profiles having a greater effect (Pihl Karlsson *et al*, 1995a). By examining variations in the number of days exceeding concentrations of 40 ppb (Figure 5.3) and 60 ppb (Figure 5.4) between sites and years information on the changes in ozone profile over the period could be determined. The year with the highest number of days where ozone concentrations exceeded 40 ppb was not the same at each site (Figure 5.3). The greatest number of days where ozone concentrations exceeded 40 ppb occurred in 1995 for UK-Nottingham, in 1996 for France-Pau and in 1997 for Finland-Jokioinen and Austria-Seibersdorf, in 1998 for Switzerland-Cadenazzo and Italy-Milan/Isola Serafini and in 1999 for Sweden-Östad, Belgium-Tervuren and Germany-Giessen. Overall, the highest number of days where ozone concentrations exceeded 40 ppb occurred in 1998, at Italy-Milan/Isola Serafini and Switzerland-Cadenazzo. A general north-south trend was identified, with the number of exceedences being higher at the more southern latitudes.

The number of days in which the ozone concentration exceeded 60 ppb showed similar patterns to the number of days in which the concentration exceeded 40 ppb (Figure 5.4). Again Italy-Milan/Isola Serafini showed the greatest number of days when exceedence of this level occurred. A clearer north-south trend existed for the number of days where 60 ppb was exceeded than for the number of days on which 40 ppb was exceeded.

5.4.3 Three-month AOT40.

Three-month AOT40 results showed no clear temporal trends (Figure 5.5), with large fluctuations over the six-year period at some sites. Spatial trends could be seen, with the more southern and central sites showing higher AOT40s than the more northerly sites. The highest AOT40s were seen in 1994 for Austria-Seibersdorf, Germany-Giessen and UK-Nottingham, in 1995 for France-Pau and Belgium-Terveuren, 1996 for Sweden-Östad and Switzerland-Cadenazzo and in 1997 for Finland-Jokioinen and Italy-Milan.

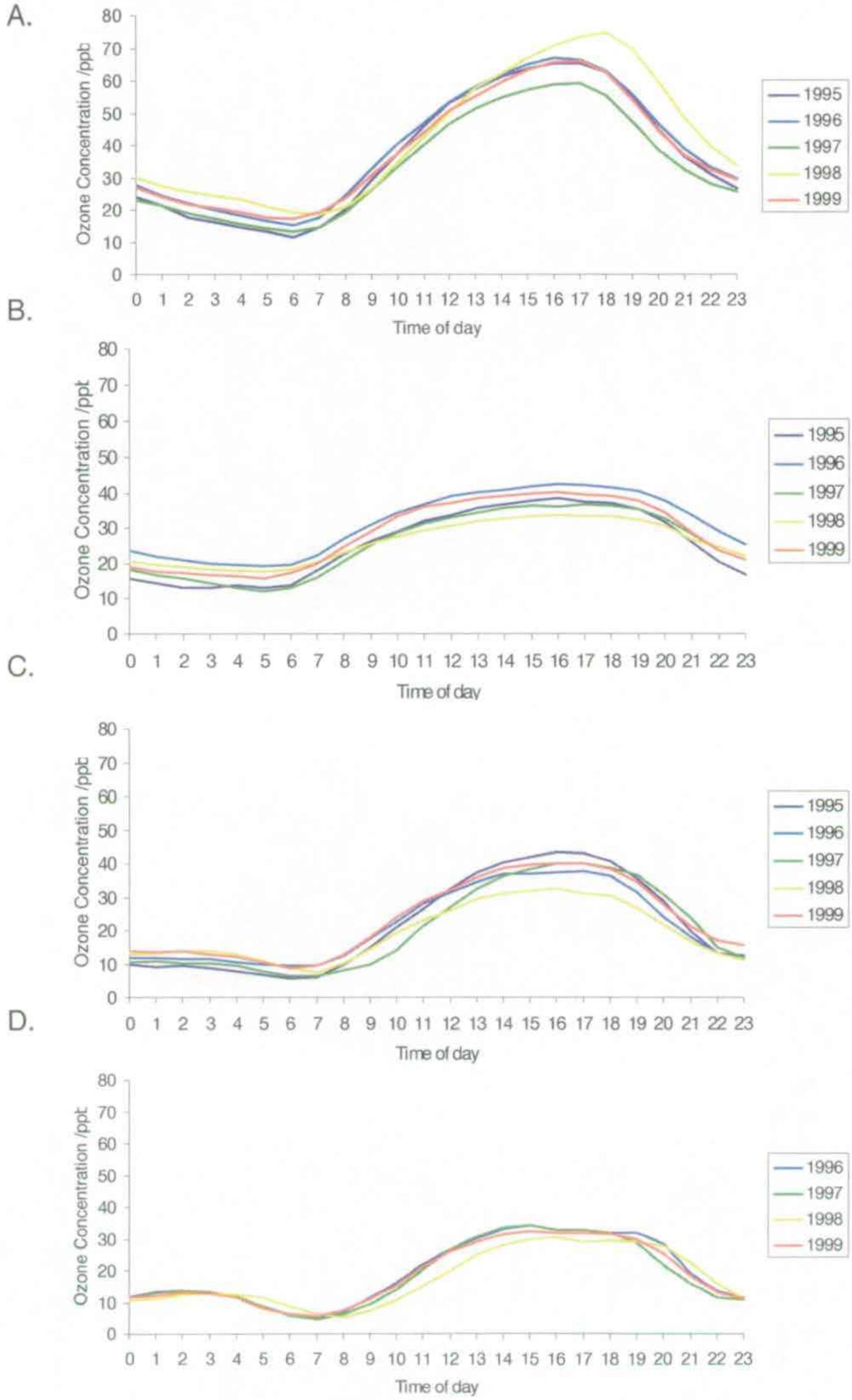

Figure 5.2: Daily ozone profiles from 1995 to 1999 for A. Switzerland-Cadenazzo, B. Sweden Ostad, C. Belgium-Terveuren and D. Germany-Trier City.

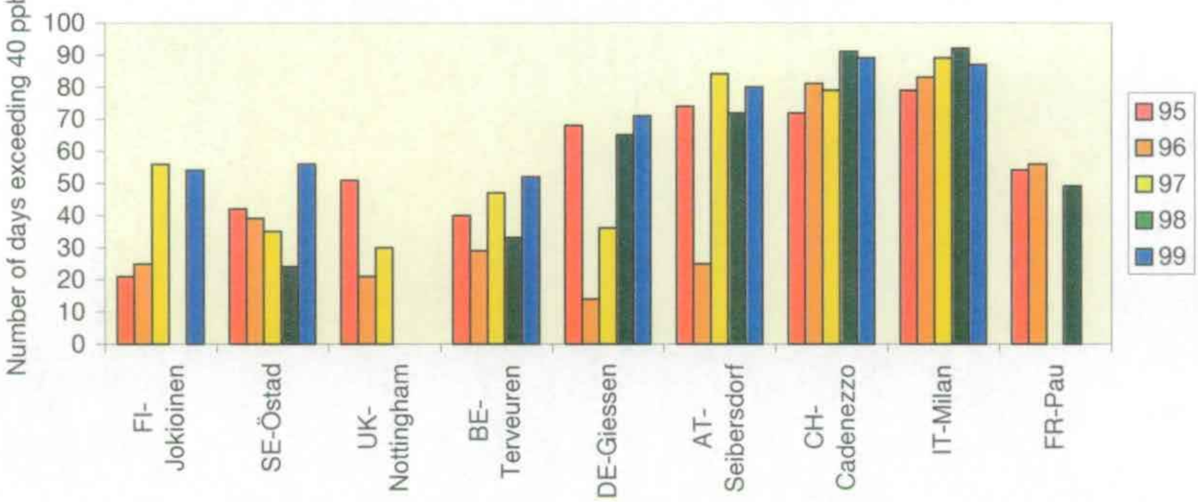

Figure 5.3: Number of days on which the ozone concentration exceeded 40 ppb (June-August) from 1995-1999. Sites ordered by latitude.

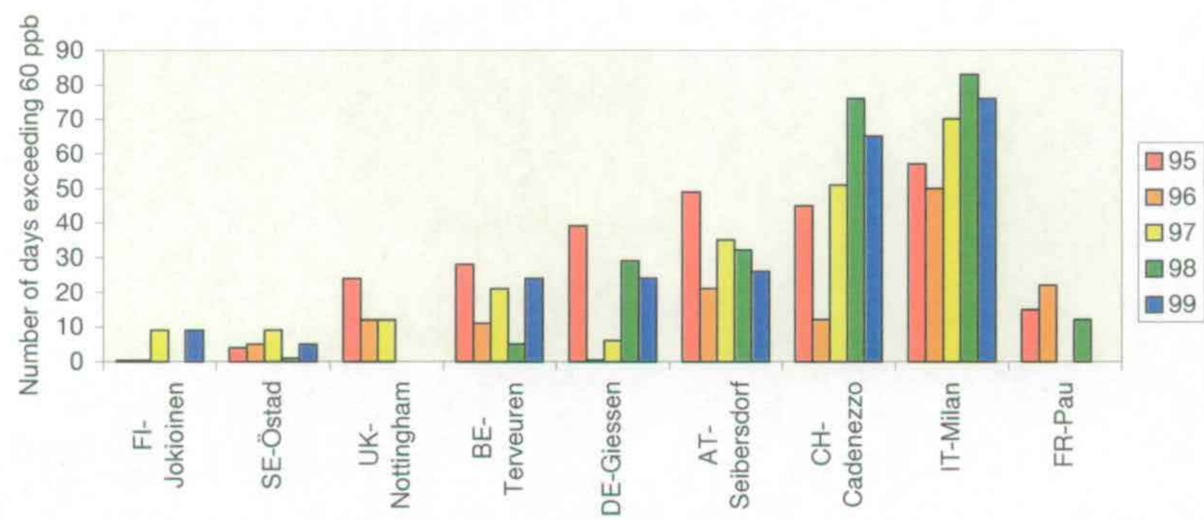

Figure 5.4: Number of days on which the ozone concentration exceeded 60 ppb (June-August) from 1995-1999. Sites ordered by latitude.

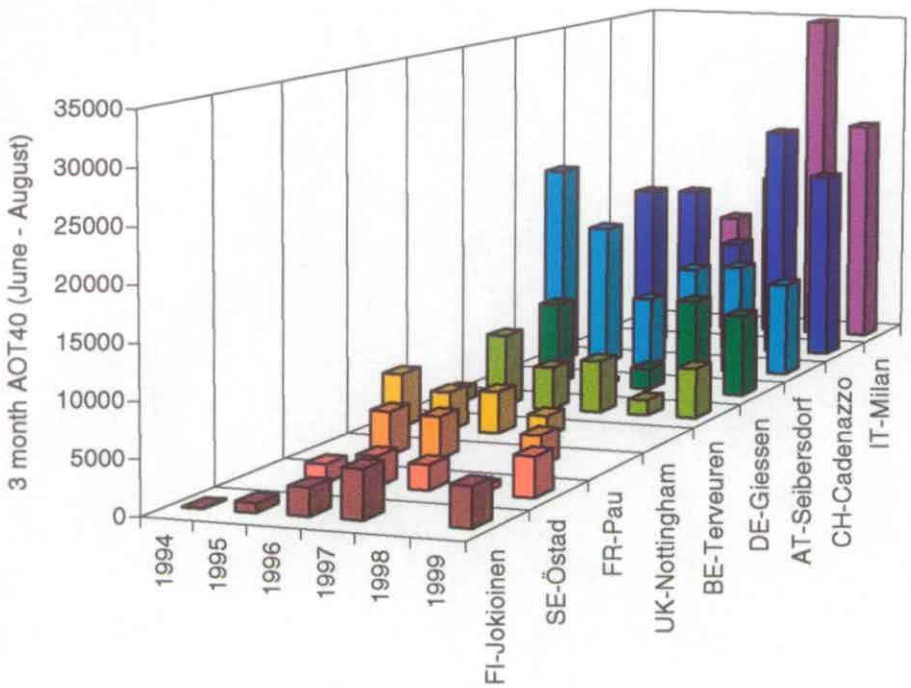

Figure 5.5: Three-month AOT40 values (1994-1997) for a range of countries calculated for June to August during daylight hours. Sites ordered for clarity of presentation.

Table 5.1: Occurrence of visible injury on any of the species monitored at 25 sites from 1992-1999 on a range of species. Key: + present, - absent, • not monitored.

Year	1992	1993	1994	1995	1996	1997	1998	1999
Austria-Seibersdorf	•	+	+	+	+	+	+	+
Belgium Terveuren	+	+	+	-	+	+	+	+
Czechoslovakia-Česk Buddjovice	+	•	•	•	•	•	•	•
Denmark-Röskild	•	•	•	+	-	•	•	•
Eire-Carlow	+	•	•	•	•	•	•	•
Estonia-Tartu	•	-	•	•	-	•	•	•
Finland-Jokioinen	+	-	+	-	-	+	•	+
France-Pau	+	•	+	+	+	•	+	+
Germany-Braunschweig	+	•	+	+	+	+	+	•
Germany-Giessen	+	•	•	•	+	+	+	+
Germany-Trier University	•	•	•	•	•	+	+	+
Italy-Naples	•	+	+	+	•	+	+	+
Italy-Rome	•	•	+	-	•	•	+	+
Italy-Milan	+		+	+	+	+	+	+
Latvia	•	+	•	•	•	•	•	•
The Netherlands-Wageningen	•	•	+	+	+	•	+	+
Poland-Kornik	•	+	+	+	•	•	+	+
Russian Federation	+	+	+	+	•	•	•	•
Slovenia-Ljubljana	•	•	•	+	-	+	+	+
Sweden-Östad	+	•	+	+	+	+	+	+
Switzerland-Cadenazzo	+	+	+	+	+	+	+	+
UK-Bangor	•	•	•	•	•	•	+	+
UK-Nottingham	-	+	+	+	+	+	•	•

5.1 Occurrence of ozone injury

Trends in effects on crop species were studied at 22 sites in 18 countries from 1992 to 1999. Participants monitored up to 8 crops and 3 non-wood species for the occurrence of visible injury at their experimental sites. Visible injury occurred on at least one of the species grown at each of the sites from 1992 to 1999 (Table 5.1). Nineteen sites showed injury in all of the years. Results of biomonitoring experiments performed prior to 1992 indicated that radish (*Raphanus sativum*) did not develop ozone injury in response to ambient episodes (results not presented).

The occurrence of visible injury on white clover (*Trifolium repens*), the main biomonitor of the ICP Vegetation programme, was investigated further by analysis of the percentage of 28 day harvests in which visible injury was recorded (Table 5.2, data also presented as a map in Section 4, Figure 4.2). These data indicated that visible injury was present at all harvests, in all years at 2 of the 16 sites. Injury was least frequent at sites in Finland and the UK.

5.5 Biomass reduction

The NC-S/NC-R biomass ratio values from 1996 to 1999 are presented in Table 5.3. No definite changes with time could be identified in this data. This would be expected because of the lack of trends found in the ozone data and because of the complexity of climatic influences on the NC-S/NC-R ozone dose response. To extend

Table 5.2: Percentage of 28 day harvests of white clover (*Trifolium repens*) in which visible injury occurred from 1994 to 1999. Key • not monitored.

Site	1994	1995	1996	1997	1998	1999
Austria-Seibersdorf	100	100	100	33	33	50
Belgium-Terveuren	100	100	100	•	20	80
France-Pau	100	•	25	•	80	50
Finland-Jokioinen	50	0	0	25	•	100
Germany-Giessen	•	•	50	100	50	75
Germany-Braunschweig	75	100	0	60	100	•
Germany-Trier University	•	•	•	75	100	100
Italy-Isola Serafini	•	•	•	75	100	100
Italy-Milan	75	100	100	•	•	•
Italy- Naples	100	•	•	100	60	100
Italy-Rome	100	•	•	100	100	100
Poland-Kornik	100	50	•	•	•	•
Slovenia-Ljubljana	•	100	100	•	100	100
Sweden-Östad	100	•	100	100	80	75
Switzerland-Cadenazzo	100	100	75	75	100	83
UK-Bangor	•	•	•	•	25	25
UK-Nottingham	•	75	50	0	•	•

the database further, the response relationships from previous ICP Vegetation experiments (1994-1996) in which ethylene diurea (EDU) was used as a protectant against damage by ozone were analysed together with those from the clover clone experiment (Figure 5.6). Again, no trends with time were detected. Statistical analysis indicated that all of the years were of the same population both in terms of their gradients and their intercepts. Details of the dose response lines are presented in Table 5.4

Table 5.3: Trends in the NC-S/NC-R biomass ratio from the clover clone biomonitoring experiments from 1994 to 1997. Key: • not monitored/excluded in QA exercise.

Site	1996	1997	1998	1999
Austria-Seibersdorf	0.93	0.92	0.78	0.85
Belgium-Terveuren	0.96	0.98	0.93	1.09
Finland-Jokioinen	1.33	1.00	•	0.86
Germany-Giessen	•	0.92	0.88	0.97
Germany-Braunschweig	0.94	0.96	1.06	•
Germany-Trier City	0.97	0.93	0.81	0.91
Italy-Milan	0.61	0.67	0.49	0.56
Sweden-Östad	•	1.15	•	1.22
Switzerland-Cadenazzo	0.98	•	1.04	0.95
UK-Bangor	•	•	1.07	1.01

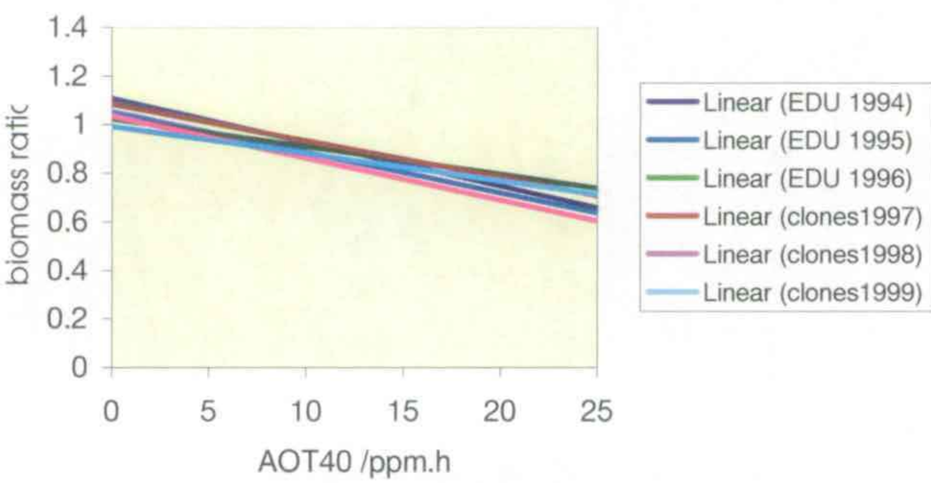

Figure 5.6: Dose response trend-lines for EDU and clover clone experiments from 1994 to 1999 (cumulative data for the 3-month harvest period). Biomass ratio refers to -/+ EDU or NC-S/NC-R.

Table 5.4: Line statistics for regression lines.

Line	Gradient	95%CI	Intercept	95%CI	r^2 for line
EDU1994	-0.018	+/-0.014	1.107	+/- 0.095	0.600
EDU 1995	-0.017	+/- 0.016	1.055	+/- 0.059	0.434
EDU 1996	-0.011	+/- 0.0095	1.024	+/- 0.056	0.087
Clones 1997	-0.015	+/- 0.013	1.083	+/- 0.084	0.540
Clones 1998	-0.017	+/- 0.013	1.036	+/- 0.084	0.844
Clones 1999	-0.011	+/- 0.012	0.992	+/- 0.150	0.359

5.6 Discussion

Analysis of the ICP Vegetation database for ozone has shown that there were no long-term trends with time over the six years for which reliable ozone data was available. This was due to the large year to year variation in both the AOT40 and in the number of days on which a given level of ozone was exceeded at individual sites, and largely reflected year to year variation in climatic conditions. This lack of trends with time was consistent with the modelled AOT40 values for eight years between 1985 and 1996 calculated from EMEP/MSC-W 6-hourly mean concentrations (Posch *et al* 1999). In both studies, a general north-south trend of increasing ozone concentration was noted.

As a consequence of the lack of trends in ozone data, there were also no trends with time in effects on vegetation. The differences in magnitude of response between sites have been considered in detail elsewhere in this report (biomass-Section 2, injury-Section 4). Although there were no trends with time, this study has confirmed the widespread occurrence of effects of ozone. For example, visible injury was detected at every site in every year on at least one of the species studied, and at some sites at every harvest on clover.

6 Impacts of Ozone on Natural Vegetation

6.1 Aims

In recent years, interest in the effects of ozone on natural and semi-natural vegetation has increased considerably. The ICP Vegetation has taken on the role of collating international databases, and has recently been named as the end-user of the models and data being produced by an EU-funded project, BIOSTRESS (BIOdiversity in Herbaceous Semi-Natural Ecosystems Under STRESS by Global Change Components). Possible biomonitoring systems for natural vegetation are being considered for future inclusion in the ICP Vegetation experimental programme. The aims of this area of study for the ICP Vegetation were:

- To review existing databases for semi-natural and natural vegetation with the intention of identifying candidate species for further study, and gaps in our current knowledge of the responses of these species to ozone.

- To conduct a pilot study at the Coordination Centre to quantify the responses of candidate species to ozone.

- To compare different modelling approaches to identify plant characteristics associated with ozone sensitivity.

- To make recommendations for further study of these species.

6.2 Introduction

One of the aims of the WGE is to map natural vegetation species and communities which are at risk from ozone pollution in the UN/ECE area by identifying areas where these species and communities coincide with high levels of ozone. In southern Europe, the Mediterranean region has a diverse and high conservation-value flora. In more northern areas, species and communities could be at risk from moderate ozone concentrations if these conditions coincide with climatic conditions which allow uptake of ozone into the plant. For example, it is suspected that communities growing where moisture is not limiting, such as wetlands, may be at a greater risk than those growing in dry areas, such as grassland.

At this stage, it is not known whether the critical levels of ozone for crops will protect natural ecosystems because there is relatively little information available on the sensitivity of these species. However, ozone injury on natural vegetation has been observed in Europe in ambient air conditions (Becker *et al*, 1989) and biomass reductions have been recorded in unfiltered compared to filtered air (Fuhrer *et al*, 1994; Evans and Ashmore, 1992; Pleijel *et al*, 1996). Obtaining reliable experimental data on which to base critical levels for natural vegetation species is an increasing priority. Unfortunately, it is not as easy to decide on the criteria for assessing the sensitivity of natural vegetation to ozone as it is for crop responses. Seed output is a possible effect-criterion for annuals and biennials, but may not be appropriate for perennial plants. In practice, visible injury and growth in ozone compared to control conditions are usually used. Visible symptoms give evidence of a biochemical response but do not necessarily indicate sensitivity in terms of growth reduction and are not evidence of an ecological impact. Implications of effects of ozone pollution on community structure and functioning have still to be determined.

6.3 Review of literature and existing data

The first stage in assessing existing knowledge of the effects of ozone on semi-natural and natural-vegetation was to review the existing literature on this subject. The full text was submitted to DETR in year 2 of this contract (Hayes and Mills, 1999). The salient points are reproduced in condensed form in this section.

6.3.1 Visible injury on natural vegetation

Ozone injury is less specific and easy to identify on natural vegetation than on crops as symptoms range from stippling or chlorotic flecks to non-specific reddening of the leaves (a general stress response in plants). The causal agent of injury on naturally-growing species should always be confirmed by exposure to ozone in controlled conditions. Confirmed reports of visible injury after ambient ozone episodes or short-term artificial exposures of up to 80 ppb are listed in Table 6.1. Working at higher ozone concentrations, Nebel and Fuhrer (1994), assessed the sensitivity of 31 native species of Switzerland, and classified 6 species as sensitive (visible injury symptoms appeared after 6 days exposure to 100ppb), 15 species as intermediate (injury occurred after an additional 3-day exposure to 150 ppb) and 10 species as resistant (no visible injury). In a longer-term study, Bergmann *et al.* (1996) recorded visible injury on 11 out of 16 natural vegetation species exposed to 70 ppb for 7h d^{-1} in open-top chambers. Approximately half of the species exhibiting visible injury also exhibited growth reductions.

When a species responds by developing visible injury, it shows that there has been an effect at the biochemical level, but it does not necessarily have any ecological significance because there is usually little relation between sensitivity in terms of visible symptoms and growth or seed production. For example, Mortensen and Nilsen (1992) and Chappelka *et al* (1997) showed that there are many species which have significant effects of ozone on growth, but which show no visible injury symptoms. Similarly, there are other species that develop visible injury symptoms, but do not show a reduction in growth (e.g. *Phleum alpinum*, *Dactylis glomerata* and *Dactylis aschersoniana* (Pleijel and Danielsson, 1997)).

6.3.2 Biomass changes, partitioning of assimilate and reproductive output

The response of plants to ozone exposure is very varied between species. Different screening experiments also show variations in response for the same species. Many species show decreased biomass production with ozone exposure, whereas others show no effect and some appear to be stimulated, e.g. sheeps fescue (*Festuca ovina*) (Pleijel and Danielsson, 1997). The degree of response depends on whether total biomass or above ground biomass was determined due to possible differences in biomass partitioning. Species showing a significant change in biomass with ozone exposures up to 80 ppb are listed in Table 6.2.

Some species may show biomass reductions due to ozone at near ambient levels. Exposure to ozone at 70-80 ppb for six weeks caused a significant reduction in the growth of the mosses *Sphagnum recurvum* and *Polytrichum commune* (Potter *et al.* 1996). Eighteen out of 27 species investigated in Sweden had increased growth in filtered air compared to non-filtered air with added ozone (Pleijel and Danielsson, 1997). In Germany some species, e.g. black nightshade (*Solanum nigrum*), common mallow (*Malva sylvestris*) and groundsel (*Senecio vulgaris*) had a loss of leaf biomass of more than 30% with 70 ppb ozone treatment within about 6 weeks, whereas fat-hen (*Chenopodium album*) and common nettle (*Urtica dioica*) showed no changes in biomass or any other parameter measured (Bergmann *et al*, 1996).

Table 6.1: Species showing visible injury symptoms in ambient air conditions or screening experiments up to 80ppb ozone

Achillea millefolium	Yarrow
Alchemilla vulgaris	Ladys mantle
Anthoxanthum odoratum	Sweet vernal grass
Anthriscus silvestris	Cow parsley
Anthyllis vulneraria	Kidney vetch
Centaurea jacea	Brown knapweed
Cerastium arvense	Field mouse-ear
Chrysanthemum leucanthemum	Ox-eye daisy
Cirsium acaule	Ground thistle
Cirsium arvense	Creeping thistle
Dactylis ashersoniana	
Dactylis glomerata	Cocksfoot
Euphrasia montanum	Mountain sticky eyebright
Filipendula ulmaria	Meadowsweet
Geranium silvaticum	Wood cranesbill
Helianthenum grandiflorum	
Hypericum perforatum	Perforate St Johns wort
Lathyrus pratensis	Yellow meadow vetchling
Lotus corniculatus	Birdsfoot trefoil
Lotus uliginosus	Marsh birdsfoot trefoil
Mentha aquatica	Water mint
Phleum alpinum	Alpine cats tail
Plantago lanceolata	Ribwort plantain
Ranunculus friesianus	
Rhinanthus minor	Hayrattle
Sanguisorba minor	Salad burnet
Scabiosa columbaria	Small scabious
Solidago virgaurea	Golden rod
Symphytum officinale	Comfrey
Taraxacum officinale	Dandelion
Trifolium montanum	
Trifolium pratense	Red clover
Trifolium repens	White clover
Trisetum flavescens	Yellow oat grass
Valeria officinalis	Valerian
Veronica chamaedrys	Germander speedwell
Vicia cracca	Tufted vetch

Sources: Ashmore *et al*. (1996); Bungener *et al* (1999c); Mortensen and Nilsen (1992); Nebel and Fuhrer (1994); Pleijel and Danielsson (1997); Reiling and Davison (1992b); Warwick and Taylor (1995).

Table 6.2: Species showing significant changes in biomass in ozone exposures of up to 80ppb.

Species		response
Arrhenatherum elatius	Oat grass	-
Campanula rotundifolia	Harebell	-
Cerastium fontanum	Common mouse ear	-
Cirsium acaule	Ground thistle	-
Cirsium arvense	Creeping thistle	-
Dactylis glomerata	Cocksfoot	-
Danthonia decumbens	Heath grass	-
Deschampsia flexuosa	Wavy hair grass	-
Desmazeria regida	Fern grass	-
Dianthus deltoides	Maiden pink	-
Festuca ovina	Sheeps fecue	+
Festuca pratensis	Meadow fescue	-
Filipendula ulmaria	Meadowsweet	-
Galinsoga parviflora	Quickweed	-
Galium saxatile	Heath bedstraw	-
Holcus lanatus	Yorkshire fog	-
Hordeum murinum	Wild barley	-
Hypericum pulchrum	Beautiful St Johns wort	-
Koeleria macrantha	Crested hair grass	-
Leontodon hispidus	Rough hawkbit	-
Lotus corniculatus	Birdsfoot trefoil	-
Malva sylvestris	Common mallow	-
Papaver dubium	Long headed poppy	-
Phalaris arundinacea	Reed canary grass	+
Pilosella officinarum	Mouse-ear hawkweed	-
Plantago coronopus	Bucks-horn plantain	-
Plantago lanceolata	Ribwort plantain	-
Plantago major	Greater plantain	-
Poa annua	Annual meadow grass	-
Poa trivialis	Rough meadow grass	-
Rumex acetosella	Sheeps sorrel	-
Rumex obtusifolius	Broad leaved dock	-
Senecio vulgaris	Groundsel	-
Solanum nigrum	Black nightshade	-
Sonchus asper	Prickly sowthistle	-
Symphytum officinale	Comfrey	-
Teucrium scorodonia	Wood sage	-
Trifolium arvense	Hare's foot clover	-
Trifolium dubium	Lesser yellow trefoil	-
Trifolium pratense	Red clover	-
Trifolium repens	White clover	-
Urtica dioica	Common nettle	-
Valeria officinalis	Valerian	-
Vicia sativa	Common vetch	-

Key + = weight gain in response to ozone; - = weight loss in response to ozone.

Sources: Ashmore *et al*, (1996); Bungener *et al* (1999c); Mortensen and Nilsen (1992); Nebel and Fuhrer (1994); Pleijel and Danielsson (1997); Reiling and Davison (1992b); Warwick and Taylor (1995).

In the presence of ozone, some species, such as sheeps fescue (*Festuca ovina*), preferentially partitioned biomass to shoots rather than to roots, which may give the plants a competitive disadvantage when they are in species mixtures, even though there appears to be no significant effect (on a total weight basis) when the plants are grown singly (Cooley and Manning, 1987). In plants which have not flowered or set fruit, at "low" ozone concentrations the remaining assimilate is generally diverted to leaves and stems at the expense of roots and crowns. At "higher" ozone levels, assimilate accumulation is greatly depressed and partitioning changes are not as obvious. However, the storage organs are most affected by ozone induced changes in partitioning when ozone concentrations are in the range commonly observed in polluted ambient air. In most species, ozone stress reduces root growth more than shoot growth (Cooley and Manning, 1987).

As a plant matures, flowers and develops seeds, these sinks receive a relatively high proportion of the available assimilate. Ozone exposure during this time can influence the biomass partitioning to these sinks. For example, long-headed poppy (*Papaver dubium*) and hare's foot trefoil (*Trifolium arvense*) showed reduced shoot biomass and increased seed/flowering allocation, whereas fat-hen (*Chenopodium album*) and *Matricaria discoidea* showed greater vegetative shoot weight and reduced reproductive allocation (Bergmann *et al*, 1995).

Although ozone may reduce the numbers of flowers or seeds, often the remaining seeds have a size and dry matter accumulation comparable to those in non-stressed plants (Cooley and Manning, 1987). Very little work has been done to assess the viability of seeds produced by plants growing in high ozone conditions, but reduced germination has been demonstrated for some annual ruderals (Bergmann *et al*, 1998).

6.3.3 Physiological effects of ozone

Fewer studies still have considered the effects of ozone on the physiology of natural vegetation species. Generalisations cannot yet be made about effects on stomatal conductance. This is partly due to the large variation in both the number and distribution of stomata on these species. Species growing in habitats with little or no shade tend to have stomata distributed over both leaf surfaces, whereas those in shaded habitats tend to have the majority of stomata on the lower epidermis (Fitter and Peat, 1994).

Chlorophyll fluorescence measurements give an indication of the efficiency of the photosynthetic apparatus of a plant. The maximum rise of induced chlorophyll fluorescence has been shown to be very sensitive to ozone, often with significant differences between treatments even when there were no differences detected in relative growth rate or root:shoot ratio (Reiling and Davison, 1992b). However this study revealed a complex pattern of response dependent on the species, making it very difficult to quantify and use changes in fluorescence for comparisons between species.

Photosynthesis rates can also be affected directly by ozone pollution. A reduction in photosynthesis due to ozone has been shown for several crops (e.g. Amundson *et al*, 1987 for winter wheat, *Triticum aestivum*; Myhre *et al*, 1988, for oat, *Avena sativa*), but photosynthetic rates have been recorded infrequently in studies of the effects of ozone on natural vegetation. In greater plantain (*Plantago major*), photosynthetic rates were significantly reduced during artificial ozone episodes (Reiling and Davison, 1994). These reductions in photosynthesis persisted so that eight weeks later the rates were still significantly different from those of plants grown in charcoal filtered air.

6.3.4 Ecological Impacts

While individual plant responses to ozone are of importance, the effects of ozone on plant communities have been highlighted as being of greater importance when establishing critical levels. This is an area where much further work is needed as it is not always possible to extrapolate from single species studies to ecosystem responses. Thus, the effects of ozone on real or artificial communities need to be studied directly.

Differences in sensitivity of species to ozone may result in selection of more tolerant species. This could influence the species composition of natural communities and could potentially eliminate the most sensitive species from an ecosystem. Furthermore, air turbulence could also provide different ozone concentrations to different species as plants growing low in the canopy could experience very different concentrations to those more exposed at the top of the canopy.

In artificial communities, ozone exposure caused a decrease in the proportion of forbs and an increase in the proportion of grasses (Ainsworth *et al*, 1994; Ashmore *et al*, 1995; Ashmore *et al*, 1996), which was consistent with the difference in ozone sensitivity when the plants were grown individually. However, in another study the reverse effect was found (Evans and Ashmore, 1992). In this particular community, it was thought that the low growing forb species were sensitive to changes in light penetration through the grass canopy due to the ozone-induced changes in growth of the grass species.

In other examples, effects on species due to ozone have been modified by competition. Studies with a field-grown grass-clover mixture exposed to four levels of ozone in open-top chambers, with the highest ozone concentration about twice ambient, showed no major ozone effect on the percentage of clover in the pasture (Pleijel *et al*, 1996). Monocultures and mixtures of crimson clover (*Trifolium incarnatum*) and annual ryegrass (*Lolium multiflorum*) were grown in filtered air and two ozone concentrations for six weeks (Bennett and Runeckles, 1977). The yield and leaf area of ryegrass in mixtures was less affected by ozone than the crimson clover. Although the proportion of ryegrass was greater at 90 ppb, the total dry weight, yield, leaf area, leaf area ratio and tiller number were less depressed by ozone in species mixtures than in monocultures. This fitted the de Wit model of competition.

Davison and Barnes (1998) have suggested that the classification of natural vegetation species as sensitive or resistant could be misleading due to intraspecific variation in response to ozone such as that found in greater plantain (*Plantago major*), alpine cats tail (*Phleum alpinum*) and white clover (*Trifolium repens*). Species may evolve ozone resistance, which has implications for both community dynamics and the derivation of critical levels. For resistance to ozone to occur, there must be a large reduction in ecological fitness so that there is a selection pressure for evolution. Reiling and Davison (1992a) showed that the relative resistance of greater plantain (*Plantago major*) could be related to the ozone exposure index of the collection site and to some of the climatic variables of the site, particularly the amount of summer sunshine. At present, the evidence suggests that ozone has led to the evolution of resistance in *Plantago major* in the southern half of the UK.

6.4 Models to identify sensitive species

Several attempts have been made to identify plant characteristics associated with ozone sensitivity in species of natural vegetation. Earlier studies had indicated that differences in ozone sensitivity between species and within species could be generally 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). In contrast, Reiling and Davison (1995) found that in populations of greater plantain (*Plantago major*) there was no relationship between mean or maximum stomatal conductance in clean air and ozone resistance. Thus it seemed likely that other factors might have additional importance in characterising sensitivity to ozone, hence the need for more complex analysis and modelling.

6.4.1 Models based on ecological characteristics and growth strategies

Initial attempts to relate ozone sensitivity to plant strategy based on Grime's classification (whether a plant is a competitor, stress tolerator or ruderal, (Grime, 1979) have had mixed results. Both Bungener *et al*, (1999a, 1999b) and Franzaring *et al*. (1999) have shown that species with a large component of competitor or ruderal strategy were more likely to have reduced growth due to ozone than stress tolerators. Indeed, those species showing growth increases due to ozone were most likely to be classified as stress tolerators.

In a collaborative study with Dr. L. Emberson (SEI-Y) and Prof. M. Ashmore (University of Bradford), Dr. G. Ball (ICP Vegetation Data Modelling Centre) used artificial neural networks to investigate factors influencing ozone sensitivity in natural vegetation (Ball *et al*, 1999). The database used for this analysis was from ozone exposure experiments that had been carried out at ICCET, to investigate the sensitivity to ozone dose (AOT40) of 42 grassland species in terms of their biomass response. ANN models, based on indices by Grime *et al*. (1988) and Ellenberg (1988), were used to test whether key ecological and physiological characteristics were associated with ozone sensitivity.

Preliminary ANN models of species sensitivity were developed using the back-propagation algorithm (for explanation, see Appendix 2). Training time and the number of hidden nodes were optimised for each model using test data performance. The importance of the input parameters was determined by analysis of the weights of the optimised ANN model and by comparison of the performance of multiple models using different input combinations.

The model using all of the Ellenberg inputs (optimum light requirement, optimum temperature requirement, continentality) produced r^2 values of 0.75 for the training data and 0.34 for test data. This model had difficulty in predicting percentage increases in biomass (represented as negative in the data set) and was unable to produce accurate predictions for unseen data.

Optimum performance was produced by a model using all possible inputs with the exception of the nitrogen requirement of the species, producing r^2 values of 0.92 for the entire dataset and 0.67 for the test dataset (Figure 6.1). Other combinations of inputs showed poorer performance. Analysis of the weights indicated that the inputs could be ranked as follows: AOT40>optimum light requirement = optimum water requirement = reaction = continentality > optimum temperature requirement = grass/forb distinction. The high ranking of AOT40 may simply represent a difference in experimental conditions since the species were screened in successive batches, with slightly different AOT40 values. The remaining inputs were all of similar importance in determining sensitivity and this could explain why the model could not be improved with further de-selection.

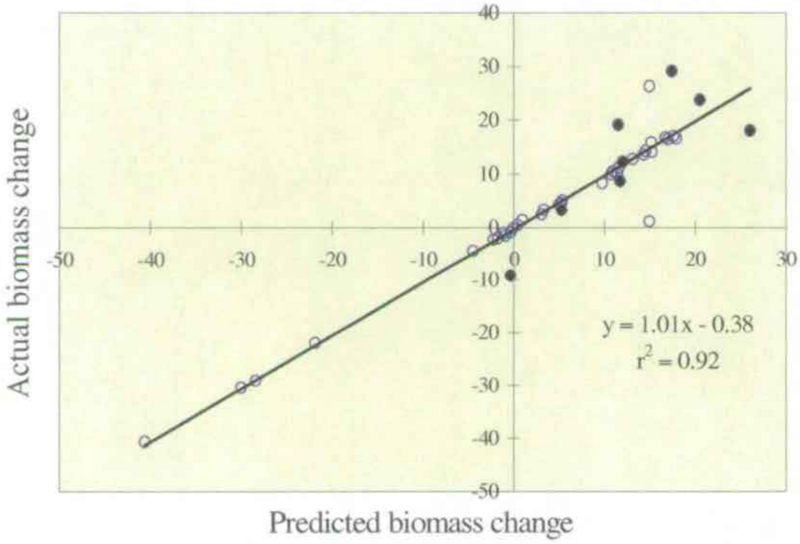

Figure 6.1: ANN modelling for the Ellenberg scheme using all classification inputs with the exception of nitrogen. Solid points indicate test data.

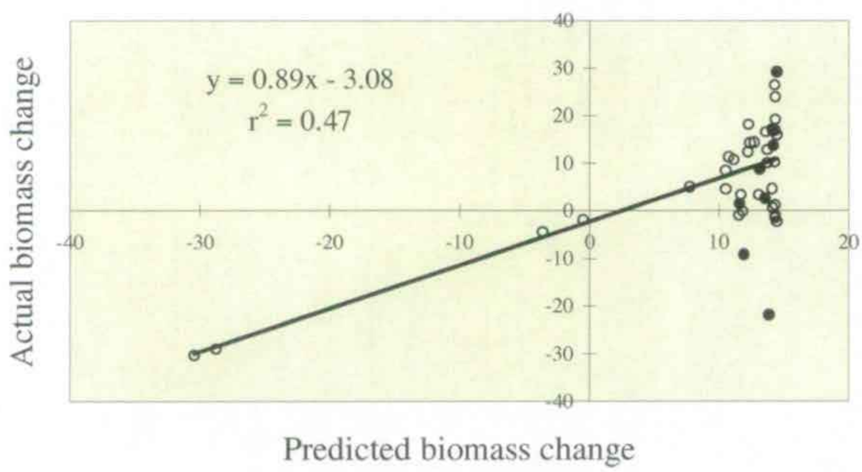

Figure 6.2: ANN modelling for the Grime scheme using all classification inputs. Solid points indicate test data

The same procedures were used to develop a model using the Grime classification, using growth strategy (competitor (C), stress tolerator (S) or ruderal (R)), AOT40, pH tolerance, and if the species was a grass or a forb, as inputs. Results indicated a poorer performance compared to the Ellenburg classification ($r^2 = 0.47$, test $r^2=0.21$, Figure 6.2).

Further analysis needs to be performed to confirm these preliminary findings and to understand the processes involved in determining response to ozone.

6.4.2 Models based on physiological responses to ozone

Dr. G. Ball also conducted an additional modelling activity in collaboration with Bungener *et al.* (1999a), in which data from the exposure of 9 species to ozone in open-top chambers was analysed using ANNs. Inputs to the ANN model were: the stomatal conductance at 1kPa VPD (GS1), relative growth rate for the species (R%); ozone dose (AOT40) and the climatic conditions of global radiation (GR) and VPD in the 5 days preceding injury and from the start of the experiment (VPD 5days). These inputs were used to predict the sensitivity of the plant by predicting the stomatal response to VPD after exposure to ozone.

The optimised ANN model had an r^2 value of 0.70 for unseen test data. The inputs to this model were ranked in order of GS1 > VPD 5 days > R% > GR from start > total AOT40 > VPD from start > light 5 days > AOT40 5 days. The importance of relative growth rate was tested by removing GS1 (the only other plant variable). In this case the r^2 fell to 0.50. Finally, only environmental variables during the 5 days before the onset of injury were used and the r^2 fell further to 0.28. Thus, this modelling approach showed that the plant species was the most important factor in predicting leaf injury. If the 'species' input was removed, then stomatal conductance was the most important factor and relative growth rate was less important. Species with a high stomatal conductance were more likely to be affected by elevated levels of ozone. With no plant-related factors included, air vapour pressure deficit and AOT40 were of highest importance.

6.5 Critical levels for natural vegetation

6.5.1 Definitions

The critical levels for natural vegetation species were reconsidered at the *Critical Levels for Ozone Workshop - Level II* (Gerzensee, Switzerland, April 1999) and the following recommendations were made (Fuhrer and Achermann, 1999):

> *For **annuals** an AOT40 value of 3000 ppb.h calculated for daylight hours over three months.*
> The critical level for annuals is the same as crops (wheat) as the response with highest ecologicial significance to these plants with a short life-cycle is seed output.
>
> *For **perennials** a provisional critical level of an AOT40 value of 7000 ppb.h over 6 months was proposed.*
> Shoot and root biomass have greatest ecological significance for long-lived species and sensitive herbaceous perennials e.g. *Trifolium repens* show a 10% reduction in shoot biomass at this level.

In both cases, the time period for calculation of the AOT40 is flexible, to relate to the times when the vegetation is most active.

No specific critical level for **biennial** species has so far been identified.

6.5.2 Consideration of Level II factors

The most important level II factors are thought to be soil moisture and nutrient status, community dynamics and structure, species and genotype, mycorrhizal interactions, deposition of nitrogen, phenology, atmospheric conductivity, VPD and air temperature, susceptibility to herbivores and plant diseases and co-occurrence of other air pollutants. Further study of the interaction between these factors and ozone is still required before they can be incorporated into the definitions for the critical level.

Vapour pressure deficit and SMD were included in the list of level II factors because both can modify the response of natural vegetation to ozone via effects on stomatal aperture. However, the degree of response and therefore protection, is species specific making generalizations difficult (Bungener *et al*, 1999a). Nutrient status is undoubtedly an important factor influencing the responses of natural vegetation communities to ozone. So far, very few studies have been performed on this subject, however, Whitfield *et al* (1998) showed that the effect of ozone on *Plantago major* was greater in root-restricted, nutrient-deficient plants than in unrestricted plants.

Semi-natural grasslands are often maintained by management such as cutting or grazing. Ashmore and Ainsworth (1995) showed that the cutting regime had a greater influence on the percentage of the two forbs (white clover (*Trifolium repens*) and germander speedwell (*Veronica chamardrys*)) than even the highest ozone concentration. The cutting or grazing regime would therefore be an important modifier of the response of these managed communities to ozone.

Of the potential level II factors which have been studied in detail so far, the modification of the ozone dose received by vegetation is species specific. Much further work is required before generalities can be made and level II factors can be incorporated into the definition of a critical level for natural vegetation.

6.6 ICP Vegetation experiments with natural vegetation

6.6.1 Experiments at the Coordination Centre

Natural vegetation species were exposed to ozone using the solardome facility at ITE-Bangor in 1999 (Figure 6.3) in order to select candidate species for use in an ICP Vegetation biomonitoring experiment. The species that were selected (Table 6.3) were ubiquitous in Europe, were easy to aquire and grow, were suitable for physiology measurements, and in some cases were known to be sensitive to ozone. The majority of the species were grown from seed obtained from commercial seed companies. Seeds of *Centaurea jacea* were collected from plants growing in Switzerland from natural populations that were sensitive (S) and resistant (R) to ozone based on expression of visible injury symptoms in ambient air.

Ozone exposure was at 100 ppb for 6.5 hours per day, four days per week for three weeks. Visible injury (ozone-specific and non-specific) was observed on bullrush, field scabious and red campion. Senescence was accelerated by ozone in birdsfoot trefoil and dark mullein. Stomatal conductance and photosynthesis were significantly reduced in the ozone sensitive variety of brown knapweed after exposure to the ozone regime for four days (Figure 6.4).

Fresh weight and dry weights were measured at the end of the three week exposure. The fresh weight was significantly less in the ozone treatment compared to the filtered air control for poppy, brown knapweed (S), dark mullein and birdsfoot trefoil (Figure

Figure 6.3: The solardome facility at Bangor

Table 6.3: Species used in the screening study at the Coordination Centre

Centaurea jacea (R)	Brown knapweed
Centaurea jacea (S)	Brown knapweed
Knautia arvensis	Field scabious
Digitalis purpurea	Foxglove
Verbascum nigrum	Dark mullein
Hypericum perforatum	St Johns wort
Lotus corniculatus	Birdsfoot trefoil
Papavar rhoeus	Field poppy
Ranunculus repens	Creeping buttercup
Sanguisorba minor	Salad burnet
Viola tricolor	Wild pansy/heartsease
Silene dioicia	Red campion
Silene alba	White campion
Typha	Bullrush

6.5), whereas the dry weight was significantly less in the ozone treatment for birdsfoot trefoil, creeping buttercup, brown knapweed (S) and poppy (Figure 6.6). Accelerated senescence had occurred in the ozone treatment for birdsfoot trefoil, brown knapweed (S) and dark mullein. Reduced biomass production had occurred in the creeping buttercup and poppy.

The effects observed in this short study occurred at similar ozone exposures to the current critical level for ozone, i.e. 3000 ppb.h. Different responses were observed in different species and there were also differences in the sensitivities of the species studied, with some species being much more sensitive to ozone than others. On the bases of these results, two comparisons were selected as suitable for further study. Firstly, the ozone-sensitive and ozone-resistant biotypes of brown knapweed would be appropriate for study because the differential response to ozone was quite marked. Secondly, the two biennials dark mullein and foxglove had similar growth habits but opposite reactions to ozone. Further experiments are planned with these species for 2000.

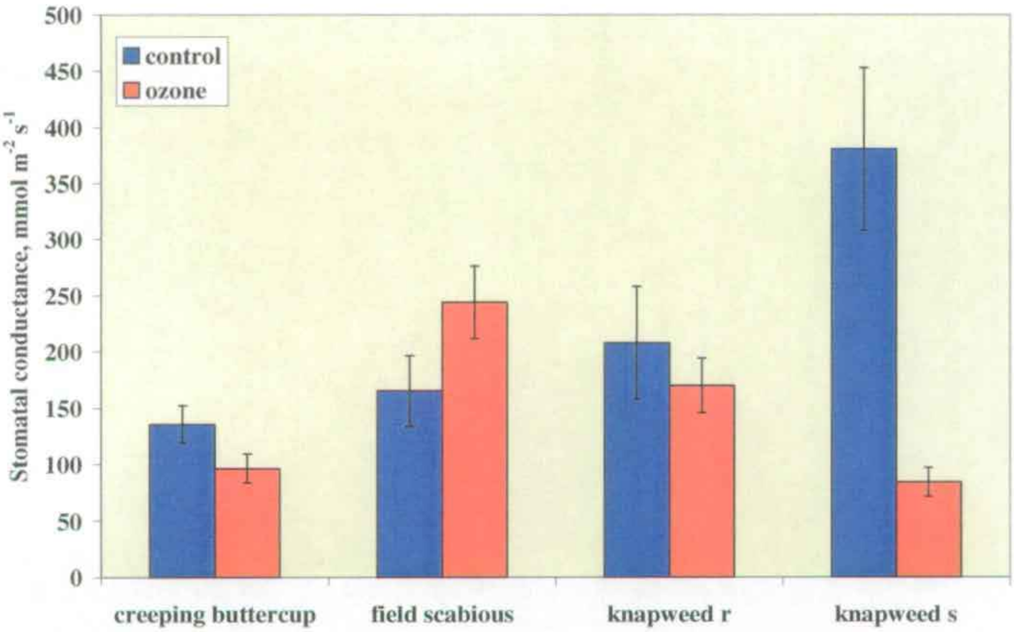

Figure 6.4: Stomatal conductance of selected natural vegetation species after exposure to the ozone or filtered air (control) regime for one week. Bars are standard errors.

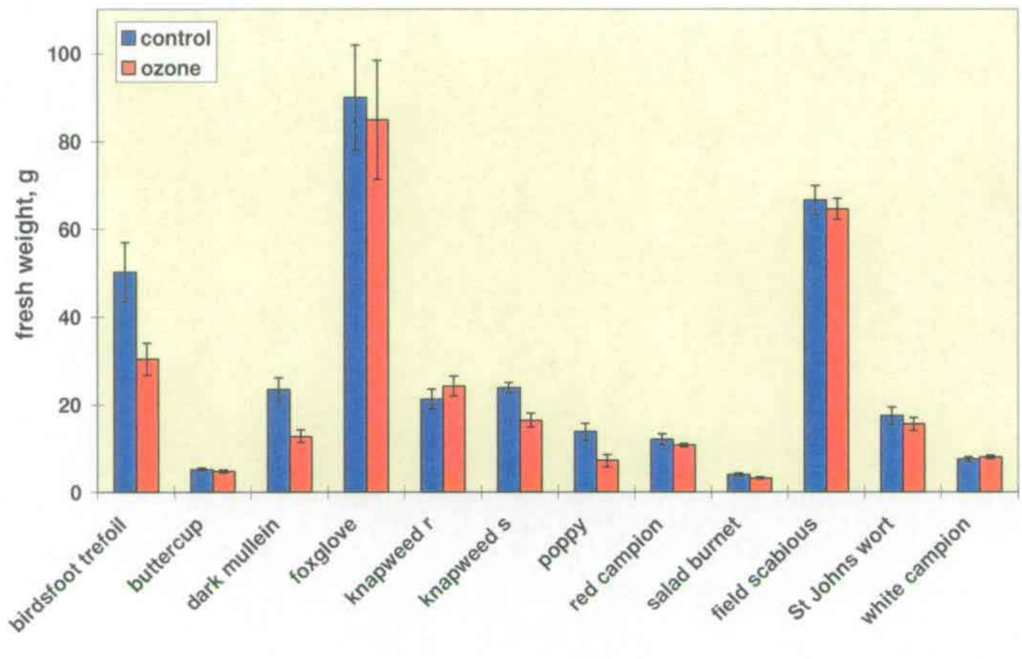

Figure 6.5: Fresh weights after three week exposure to ozone or filtered air control. Bars are standard errors.

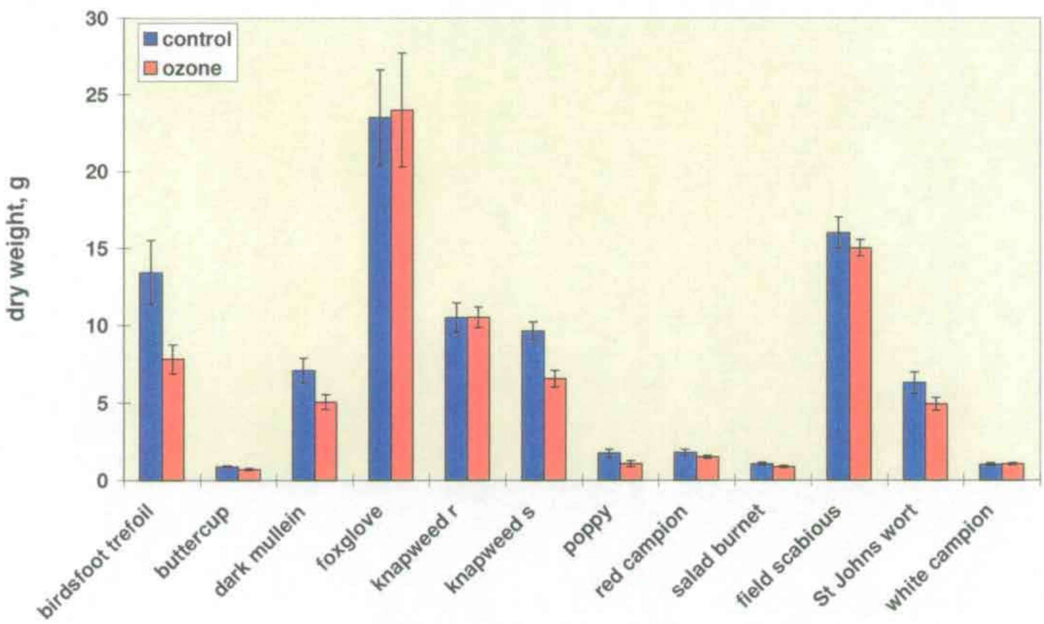

Figure 6.6: Dry weights after three week exposure to ozone or filtered air control. Bars are standard errors.

6.6.2 The EU-funded BIOSTRESS project

The BIOSTRESS (BIOdiversity in Herbaceous Semi-Natural Ecosystems Under STRESS by Global Change components) programme started on 1st January 2000 under the leadership of Dr Andreas Fangmeier (University of Giessen, Germany). This project involves participants from 8 ICP Vegetation groups, and will report annually to the ICP Vegetation as the ICP has been listed as one of the "end-users" of the findings of the project.

The project aims to provide an understanding of the general ecological processes by which global change components affect biodiversity. Tropospheric ozone will be used as a tool to perturb early-seasonal growth in several types of semi-natural, herbaceous ecosystems across Europe. The objective of BIOSTRESS is to gain a generally-applicable model of the effects of global change components on biodiversity in those ecosystems and, through a system of plant functional types, to provide a general expert system tool for vegetation science based on outputs from this model. This will be designed to be directly relevant to European and national biodiversity strategies. Output from the BIOSTRESS project will also be directly useful for helping evaluate critical levels of ozone for semi-natural vegetation.

One of the hypotheses to be tested is whether ozone affects plant species diversity. Grimes CSR strategies will be assigned to the plants tested as a general classification and this will also be used as a predictive tool for any changes. Experiments to be carried out as part of this programme range from mesocosms to field exposure systems. Species to be used include those of wet grasslands and annual weeds. Mechanistic models using physiological plant traits will be developed along with cellular automata plant community growth according to CSR strategy to look at long-term shifts.

6.6.3 Other nationally-funded research

Natural vegetation experiments are continuing at the University of Newcastle and the University of Bradford, UK and data from these experiments are being used in development of models to identify features which make a plant sensitive to ozone and to make generalised models of responses of plants to ozone. Ozone-exposure experiments on wet grassland species using open-top chambers are being conducted in the Netherlands to study the effects of competition and ozone-drought interactions. Field studies of brown knapweed (*Centaurea jacea*) and red clover (*Trifolium pratense*) are occurring in Switzerland. These and other species are being exposed in open-top chambers to identify and characterise some of the level II factors modifying the response of the plants to ozone. In Slovenia, ICP Vegetation participants are monitoring for injury in natural vegetation in ambient conditions in nearby fields and have observed natural white clover with typical ozone injury. In Spain, eco-physiological responses of Mediterranean wild plants to ozone are being investigated in ozone exposure experiments. The results of these studies are made available to the ICP Vegetation for use in analysis of critical levels for natural vegetation.

6.7 Discussion

The exposure-response experiments performed so far with natural vegetation species have shown that 27 species developed ozone injury at concentrations up to 80 ppb, 32 species responded to ozone by reducing biomass, and only 10 species exhibited both responses. The relatively small number of species that belonged to the latter group of plants may not be a true reflection of the frequency of occurrence of both effects since both responses were not always measured in each of the experiments reviewed. Nevertheless, these figures do confirm the general opinion that the presence of ozone injury is not necessarily an indication of a response that is of ecological significance to the plant. The review of the literature also revealed that there are no immediately identifiable features reported to be associated with sensitivity to ozone in natural vegetation. Ozone-sensitive species tended to have the characteristics associated with a ruderal or competitor growth strategy. However, these two categories are too broad to be used to identify ozone-sensitive species, and experiments have shown that there are several species in these groups that are ozone-tolerant.

By using artificial neural networks, the ICP Vegetation hoped to analyse exposure-response data in a non-subjective way to further identify characteristics associated with ozone injury. Initial ANN analysis confirmed that the Grime classification (ruderal, competitor, stress tolerator (Grime, 1979) was not a good indicator of ozone sensitivity as the r^2 values for both test and training data were below 0.5. Models based on the Ellenburg (1988) classification performed better (r^2 of 0.92 for the training data and 0.67 for the test data) indicating that the ecological requirements (water, temperature, nutrients) of the species were better indicators of sensitivity to ozone. The ANN Model based on physiological characteristics (e.g. relative growth rate and stomatal conductance) proved to be a good indicator of the likelihood of ozone injury development in 9 species of grassland vegetation. The modelling work presented in this section has indicated that there is scope for a much more detailed study of the nature of factors influencing ozone sensitivity. It might then be possible to identify plant communities that have a high proportion of ozone-sensitive species and that might therefore be classified as being "at risk" from ozone pollution.

For the reasons outlined above, little progress has been made in defining a definitive critical level for natural vegetation. The value for annuals is still that used for crops, and a critical level for perennial species has only tentatively been suggested. At this stage, the "effects-community" is not in a position to propose level II critical levels for natural vegetation. The newly established BIOSTRESS project, as part of the ICP Vegetation, will be an invaluable source of relevant information in the future. It is also anticipated that a biomonitoring system for natural vegetation using either ozone -sensitive and -resistant biotypes or species, will be incorporated into the ICP Vegetation experimental programme in the near future. The latter will provide data for the development of a predictive model of ozone effects on one species that incorporates the influence of level II factors.

7 Monitoring Heavy Metal Deposition to Clover

The heavy metals analysis described in this section was performed for the ICP Vegetation by Dr Ludwig DeTemmerman, Belgium, as a "contribution in kind" to the ICP.

7.1 Aims

The *Workshop on Critical Limits and Effect Based Approaches for Heavy Metals and Persistant Organic Pollutants* (Bad Harzburg, Germany, November 1997) identified a need for more information on the deposition of heavy metals to plants. In 1998, the ICP Vegetation added the study of heavy metal deposition to crops into the objectives of the programme. Following advice from a member of the ICP Vegetation Steering Committee (Dr L. De Temmerman, VAR, Belgium), it was agreed that the clover clone system used to investigate the effects of ozone on biomass could serve as a system for monitoring heavy metal deposition to crops.

The aims of this study were:

- To determine the feasibility of using the white clover clone system to assess heavy metal deposition at ICP Vegetation sites.
- To analyse clover samples from the sites for lead, cadmium, copper and arsenic content.
- To consider how this approach can be used to validate EMEP deposition models for heavy metals.
- To initiate negotiations for the transfer of the "Heavy Metals in Mosses programme" to the ICP Vegetation.

7.2 Introduction

Concern over the accumulation of heavy metals in ecosystems, and their impacts on the environment and human health, increased during the 1980s and 1990s. The LRTAP Convention responded to this concern by establishing a Task Force on Heavy Metals (and persistant 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". Shortly before the protocol was signed, methods for an effects-based approach were considered at a workshop in Bad Harzburg (October, 1997). The Workshop concluded that more research was needed to establish methods for deriving critical values for heavy metals. A shortage of information on deposition to crop plants was noted, and the ICP Vegetation responded by including such measurements in its workplan in 1998. The results were presented at the follow-up *Workshop on Effects-Based Approaches for Heavy Metals* (Schwerin, Germany, October, 1999). A new sampling and analysis regime is planned for the summer of 2000. Furthermore, in recognition of the progress made with the clover analysis, the ICP Vegetation has been asked to take over an existing programme investigating heavy metal deposition to mosses in over 30 countries.

The heavy metals protocol of the LRTAP Convention targets three particularly harmful metals: cadmium, lead and mercury. Parties will have to reduce their emissions for these three metals to below their levels in 1990 (or an alternative year between 1985 and 1995) by cutting emissions from industrial sources (iron and steel industry, non-ferrous metal industry), combustion processes (power generation, road transport) and waste incineration. Lead and cadmium analysis were included in the analysis of the clover clones used in the ozone experiment of the ICP Vegetation. The mercury content was not measured because its volatility can lead to its re-release from plants, making quantification from dried material difficult and unreliable. Instead, the arsenic and copper content was determined as these two elements can also be harmful if accumulated in the environment or foodstuffs, and are included on the list of heavy metals requiring consideration by the Convention.

Data on the deposition to growing plants is essential for validation of heavy metal deposition maps that have been developed from data from direct deposition to rain gauges or their equivalent. Values from such deposition gauges do not necessarily relate to the uptake by vegetation. First, the deposition must be intercepted by plants, either directly through the leaves or indirectly by deposition to the soil followed by root uptake. The deposits may then be removed by rain, wind or dew formation etc. and any accumulated metals may subsequently be lost by litter fall. Thus, plants accumulate heavy metals by root uptake and by leaf interception of dust particles originating from local or long-range transport. Accumulation of dust deposits by living plants is a dynamic process. Biomass increase has a diluting effect on the metals, but at the same time covering of the soil surface increases, allowing more interception of the particles. From the concentration of heavy metals in the plant biomass, the covered soil surface at harvest, and the exposure time, the part of the vertical flux accumulated by the plants can be calculated. This flux can then be compared to dust deposits measured with NILU rain gauges.

7.3 Using the clover clone system to monitor heavy metal deposition

7.3.1 Sampling regime

Samples from the 1998 experimental season (plus one from the 1999 season) of the ICP Vegetation were used to assess heavy metal depositon at 18 of the experimental sites. Participants grew the NC-S (ozone-sensitive) and NC-R (ozone-resistant) clones according to the standard protocol (Section 2.3). Two samples of the dried harvested material (leaves and stems) per clone per 28d harvest were sent to the Veterinary and Agrochemical Research centre (VAR) for analysis. Samples from the first harvests were excluded from the analysis because the plants were establishing outdoors at this stage. Material from 3 to 4 succesive harvests per site were analysed for their lead, cadmium, copper and arsenic content. The soil substrate was not standardized at each site, and thus samples from the different locations were also included in the analysis.

7.3.2 Analysis of heavy metal content

The analysis was carried out at VAR, Belgium using a technique based on the CII method (Comité Inter-Instituts d'études des techniques analytiques) of ashing the dried material, dissolution in HNO_3, and measurement with graphite furnace atomic absorption spectrometry (GF-AAS). The same method was used for peat based organic soils. Mineral soils were extracted with HNO_3/HCl 1:3 (*aqua regia*) and measured with GF-AAS. The detection limits were 0.08, 0.025, 1 and 0.009 µg g^{-1} d.m. for lead, arsenic, copper and cadmium respectively.

7.3.3 Validation

From the analysis of the duplicate samples (two containers per clone) it appeared that the reproducibility of the measurements was satisfactory for the different elements. In spite of a different growth rate for the NC-S and NC-R clones at some of the sites (see Section 2), there was no clear difference in bioaccumulation of arsenic, cadmium, copper and lead between the clones (e.g. Pb, Figure 7.1). When comparing concentrations of heavy metals in clover plants, the amount of growth is not of major importance as an increase in biomass is related to an increase in leaf surface and a better interception of the dust fallout. As a precaution though, the data presented is this report is only from the NC-R clone.

A further consideration was the influence of root uptake on the content of the foliage given that different substrates were used at each site. Organic and mineral substrates were considered separately (Table 7.1). No clear relationships were found between the lead and cadmium contents in the growth media and the clover forage indicating that the cadmium and lead content in the soil is not the predominant source of those elements in the above-ground biomass. For copper, there was a relationship between the content in mineral soil and the forage (Figure 7.2), but no such relationship existed for the organic soils. Thus, with the possible exception of measurements of the copper content of clover grown in mineral soils, there was little input from the soil substrate and the measured heavy metal contents can be considered to have been mainly deposited on to the foliage from the atmosphere.

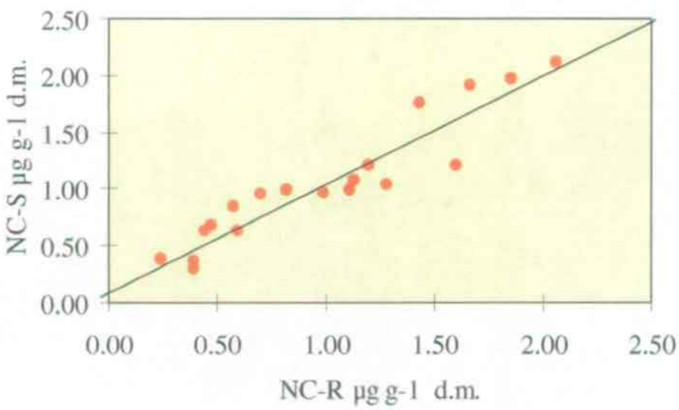

Figure 7.1: The lead content of the NC-R clone versus that of the NC-S clone.

Table 7.1: Regression of the heavy metal content of the soil substrate against that of the clover forage.

	r^2 from linear regression	
	Organic substrates	Mineral substrates
Lead content	0.04	0.01
Cadmium content	-0.2	0.01
Copper content	0.32	0.60

*not assessed for arsenic because the levels were below the detection limit at most sites.

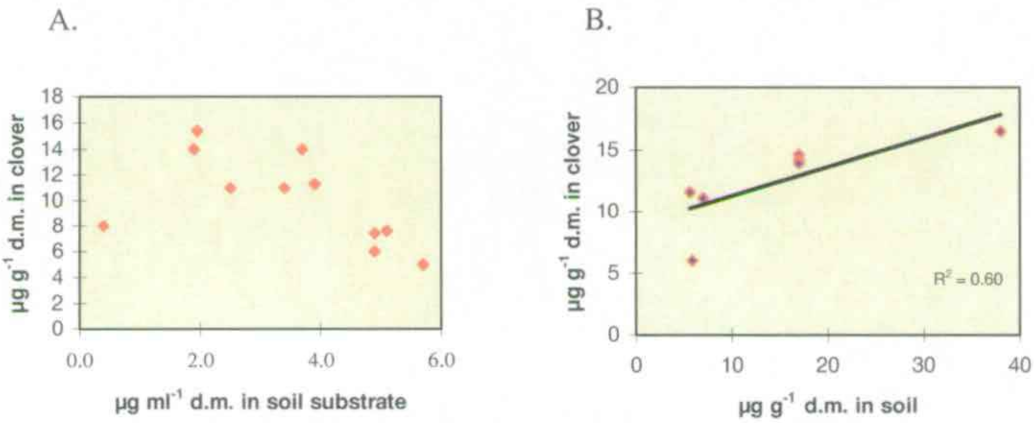

Figure 7.2: The relationship between the copper content of the clover forage with that of the (A) organic and (B) mineral soil substrates used at the sites.

7.4 Patterns in the Heavy Metal Content of Clover

The mean heavy metal content of the forage at the participating sites is presented in Figure 7.3.

The natural heavy metal contents, originating from root uptake, were low in the clover foliage, and in a few cases were below the detection limit of 0.08, 0.025 and 0.009 µg g^{-1} d.m. for lead, arsenic and cadmium respectively. Copper, being an essential element was present at higher concentrations in the forage; plants can suffer from copper deficiency when there is not sufficient copper available.

Lead

The seasonal mean lead concentration in clover ranged from 0.24 µg g^{-1} d.m. in the coastal areas of France, Wales and Germany to 2 µg g^{-1} d.m. in Central Europe. The highest lead depositions were found in Germany-Cologne, Belgium-Tervuren, Switzerland-Cadenazzo and Italy-Rome and can, to some extent, be linked to the high traffic density in those areas.

Cadmium

For cadmium, the concentrations ranged from 0.019 to 0.12 µg g^{-1} d.m.. The highest cadmium concentrations in clover were found in Belgium-Tervuren, Germany-Cologne and Germany-Trier and their surrounding areas. Emissions maps for cadmium also indicate high levels in these areas.

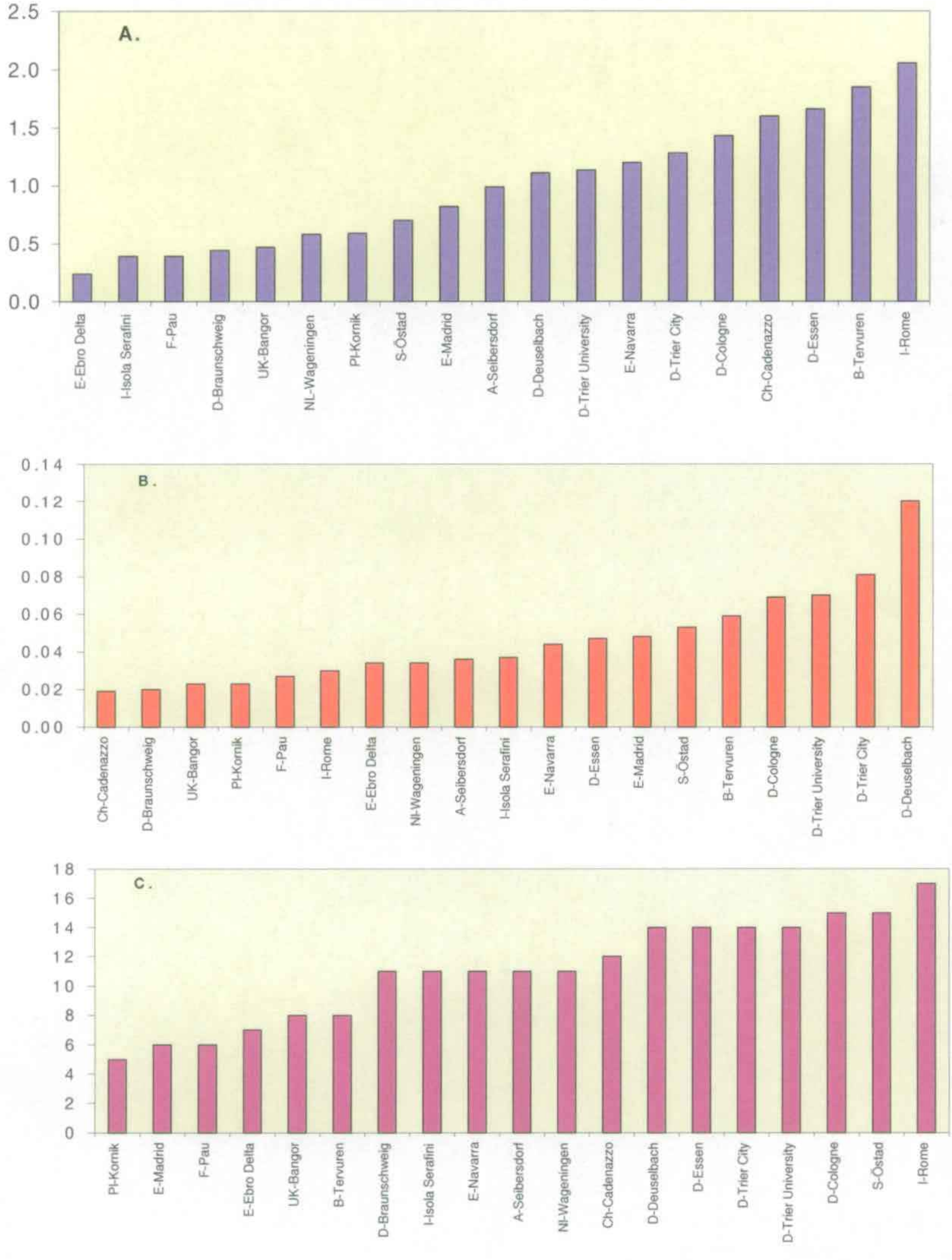

Figure 7.3 The content of (A) lead, (B) Cadmium, and (C) Copper in the forage of the NC-R clone of white clover. Each value represents the mean content per 28d harevest interval in $\mu g\ g^{-1}$ d.m. The sites are ordered by increasing content.

Arsenic

The natural arsenic content of clover is very low, and was below or very close to the detection limit of 0.025 $\mu g\ g^{-1}$ d.m. at over half of the sites. The seasonal mean values were only clearly above the natural content expected from root uptake at the following sites: Italy-Isola Serafini (0.074 $\mu g\ g^{-1}$), Spain-Madrid (0.090 $\mu g\ g^{-1}$), Spain-Navarra (0.081 $\mu g\ g^{-1}$), and Italy-Rome (0.114 $\mu g\ g^{-1}$).

Copper

It is difficult to draw conclusions from the copper contents in clover because root uptake could have markedly influenced the clover content. Nevertheless, the highest values were found at sites near to large cities or affected by major roads.

For most of the experimental sites, the heavy metal accumulation can be attributed to dust deposits on the leaves from long- or mid-range transport of heavy metals as there were no known large local sources. A contribution from short distance transport was likely at the sites in large towns or in the neighbourhood of areas with high traffic density.

7.5 The Heavy Metals in Mosses Project

A new development for the ICP Vegetation was agreed at the 18th Session of the WGE (August, 1999). Following on from the success of the above pilot study on the deposition of heavy metals to, the ICP Vegetation has been asked to take 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, has grown in size to include 30 European countries in the last survey in 1995. Some 64,000 measurements were made in the latest survey thus providing a comprehensive picture of metal deposition across Europe (Rühling and Steinnes, 1998). Use of mosses for this type of survey has several advantages over conventional precipitation analysis as sampling is easier without the need for expensive equipment and the higher trace element concentrations in mosses make analysis more straightforward and less prone to contamination. Several regression approaches have been used to relate the results from moss surveys to precipitation monitoring data (Berg and Steinnes, 1997). Whilst the Nordic Council of Ministers via the Nordic Working Group on Monitoring and Data (NMD) are currently supporting the early phases of the year 2000 survey, their initial remit of supporting the development and harmonisation of methodologies has now been fulfilled, especially as the programme has considerably outgrown its original framework as an entirely Nordic project. In view of this, the ICP Vegetation has been invited to incorporate the programme within its remit in order to utilise this important source of data for the heavy metals protocol. This will be in collaboration with EMEP, the Coordination Centre for Effects, the ICP Mapping and the ICP Integrated Monitoring, and will take place officially on 1 April, 2001. In the meantime, the year 2000 sampling survey will be conducted by Professor Rühling, and the ICP Vegetation will begin to collate the data.

7.6 Discussion

The results presented have shown that the clover clone system for detecting effects of ozone can have the dual purpose of being used to monitor heavy metal deposition to crops. Concerns over the contribution from root uptake were largely unsubstantiated for arsenic and lead because these elements are not readily taken up by the plant and thus there was no relationship between soil and forage content. However, the soil type and acidity were important for the uptake of copper and to a lesser extent cadmium. It would be preferable to have a standard soil mixture at all sites to increase the comparability. Unfortunately, this was not found to be feasible in earlier ICP Vegetation experiments as it was not possible to grow plants equally well in a standard substrate at all sites in the different climates experienced in the network. Furthermore, the basic substances for the substrate were not found to be identical in the different countries. The possible contribution from soil uptake will be taken into account in the following interpretation of the data.

Some patterns have emerged in the data. For example, the site at Italy-Rome had the highest detected content of lead, copper, and arsenic. Since this site is close to the centre of Rome, it seems likely that dust deposition from local sources might be responsible for these high values. Other sites close to city centres e.g. Spain-Madrid, Germany-Trier City, and Germany-Cologne also feature in the group of sites with the highest levels of each heavy metal. It is more appropriate to consider the metal content of the rural sites that are away from local sources such as motorways in order to gain an impression of the input from long-range transport. Sites such as Austria-Seibersdorf and Netherlands-Wageningen fall into this category and had similar mid-range heavy metal contents for all four metals. However, a similarly rural site at Germany-Deuselbach had comparable contents for three of the metals, but had the highest cadmium content of all sites with no obvious root uptake. This suggests that emissions from a local cadmium source might be being deposited on the clover at this site.

Excluding inputs from local sources, the broad patterns of lead and cadmium content at the ICP Vegetation sites largely reflected those predicted by ESQUAD (1994). For example, the lead content was "high" at sites in the Benelux countries, Switzerland and northern Germany that were predicted to have high deposition rates. Similarly, patterns in the concentrations of heavy metals in mosses in the Nordic Council-funded project (NORD, 1998) broadly reflected those determined by the ICP Vegetation. However, the concentrations in the mosses used by the Nordic Council were approximately a factor of 10 higher then in the clover clones. This could reflect the time period of exposure (28 days compared to an undefined period covering the age of the moss), physiological differences between the receptors, chemical differences in the substrate used (growing media versus forest soils) or the location sampled (open field versus forest clearing).

This provisional study has indicated that the clover clone system can be used to monitor heavy metal deposition at ICP Vegetation sites. The next stage is to repeat the sampling in 2000 using a revised Protocol. Additional measurements will be made such as leaf area index that will allow deposition rates to be calculated. The results, together with those from the 2000 mosses survey, will ultimately be used to validate EMEP and ESQUAD deposition maps.

8 Conclusion and further work

8.1 An overview of the main results

The number of sites contributing to the ICP Vegetation network has doubled in the last three years indicating the widespread interest in the programme. National networks have been established in countries such as Italy, Germany, Spain and Slovenia, and the Russian Federation, Greece and Ireland have joined or re-joined in the last three years. With such a diversity of sites, it has been possible to monitor both the responses of sensitive species of plants to ambient ozone and the heavy metal content of clover across most of Europe.

Measurement of the ozone concentrations at the ICP Vegetation sites have shown that the long-term critical level of an AOT40 of 3 ppm.h was exceeded at over 70% of the sites in each of the three years, with the greatest exceedance occurring in 1999 (86% of sites). The rural ozone concentrations increased on a north-south transect with the highest rural concentrations being recorded at Switzerland-Cadenazzo and Italy-Isola Serafini. Local sources of NO_x reduced the ozone levels at some semi-urban sites such as Germany-Trier, Germany-Cologne, and Spain-Madrid. The highest AOT40 values were recorded in northern Italy and North-Carolina (USA) where values were 7 - 11 times the current critical level. On the basis of AOT40 alone, the measurements have shown that there was considerable potential for impacts on vegetation in Europe, especially in the more sourthern countries.

Two effects on vegetation were detected. First, the ozone climate of Europe caused visible injury to occur on the test species (white clover, *Trifolium repens* cv Regal) at least once at every site in the network in 1997, 1998 and 1999 with a higher incidence of injury-causing episodes occurring in southern Europe. Ozone injury was also quite prevalent at the northern site of Sweden-Östad where a low mean VPD of 0.83 kPa (28d mean, 1997 and 1999 season) increased ozone uptake. Surveys of commercial fields revealed the presence of ozone injury on many of Europe's most important agricultural and horticultural crops (e.g. wheat, maize, soybean, grapevine and tomato). Although injury might not necessarily be associated with changes in yield, these surveys have shown that 22 agricultural and horticultural crops are "at risk" from ozone pollution. Secondly, the biomass of an ozone-sensitive clone of white clover was regularly reduced by ozone in southern Europe with occasional reductions occurring in central and northern Europe. When all of the biomass data were combined together, AOT40 was found to be the parameter with the best fit to the data but there was some scatter ($r^2 = 0.4$).

The influence of modifying (level II) factors on the biomass response to ozone was studied by developing a parsimonious model that only contained the most important influencing factors as inputs. Over 240 input combinations were tested. The ozone conditions at the sites were described in the best performing ANN model, PROBE, by AOT40 and $O_{3\ 24h}$ which together provide information on the "peakiness" of the ozone climate. An NO parameter was also included and appeared to only be important at sites with a strong local influence from traffic. In general, the input selection process indicated that parameters describing the ozone and NOx conditions were more important than those describing climatic conditions. Of the numerous temperature and VPD parameters tested, only T_{day} and T_{24h} were found to be important in the model suggesting that temperature effects on conductance and growth might be contributing to the biomass response. The lack of importance of VPD parameters may be because the effects of this parameter on flux are instantaneous and are lost in the averaging process. Repetition of the input selection process following inclusion of the 1999 data led to a model of similar structure and further improved the performance to an r^2 of 0.85 for previously unseen data compared to an r^2 of 0.4 for an AOT40-only model.

A unique feature of the ANN model, PROBE, was that it was developed from experiments in which plants were exposed to ambient air in a diverse range of pollutant and climatic conditions. More usually, models are based on data from artificial exposure experiments using facilities such as open-top chambers. Furthermore, the input selection process described in Section 2 was performed without the influence of prior knowledge. This approach is in contrast to that used by Dr L Emberson and colleagues, in which only those factors known to influence flux were considered in the development of the model. Another feature of PROBE is that it was developed from data from well-watered plants and thus avoids any modifying effect of SMD. Because the ANN model had been developed from data from ambient conditions, it could be used to predict responses to ozone for the range of conditions experienced in Europe. For example, the model predicted that the AOT40 required to reduce clover biomass by 5% over 28d ranged from 0.9 ppm.h to 1.65 ppm.h at an average daylight mean temperature of 19 °C depending on the level of NO_{1700} and $O_{3\ 24h}$. By extracting an equation from the model, it has been possible to use PROBE to predict clover biomass for Europe using a 150 x 150 km grid. $PROBE_{equation}$ predicted that reductions in biomass ratio were highest in parts of Italy and central Spain, with reductions of 10% or more predicted for most of Portugal, Spain, France, Italy and Greece. This zone of highest effect was further south than that predicted for wheat suggesting that when growth stage and SMD are not limiting, significant impacts of ozone can occur in these countries.

The flux models of Dr L Emberson and colleagues have suggested that the ozone flux in June is high in Europe in the northern half of France, most of Germany, The Netherlands and Belgium. For most of these areas, anthesis falls in early-mid June; high ozone fluxes in June would thus be occurring at the time when the crop was most sensitive to ozone and would be likely to have a large impact on the final yield (Soja *et al*, in press). The same areas of Europe were identified by Dr M. Posch and colleagues as having the highest "modified AOT40" when modifying factors for phenology and SMD were incorporated into the AOT40 response function for wheat. It was less appropriate to compare the two models for more southern countries such as Spain, Italy and Greece since ICP Vegetation participants have shown that anthesis occurs in Spain (and possibly Greece and Italy) in early May. Thus, wheat would be senescing for a large part of June, and ozone flux would, as predicted by Emberson and colleagues, be significantly reduced. By shifting the timing of the three-month window according to phenological considerations, the method employed by Posch and colleagues allows the "modified AOT40" to be established for an appropriate time period for these countries. The modelling suggests that crops grown in Italy and Slovenia are more at risk from ozone than those grown in Greece and Spain. In summary, both flux-modelling and modified dose-response modelling indicated that ozone effects may not be greatest in those areas with highest AOT40 because high SMDs and VPDs in these areas are predicted to reduce the absorbed dose of ozone, and hence the effect.

Recent progress with all three modelling approaches was considered at the Gerzensee Critical Levels Workshop (April, 1999). It was agreed that the ability to estimate absorbed ozone dose makes an important step towards establishing level II values for ozone. However, the link between absorbed dose and damage needs to be made before reliable estimates of actual ozone damage can be determined. The application of the basic concepts used in modelling ozone uptake, to the process of estimating of ozone deposition, should result in more reliable calculations of ambient ozone concentrations as predicted with the EMEP photochemical ozone model. As these methods will take some time to be developed, it was agreed that important progress could be made in the meantime using the modified AOT40 approach. However, concern was raised about reliance on the data from open-top chamber experiments due to the influence of the chamber system on ozone uptake (see Section 3.6.2). By using data from the ICP Vegetation clover clone experiments, especially those planned with field-grown plants, a flux-effect relationship can be established that is relevant for plants grown in such a wide range of ambient conditions.

A review of the literature on the effects of ozone on natural vegetation has confirmed a range of sensitivity with the most sensitive species being at least as sensitive to ozone as the most sensitive crops. The rate of uptake of ozone into the plants is likely to be species specific as a wide range of stomatal conductance has been reported for optimum climatic conditions. A pilot study has shown that it is feasible to identify the factors associated with ozone sensitivity in natural vegetation using ANNs. Using a limited data set, the two models based on growth requirements (Ellenberg classification) and plant parameters performed better than that based on ecological strategy (Grime classification). This approach will be expanded by pooling data from other nationally-funded research programmes with the aim of identifying natural vegetation communities that are at risk from ozone pollution. Natural vegetation experiments are currently being planned for use in future years of the ICP Vegetation experimental programme with the ultimate aim of suggesting an improved definition for the critical level for these species.

Concern over the impacts of heavy metals on the environment and health led to the development of the Heavy Metals Protocol (signed in 1998), which commits countries to a reduction in emissions. Analysis of the lead, cadmium, copper and arsenic content of clover at 18 ICP Vegetation sites showed that concentrations were highest at sites like Italy-Rome and German-Cologne where dust deposition was likely to be affected by local traffic and industrial sources. Plants grown at sites in rural areas of Austria, The Netherlands and Germany were away from local influences and thus their mid-range heavy metal contents were more likely to have resulted from long-range transport. Since these sites were in the areas predicted by ESQUAD (1994) to have relatively high heavy metal deposition, it seems reasonable to accept that the clover clone system can be used to validate these maps. Additional leaf area measurements planned for the year 2000 sampling season will facilitate calculation of deposition rates for clover. The results will also be compared with those from the year 2000 mosses survey.

In conclusion, the ICP Vegetation has shown that the ozone pollution climate of Europe is having an impact on vegetation by causing both visible injury and reductions in biomass in sensitive species. Incidences of injury occurred at every site in every year of the experiment on the test species, and were frequently reported on commercial crops. Biomass reductions in white clover were less widespread, but were common in the southern countries. Changes in biomass were best described by inclusion of 24h mean ozone concentration, temperature and NO in the response model for AOT40. Level II modelling for wheat showed that low soil moisture content, low humidity, and early maturity reduced the impact of ozone in southern countries, with greater effects expected in central and more northern areas where these factors were less limiting to ozone uptake. Initial studies have shown that a large number of species of natural and semi-natural vegetation are sensitive to ozone, but that further work is necessary in order to identify specific communities at risk from ozone pollution in Europe. The heavy metal content of clover was highest in areas influenced by local industrial and traffic sources, but was also significant in rural areas suggesting an input from long-range transport.

8.2 Further work

The ICP Vegetation agreed at its 13th Task Force Meeting (January, 2000) to further develop the programme in the following ways. This will allow the programme to continue to take a leading role in level II modelling and mapping for ozone effects on crops and natural vegetation, whilst expanding further the work on heavy metals by consideration of deposition to both clover and mosses.

8.2.1 Critical Levels for Ozone

- Conduct experiments with pot- and soil-sown clover to monitor the spatial differences in response to ozone, and use the data to develop a flux-effect model.

- Further development of the incorporation of modifying factors into the AOT40 response function for wheat, and compilation of a database for sources of information on modifying factors and dose-response functions for other commercially important crops in the UN/ECE area.

- Further parameterisation and validation of the ozone flux-effect model for wheat, and efforts to establish the link between absorbed dose and plant response for other important European species.

- Studies to establish how absorbed ozone dose should be quantified to give the best relationship with species response (i.e. cumulative ozone dose throughout the growing season, cumulative dose above a threshold throughout a growing season, phenologically-weighted cumulative ozone dose).

- Conduct surveys of the use of irrigation in commercial fields near ICP-Vegetation experimental sites.

- Use the above information to assess the economic cost of losses in crop production due to ozone.

- Continue to develop methods to identify ozone-sensitive natural vegetation species and functional types by pooling and analysis of existing data.

- Conduct nationally- and EU-funded studies of the factors associated with ozone sensitivity in species of natural vegetation, like water and nutrient status, and contribute the data to a central database.

- Develop a monitoring system for natural vegetation that is similar to the clover clone experiment and is suitable for use at a range of locations in Europe.

- Develop methods to facilitate the mapping of ozone sensitive plant species, populations and communities in Europe.

8.2.2 Deposition of heavy metals to clover

- Conduct a new sampling programme in the summer, 2000, followed by analysis of lead, cadmium, arsenic and copper content.

- Calculate deposition rates to clover by inclusion of total area of the clover foliage.

- Compare deposition to clover at ICP Vegetation sites with ESQUAD deposition maps and maps of heavy metal content of mosses.

8.2.3 Deposition of heavy metals to mosses

- Assist Professor Rühling (Lund, Sweden) with the administration for the year 2000 survey for heavy metals content, and facilitate the transfer of the programme to the ICP Vegetation on April 1st, 2001.

- Collate and analyse data from the year 2000 survey and use the data to produce a colour report illustrating the main results.

- Convert existing data from the previous four surveys into a usable format for mapping and analyse trends in the data.

Investigate ways of estimating actual deposition of heavy metals to terrestrial surfaces from the moss survey data allowing deposition maps to be produced.

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Appendix 1. Publications list for the ICP Vegetation

1. Reports to the DETR

1.1 Annual Reports

Mills, G., Palmer-Brown, D. Ball, G. (1998). First Year Report to the DETR for Contract EPG 1/3/96. The UN/ECE ICP-Crops International Cooperative Programme on Crops. April, 1997 – March, 1998. 54 pages. ITE-Bangor Research Unit.

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Mills, G. and Hayes, F. (1999). Ozone and natural vegetation: An overview of existing knowledge and recommendations for further work. The UN/ECE ICP-NWPC International Cooperative Programme on Non-wood plants and Crops. 24 pages. ITE-Bangor Research Unit.

1.2 Short reports

Quarterly reports sent June, 1997, September, 1997, January, 1998, June, 1998, September, 1998, and January, 1999.

Minutes of the 11th, 12th and 13th Task Force Meetings of the ICP Vegetation (January 1998, 1999 and 2000 respectively).

Oral or written reports provided at:

14th and 15th Task Force on Mapping Meetings (May 1998 and June 1999).

16th and 17th Sessions of the UN/ECE Working Group on Effects (August 1997 and 1998).

Critical Levels for Ozone - Level II Workshop, Gerzensee, Switzerland (April, 1999).

1.3 Reports to the UN/ECE

Annual Progress Report for the ICP-Crops (August 1994 - July 1995). ICP-Crops Coordination Centre, The Nottingham Trent University, UK.

Annual Progress Report for the ICP-Crops (August 1995 - July 1996). ICP-Crops Coordination Centre, The Nottingham Trent University, UK.

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Annual Progress Report for the ICP-Crops (September 1997 - August 1998). ICP-Crops Coordination Centre, Institute of Terrestrial Ecology, Bangor Research Unit, UK.

Annual Progress Report for the ICP-NWPC (September 1998 - August 1999). ICP-Crops Coordination Centre, Institute of Terrestrial Ecology, Bangor Research Unit, UK.

Technical Report: Artificial neural networks - a novel computer modelling approach for predicting the effect of ozone on crops and non-wood plants. (EB.AIR/WG.1R.120). Prepared for the Fifteenth Session of the Working Group on Effects, July, 1996.

Technical Report: Improving the short and long-term critical levels of ozone for crops by incorporating Level II factors. (EB.AIR/WG.1/1997/9). Prepared for the Sixteenth Session of the Working Group on Effects, August, 1997.

Technical Report: Progress Report on the incorporation of level II factors into the critical level of ozone for yield reduction in crops and its subsequent mapping (EB.AIR/WG.1/1998/10). Prepared for the Seventeenth Session of the Working Group on Effects, August, 1998.

Technical Report: Further progress with level II modelling and mapping of ozone for crops and natural vegetation (EB.AIR/WG.1/1999/8). Prepared for the Eighteenth Session of the Working Group on Effects, August, 1999.

UN/ECE (1995) ICP-Crops Experimental Protocol for 1995. ICP-Crops Coordination Centre, the Nottingham Trent University, UK.

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1.3.1 Contributions to UN/ECE Working Group on Effect Documents

UN/ECE Substantive Report on Trends in the Impacts of Long-Range Transboundary Air Pollution. 1st and 2nd draft (1998 and 1999).

UN/ECE (1996) Joint Report of the International Cooperative Programmes and Mapping Programme. EB.AIR/WG.1/R.114.

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1.4 Publications produced by the Coordination Centre of the ICP Vegetation

1.4.1 Journals

Ball, G., Benton, J., Fuhrer J., Gimeno, BS., Skärby, L., Palmer-Brown, D., Roadknight, C. and Mills, G. (1998). Identifying factors which modify the effects of ambient ozone on white clover (*Trifolium repens* L.) in Europe. Environmental Pollution. 103: 7-16

Ball, G., Palmer-Brown, D., Fuhrer, J., Skärby, L., Gimeno, B.S. and Mills, G. Identification of non-linear influences on the seasonal ozone dose response of sensitive and resistant clover clones using artificial neural networks. Accepted by Ecological Modelling.

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Mills, G., Ball, G.R., Hayes, F., Fuhrer, J.,Skärby, L., Gimeno, B., De Temmerman, L. and Heagle, A., Members of the ICP Vegetation programme. Development of a multi-factor model for predicting the effects of ambient ozone on the biomass of white clover. Accepted by Environmental Pollution.

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1.4.2 Book Chapters

Ball, G., Palmer-Brown, D. and Mills, G.E. A comparison of artificial neural network and conventional statistical techniques for analyzing environmental data. Chapter 14 in: Artificial Neural Networks Eds S LEK and JF Guigan, Springer Verlag, 2000.

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1.4.3 Conference Proceedings

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

Benton, J., Fuhrer, J., Gimeno, B.S., Skärby, L., Balls, G.R., Palmer-Brown, D., Roadknight, C. and Sanders, G.E. (1995). ICP-Crops and critical levels of ozone for injury development. In: M. Knoflacher, J. Schneider and G. Soja (eds). Exceedance of Critical Loads and Levels. Umweltbundesamt (Federal Environment Agency) Wien, Austria. pp 97-112.

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1.4.4 Publicity brochures

Sanders, G. and Benton, J. (1995). Ozone pollution and plant responses in Europe - an illustrated guide. ICP-Crops Publication, The Nottingham Trent University. 15 pages.

Mills (1999). The International Cooperative Programme on Effects of Air Pollution and Other Stresses on Non-wood Plants and Crops. Institute of Terrestrial Ecology - Bangor Research Unit. 4 pages.

1.5 Publications by published by participants of the programme that describe the experiments of the ICP-Vegetation.

1.5.1 Journal Papers

Astorino, G., Margani, I., Tripodo, P. and Manes, F. (1995). The response of *Phaseolus vulgaris* L. cv. Lit to different doses of the anti-ozonant ethylenediurea (EDU) in relation to chronic treatment with ozone. *Plant Science*, 111: 237-248.

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Bungener, P., Nussbaum, S., Grub, A. and Fuhrer, J. (1998) Ozone sensitivity of grassland species. In: Breeding for a multifunctional agriculture. Eds: Boller, B and Stadelmann, F.J. Proc. Of the 21st Meeting of the Fodder Crops and Amenity Grasses Section of EUCARPIA, Ittingen, Switzerland, pp. 202-204. FAL Reckenholz-Zürich.

Fuhrer, J. (1995). Luftreinhaltung im Interesse des Pflanzenbaus. *Agarforschung*, 2 (6): 209-212.

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Appendix 2. UNDERSTANDING NEURAL NETWORKS

Introduction

Artificial neural networks (ANNs) are a useful tool for the modelling of plant-climatic interactions (Hirafuji and Kubota, 1994, Balls *et al*, 1995). They can be used to identify patterns within data and determine the influence of many interacting factors (Kothari and Heekuck, 1993), and are particularly good at analyzing noisy data containing non-linear interactions. Several studies have indicated that they can produce generalized models of environmental systems with greater accuracy than conventional statistical techniques (Comrie, 1997, Paruelo and Tomasel, 1997, Lek *et al*, 1996). ANNs do not rely on predetermined relationships as in the case of mechanistic modelling but derive their own relationships based on the patterns being modelled. These characteristics make ANNs very well suited to modelling biomonitoring data which contains complex interactions and influences and data from sites experiencing wide ranging conditions.

The structure and functioning of back-propagation artificial neural networks

ANNs can be run on a conventional PC using commercially available software such as Neuroshell 2 (Ward Systems Group). The ANN approach commonly used by the ICP Vegetation is based on back propagation, a technique that uses the errors associated with predictions as part of the network training (section 4). ANNs comprise three layers of several neurons, called the input layer, the hidden layer and the output layer (Figure 1).

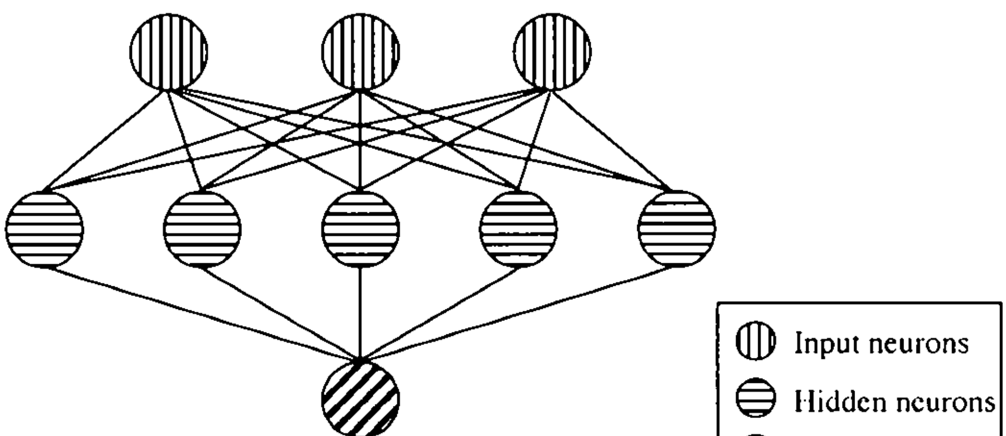

Figure 1: The structure of the multi-layer ANN

The input layer is where the causal agents (e.g. AOT40, humidity) of the network are represented. These are equivalent to the independent variables used in multiple linear regression. Each neuron of the input layer represents an individual variable. So, if a model were being constructed in which temperature was thought to have an influence, one of the input neurons would represent temperature. Each of the input neurons has a scaling function associated with it, which scales the inputs to a value in the range of 0 to 1. This serves two functions: firstly it converts the input numbers into a form the neural network can handle; and secondly it ensures all of the numbers are in the same range. Normally a linear scaling function is used.

The hidden layer of the network mathematically connects the input layer and the output layer. It is called the hidden layer because it has no connections out of the network. This layer serves as a feature detector for patterns in the data.

Finally, the output layer represents the effect(s) that the causal agents have brought about (e.g. biomass change) and is equivalent to the dependant variable(s) in a multiple linear regression. Thus, each of the output neurons represents an effect. For example, in the model mentioned earlier, if temperature were to influence the growth rate, then one of the output neurons would represent growth rate.

Each of the neurons of the three layers is connected to each neuron of the subsequent layer. So, the humidity neuron mentioned earlier would have a connection with all of the hidden neurons, and the same would be true for all of the other inputs. Each of the hidden neurons are also connected to each of the outputs. This creates the structure of the neural network (Figure 1).

How the network functions as a model

As has been mentioned earlier each connection of the network is represented by a weighting. The network produces an output value from an input value by using these weightings and the transfer functions of the hidden neurons and output neurons, to modify the input values to form the output values. Thus, when a set of inputs is presented to the network it mathematically modifies them to produce a value for each of the outputs of the network.

In practice this is achieved as follows: Each of the hidden neurons is connected to all of the input neurons by weighted links. Each hidden neuron receives values from each of the inputs in the form of a value that is scaled in the range 0 to 1 and then multiplied by the weight of the connection. The hidden neuron then takes all of the values it receives and sums them. This can be represented by the equation:

$$X = \sum_{i} Oi \,.\, Wij$$

Where: O is the output of the neuron; i is the neuron the output came from, j is the current neuron which is producing the output; Wij is the weight of the connection between the ith and jth neuron. The value produced is then applied to the transfer functions, which generates an output value. This may in the case of a sigmoidal transfer function (used in this study), be represented by the equation:

$$Oj = \frac{1}{1 + e^{-x}}$$

The output value is then applied to the weightings of the connections to the next layer of the network. The neurons of this layer again take the sums of these weighted values and apply them to a transfer function to produce an output as in the previous layer. If this layer is the output layer, then the output values are converted by a scaling function to produce an output value that is meaningful. There is an error value associated with each of the neurons of the network for each of the input patterns. This is a measure of the accuracy of the predictions made and is used in training the ANN model.

The learning process

Artificial neural networks using a back propagation algorithm, "learn" by making comparisons between their predicted output and the actual value being modelled (Dayhoff, 1990). When trained, the network should be able to produce an accurate prediction for all of the possible input patterns it will encounter, assuming the training data is fully representative of the whole set of possible data.

The network trains by multiple iterative feed-forward/back-propagation steps (Figure 2). In the first feed-forward step output values for a given data point are generated by presenting the network with its associated inputs. Next, an error value based on the differences between predicted and actual values is calculated.

Following this, in the back-propagation stage, the error value is sent back to the hidden layer (Figure 2). The hidden layer then updates the weights to the output layer, so that in the next iteration the error for the point will be reduced. This procedure is also repeated for the error values generated from the hidden layer, updating the weights leading from the input layer to the hidden layer. The rate at which weights are updated is controlled by the learning rate of the network. The greater the learning rate the faster the error is reduced in the next epoch (one complete cycle of training using all of the data points).

Associated with the learning rate of the network is a momentum value. This determines the magnitude of influence the error has on the weight and effectively smoothes changes in the weights of the network, preventing oscillations in the changes in weights and preventing minimisation of error. Both the learning rate and the momentum of the network are important factors that influence the way the network learns.

When the cycle of feed forward followed by back propagation is completed for all of the data, one "epoch" is completed and a mean error value is determined. If this mean error has improved, the network is saved to disk.

Potential problems with ANNs

One disadvantage of the back propagation algorithm is that it takes a long time to train and reach convergence (Dayhoff, 1990). Learning time depends on the complexity of the data being modelled and the number of input patterns in the data set. Another problem with back propagation networks is that training may fail, producing inaccurate results, because the network reaches what is called a local minimum. In this case the solution produced by the model does not represent the best solution available i.e. the network fails to accurately model the data and fails to reach an absolute minimum error. Local minima represent points in the training of the network where accurate prediction is made for a subset of the data, i.e. the network has found a partial solution to the problem. They are more likely to occur in noisy data, but can be overcome by giving the ANN a low learning rate, a high momentum rate, and by rigorously testing the model for a wide range of conditions.

Over-training is another problem associated with the training of ANN models. This occurs when the ANN learns the training data too well, modelling the errors in the data, without reflecting the general trends. Over-training reduces the ability of the network to make generalisations of the real-world solution and thus accurate predictions, based on data it has not previously been presented with. Over-training may occur, if the training is not halted soon enough, or if the network has too many hidden neurons, making it find more features in the data than it needs to be able to generalise well.

Over-training may be prevented by having a proportion of the data set that is not used for training the network ie a test data subset and training multiple sub-models using a range of numbers of hidden neurons. The test data subset is used to repeatedly test the progression of the training of the network, and gives an indication of the ability of the network to generalise. This can be represented by an error value for the test data. To prevent over-training the training process is stopped when this error value fails to decrease. Selection of the sub-model with the best test data performance (for a range of hidden neurons) further ensures the optimum parsimonious solution of the network is reached.

Advantages of using ANNs

Despite these potential problems, back propagation neural networks have a number of advantages. Firstly, they may be applied to a wide range of problems and situations, where they generally find an accurate solution. Back-propagation networks also have the ability to produce a predictive model when they have fully trained. This model may act as a stand-alone expert system. Finally, the weights of a trained back-propagation network may be analysed to determine the relative importance of the input factors of the network. The latter provides a powerful analysis tool.

Uses of ANNs

Weightings analysis

Weightings analysis is an analysis method which indicates the relative importance of each input to a trained ANN model. This is a useful tool for the identification of the strength of influences on environmental systems. For example, this technique was used to identify the important microclimatic influences on the AOT40 biomass dose response of clover. The relative importance of an input is calculated by taking the sum of the absolute weight values leading from each input.

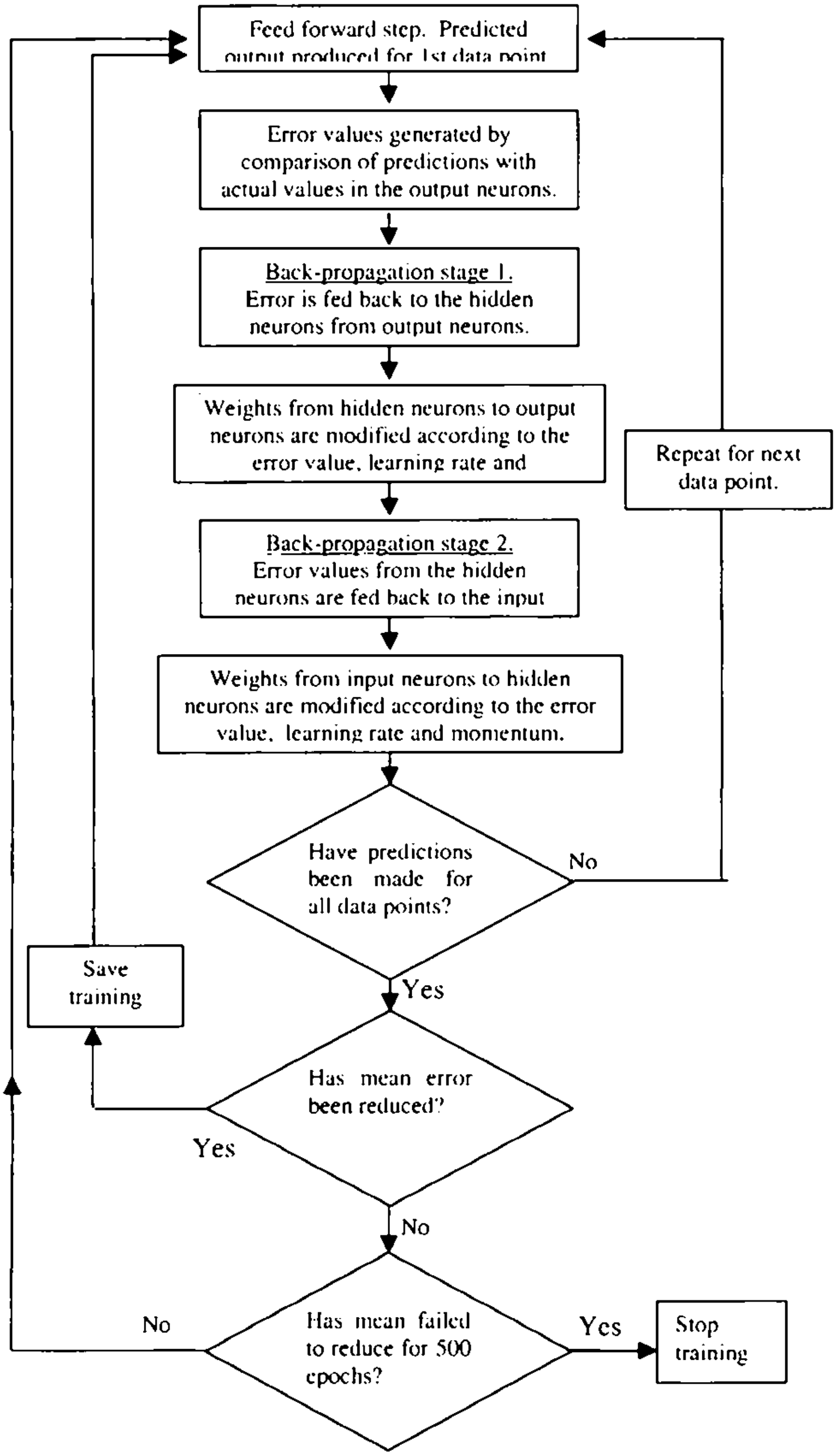

Figure 2: The ANN learning process using a back propagation algorithm

Predictive modelling

One of the most important applications is the predictive modelling of complex systems. This helps in the understanding by allowing visualisation of the processes occurring within the system and has proved useful in the prediction of the critical levels of ozone under different climatic conditions. Predictions can be made easily by embedding trained ANN models within spreadsheet packages such as MS Excel.

Extracting empirical equations from ANNs

Empirical equations can be extracted from optimized ANN models based on the weights of the ANN models (Roadknight *et al*, 1997). The equation extracted is a simplification of an equation containing multiple components (each representing a hidden neuron) based on sigmoidal functions, where the weights provide the constant terms. Extraction of empirical equations from the ANN allows presentation of the models to a wider audience and adds a greater transparency. The equations can also be used within other applications for example Geographical Information Systems or decision support systems.

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